

**CENTER FOR DRUG EVALUATION AND  
RESEARCH**

*APPLICATION NUMBER:*

**208623Orig1s000**

**MULTI-DISCIPLINE REVIEW**

**Summary Review**

**Office Director**

**Cross Discipline Team Leader Review**

**Clinical Review**

**Non-Clinical Review**

**Statistical Review**

**Clinical Pharmacology Review**

### NDA Multi-Disciplinary Review and Evaluation

<b>Application Type</b>	NDA
<b>Application Number(s)</b>	208623
<b>Priority or Standard</b>	priority
<b>Submit Date(s)</b>	December 13, 2017
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<b>PDUFA Goal Date</b>	August 13, 2018
<b>Division/Office</b>	Division of Gastroenterology & Inborn Errors Products (DGIEP)/ Office of Drug Evaluation III (ODE III)
<b>Review Completion Date</b>	August 10, 2018
<b>Established Name</b>	Migalastat
<b>Proposed Trade Name</b>	Galafold
<b>Pharmacologic Class</b>	alpha-galactosidase A (alpha-Gal A) pharmacological chaperone
<b>Code name</b>	AT1001
<b>Applicant</b>	Amicus Therapeutics
<b>Formulation</b>	capsule
<b>Dosing Regimen</b>	123 mg orally once every other day (QOD)
<b>Applicant Proposed Indication/Population</b>	treatment of patients [REDACTED] <sup>(b) (4)</sup> with Fabry disease and who have an amenable mutation
<b>Recommendation on Regulatory Action</b>	Approval
<b>Recommended Indication/Population</b>	treatment of adults with a confirmed diagnosis of Fabry disease and an amenable galactosidase alpha gene ( <i>GLA</i> ) variant based on in vitro assay data

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OPQ = Office of Pharmaceutical Quality  
 OPDP = Office of Prescription Drug Promotion  
 OSI = Office of Scientific Investigations  
 OSIS = Office of Study Integrity and Surveillance  
 OSE = Office of Surveillance and Epidemiology  
 DEPI = Division of Epidemiology  
 DMEPA = Division of Medication Error Prevention and Analysis  
 DRISK = Division of Risk Management  
 DMPP = Division of Medical Policy Programs  
 DPMH = Division of Pediatric and Maternal Health  
 DCRP = Division of Cardiovascular and Renal Products  
 IRT = Interdisciplinary Review Team for QT studies  
 CSS = Controlled Substance Staff

## Glossary

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4-MUG	4-methylumbelliferyl-D-glucuronide
AC	advisory committee
ACE	angiotensin converting enzyme
ADME	absorption, distribution, metabolism, excretion
AE	adverse event
AH	available histology
alpha-Gal A	alpha-galactosidase A
alpha-MDG	alpha-methyl-D-glucopyranoside
AUC	area under the curve
BID	twice daily
BLA	biologics license application
bp	base pair(s)
CBER	Center for Biologics Evaluation and Research
CDER	Center for Drug Evaluation and Research
cDNA	complimentary DNA
CDRH	Center for Devices and Radiological Health
CFR	Code of Federal Regulations
CHO	Chinese hamster ovary
CI	confidence interval
CL/F	oral clearance
CRT	clinical review template
CSR	clinical study report
CV	coefficient of variation
DGIEP	Division of Gastroenterology and Inborn Errors Products
ECG	electrocardiogram
eGFR	estimated glomerular filtration rate
ERT	enzyme replacement therapy
ESRD	end-stage renal disease
FD	Fabry disease
FDA	Food and Drug Administration
FDAAA	Food and Drug Administration Amendments Act of 2007
FDASIA	Food and Drug Administration Safety and Innovation Act
Gb3	globotriaosylceramide
GD	gestation day
GI	gastrointestinal
GL-3	globotriaosylceramide
GLA	galactosidase alpha gene
GLP	good laboratory practice
HEK-293	human embryonic kidney cell line 293

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hERG/Kcnh2	human ether-a-go-go related gene/potassium voltage-gated channel subfamily H member 2
IC	interstitial capillary
ICH	International Conference on Harmonization
IND	Investigational New Drug
ISE	integrated summary of effectiveness
ISS	integrated summary of safety
ITT	intent to treat
KIC	kidney interstitial capillary
LC/MS/MS	liquid chromatography coupled tandem mass spectrometry
LLN	lower limit of normal
lyso-Gb3	globotriaosylsphingosine
lyso-GL3	globotriaosylsphingosine
MedDRA	Medical Dictionary for Regulatory Activities
mITT	modified intent to treat
NCI-CTCAE	National Cancer Institute-Common Terminology Criteria for Adverse Event
NDA	new drug application
NME	new molecular entity
NOAEL	no observed adverse effect level
NOEL	no observed effect level
OCP	Office of Clinical Pharmacology
OIR	Office of In Vitro Diagnostics and Radiological Health
OPQ	Office of Pharmaceutical Quality
OSE	Office of Surveillance and Epidemiology
OSI	Office of Scientific Investigation
PCS	potentially clinically significant
PD	pharmacodynamic
PI	prescribing information
PK	pharmacokinetic
PMC	postmarketing commitment
PMR	postmarketing requirement
PND	postnatal day
PP	per protocol
PREA	Pediatric Research Equity Act
PRO	patient reported outcome
QD	daily
QOD	every other day
REMS	risk evaluation and mitigation strategy
SAE	serious adverse event
SAP	statistical analysis plan
SD	standard deviation
SE	standard error
SPA	special protocol assessment

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TEAE	treatment emergent adverse event
Tg/KO	transgenic knock-out
TK	toxicokinetics
UTI	urinary tract infection
WBC	white blood cell
WT	wild type

## 1 Executive Summary

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### 1.1. Product Introduction

The active ingredient in Galafold is migalastat, a new molecular entity (NME), which is a small molecule pharmacological chaperone designed to bind selectively and reversibly to the active site of the enzyme alpha-galactosidase A (alpha-Gal A), which is deficient in patients with Fabry disease. This binding stabilizes alpha-Gal A in the endoplasmic reticulum facilitating its proper trafficking into lysosomes. When in lysosomes, in an acidic pH and in a higher relative concentration of the relevant substrates, migalastat dissociates from alpha-Gal A, thereby allowing it to catalyze the breakdown of globotriaosylceramide (GL-3) and globotriaosylsphingosine (lyso-Gb3), the glycosphingolipids which accumulate in Fabry disease. In Galafold, migalastat is formulated as a 123-mg tablet to be administered orally once every other day.

### 1.2. Conclusions on the Substantial Evidence of Effectiveness

The Applicant has demonstrated the effect of Galafold on a surrogate endpoint (renal globotriaosylceramide, GL-3, deposition in kidney interstitial capillaries) that is reasonably likely, based on pathophysiologic data, to predict clinical benefit in adults with Fabry disease. This endpoint has previously formed the basis for accelerated approval of agalsidase beta (Fabrazyme) in patients with Fabry disease. The Applicant has demonstrated that Galafold produced a greater reduction in the disease-specific substrate (globotriaosylceramide, GL-3) deposition in kidney interstitial capillaries, as assessed in renal biopsy samples after 6 months of treatment, compared to placebo in adults with Fabry disease. This reduction was seen in males and in patients with higher baseline histological disease burden. In addition, available data in a limited number of males suggest a larger pharmacodynamic response (reduction in plasma lyso-Gb3) in Galafold-treated patients compared to placebo-treated patients, which provides supportive evidence of a potential treatment benefit. As such, Galafold is approved under the accelerated approval regulations as described in 21 CFR 314.510 (subpart H). The Applicant is required to conduct a postapproval study to verify and describe the clinical benefit of Galafold in adult patients with Fabry disease and amenable *GLA* variants.

## Benefit-Risk Summary and Assessment

Galafold (migalastat) is an alpha-galactosidase A (alpha-Gal A) pharmacological chaperone intended to be used as an oral monotherapy in adults with Fabry disease. The granted indication is for the treatment of adults with a confirmed diagnosis of Fabry disease and an amenable galactosidase alpha gene (*GLA*) variant<sup>1</sup> based on in vitro assay data. Treatment with Galafold 123 mg orally once every other day resulted in reductions in substrate deposition in kidney interstitial capillaries over 6 months in adults with Fabry disease treated in the placebo-controlled phase 3 trial AT1001-011. After thorough review of this application, the review team recommends approval for Galafold 123 mg orally once every other day under the accelerated approval regulations for the granted indication.

Fabry disease (FD) is a rare, X-linked, inborn error of glycosphingolipid metabolism caused by partial or complete deficiency of the lysosomal enzyme alpha-galactosidase A (alpha-Gal A). This enzyme deficiency results in the progressive intralysosomal accumulation of glycosphingolipids (mainly GL-3) in the kidneys, cardiovascular system, peripheral nerves, and the gastrointestinal tract. Despite its X-linked inheritance, both males and females can be affected, but with variable disease severity. In general, affected males present earlier and display more severe clinical manifestations than affected females. Even among affected males with FD, there is variability in disease onset and disease severity, largely depending on the amount of residual alpha-Gal A enzyme activity in relevant tissues, genotype, and other, yet unidentified, contributing factors. Clinical symptoms and organ damage in FD progress slowly over many decades. In males with classic FD (early age of onset, undetectable residual enzyme activity), symptoms appear in early childhood, typically starting with gastrointestinal complaints (diarrhea, abdominal pain) and neuropathic pain (acroparesthesias). Chronic kidney disease (leading to end-stage renal disease and renal failure), as well as cardiovascular (arrhythmias, myocardial infarction) and cerebrovascular disease (stroke, transient ischemic attacks) develop slowly over time and cause chronic morbidity, disability, and early mortality. In contrast, patients with late-onset FD may not show clinical manifestations until adulthood, which consist primarily of renal insufficiency and/or cardiac hypertrophy/myocardial infarction without multiple other FD-specific symptoms. The leading cause of death in both the classic and late-onset FD forms is cardiac disease followed by end-stage renal disease. Concentrations of alpha-Gal A substrates, such as GL-3 and lyso-Gb3, are typically elevated in affected tissues and in plasma and are used both for diagnosis and to gauge baseline disease severity. Males with classic FD typically have the highest levels of disease substrate accumulation as compared to affected females and to patients with late-onset FD.

The Galafold clinical trials assessed the effect of Galafold on alpha-Gal A specific substrates in the kidneys and plasma (GL-3 inclusions in kidney interstitial capillaries and lyso-Gb3 in plasma). The primary efficacy outcome, reduction in number of GL-3 inclusions in kidney biopsy (assessed via light microscopy of histology samples), was the same endpoint that had previously formed the basis for accelerated approval for agalsidase beta (Fabrazyme) in patients with FD. This histological endpoint assesses

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<sup>1</sup> "Variant" refers to any *GLA* DNA sequence change, more commonly referred to as a "mutation."

changes in disease-specific substrate burden in one of the major organs affected in FD, the kidneys. The choice of such an endpoint is appropriate as it is based on the known fundamental underlying pathophysiologic mechanism of Fabry disease and, in addition, is an endpoint that has been previously accepted by the Agency as reasonably likely to predict clinical benefit in this patient population.

The demonstration of efficacy of Galafold comes from the phase 3 clinical trial AT1001-011. This placebo-controlled clinical trial assessed the change from baseline in the average number of GL-3 inclusions in kidney interstitial capillaries (KIC) after 6 months of treatment. Patients 16 years and older with Fabry disease were randomized 1:1 to receive either Galafold 123 mg orally once every other day or placebo for 6 months. Renal biopsy samples were obtained at baseline and at month 6, and changes in the number of GL-3 inclusions were counted using light microscopy. The trial enrolled 67 patients (43 females, 24 males) with FD (ITT population) who had a “responsive” *GLA* variant using an in vitro assay, the “CT HEK-293” assay, which demonstrated an increase in residual alpha-Gal A enzyme activity from pre-treatment levels following incubation of the cells with migalastat. As the CT HEK-293 assay underwent validation in parallel with the phase 3 trial, 17 of the 67 enrolled patients were determined to have “non-amenable” *GLA* variants using the validated good laboratory practice (GLP) human embryonic kidney (HEK) assay. As such, the final efficacy population (“ITT-amenable”) was reduced from 67 to 50 patients. Furthermore, among the 50 patients in the ITT-amenable population in trial AT1001-011, 45 had available histology data both at baseline and at month 6. As such, the final population used for the analysis of efficacy consisted of 45 patients with amenable *GLA* variants and available histology data, of whom 29 were females and 16 were males. (b) (4)

(b) (4)

The primary efficacy outcome, the proportion of patients with  $\geq 50\%$  reduction from baseline to month 6 in the average number of GL-3 inclusions per kidney interstitial capillary (KIC) in biopsy samples, was assessed in the ITT (all enrolled patients) and the ITT-amenable (patients with amenable *GLA* variants and available histology) populations. In the ITT population, which included 17 patients with non-amenable *GLA* variants, the primary efficacy outcome was achieved in 13 of 34 (38%) Galafold-treated patients as compared to 9 of 33 (27%) placebo-treated patients ( $p = 0.3$ ). In the ITT-amenable population with available histology data ( $N = 45$ ), 13 of 25 (52%) patients on Galafold achieved the primary efficacy outcome as compared to 9 of 20 (45%) patients on placebo. Of note, 28 of the 45 evaluable patients in trial AT1001-011 had a relatively low baseline GL-3 inclusion burden (defined as GL-3 inclusions  $< 0.3$  based on the histological methodology used in the migalastat program, the BLISS methodology, as described in Appendix 1), indicative of low substrate burden and, thus, mild disease at baseline. In those patients with low baseline substrate burden, changes in GL-3 inclusions after 6 months of treatment were minimal and comparable between the Galafold group and the placebo group. However, analyses of the treatment effect in the remaining 17 of 45 patients with higher substrate burden at

baseline (GL-3 inclusions  $\geq 0.3$ ) demonstrated a treatment effect in treated patients in terms of reduction in substrate deposition in the renal tissues; in this group of patients with more severe disease at baseline, 7 of 9 Galafold-treated patients achieved the primary efficacy outcome as compared to two of eight placebo-treated patients. The efficacy results appear to be driven by the treatment effect in males who showed a greater reduction in GL-3 inclusions after 6 months of treatment with Galafold as compared to treated females; the treated males had a higher burden of GL-3 inclusions at baseline than the females, which is in line with the anticipated higher disease severity in males with FD. In fact, among the 29 females with amenable *GLA* variants in trial AT1001-011, 8 of 18 (44%) in the Galafold group achieved the primary efficacy outcome versus 5 of 11 (45%) in the placebo group. In contrast, among the 16 males with amenable *GLA* variants, 5 of 7 (71%) in the Galafold group achieved the primary efficacy outcome as compared to 4 of 9 (44%) in the placebo group.

The safety of Galafold was assessed throughout short-term and long-term treatment in the phase 3 trials including the two randomized controlled trials AT1001-011 and AT1001-012 and the two long-term, open-label extension trials AT1001-041 and AT1001-042. The overall median duration of exposure in the phase 3 trials was 4 years (range 0.1 to 6.8 years). Over 50% of exposed patients in the phase 3 trials had a duration of exposure between 48 and 84 months. In the context of the disease rarity, the submitted safety database for Galafold appears sufficient for assessment of safety. During phase 3 trials, there were no deaths or serious adverse events convincingly attributed to Galafold treatment. The most frequently reported adverse events in patients treated with Galafold in trial AT1001-011 over the first 6 months (and with a higher incidence than placebo) included headache, nasopharyngitis, urinary tract infection, nausea, and pyrexia. No clinically significant laboratory or vital sign changes were observed in Galafold-treated patients in the phase 3 trials. A thorough QT study of Galafold showed no effects on the QT interval and electrocardiograms performed during the phase 3 trials revealed no clinically significant changes from baseline during Galafold treatment.

The Applicant is required to conduct a postapproval adequate and well-controlled trial as a postmarketing requirement to verify and describe the clinical benefit of Galafold in adults with confirmed Fabry disease and amenable *GLA* variants as part of the accelerated approval regulations (21 CFR 314.510, subpart H). The Applicant has also proposed to utilize additional efficacy data as supportive evidence of clinical benefit (as part of the postapproval subpart H requirement) collected through a postapproval prospective, observational, registry-based study, which will form a separate postmarketing requirement under subpart H approval. Furthermore, remaining uncertainties regarding the appropriate dosing regimen of Galafold in patients with Fabry disease and severe renal impairment will be evaluated through a postapproval pharmacokinetic/pharmacodynamic (PK/PD) trial as part of a postmarketing commitment. Postapproval long-term safety data in pregnant women and their offspring treated with Galafold will be collected and analyzed through a registry-based collection (postmarketing commitment). Lastly, the Applicant commits to

verifying all *GLA* complimentary DNA (cDNA) constructs used in the GLP HEK-293 assay by collecting and submitting to the Agency additional sequencing data which will cover the entire length of the *GLA* cDNA in both directions (postmarketing commitment).

In summary, Fabry disease is a serious, slowly-progressive, and chronically disabling lysosomal storage disease with an unmet medical need for additional effective treatments. The only approved therapy for this indication, enzyme replacement therapy with agalsidase beta, is associated with important safety risks from immunogenicity to the product and imposes considerable patient burden from the need for intravenous infusions every 2 weeks. Galafold appears to provide an alternative therapeutic option in adults with Fabry disease as it is orally administered and has an acceptable safety profile. Based on the data presented in this NDA, the review team believes that the Applicant has demonstrated that Galafold 123 mg once every other day has a favorable benefit-risk profile and recommends it for approval under the accelerated approval regulations (21 CFR 314.510 subpart H) based on a surrogate endpoint reasonably likely to predict clinical benefit in adults with Fabry disease. The Applicant is required to conduct a postapproval adequate and well-controlled trial to verify and describe the clinical benefit of Galafold in adults with Fabry disease. Agreement has been reached with the Applicant on the prescribing information, patient labeling, and post-marketing requirements as part of accelerated approval. Remaining uncertainties regarding *GLA* variant confirmation via appropriate sequencing techniques, long-term safety during pregnancy and lactation, and dosing in patients with severe renal impairment (which is part of Fabry disease progression) will be further evaluated in the post-marketing setting through the planned postapproval studies.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Analysis of Condition	<ul style="list-style-type: none"> <li>Fabry disease (FD) is a rare inborn error of glycosphingolipid metabolism caused by partial or complete deficiency of the lysosomal enzyme alpha-galactosidase A (alpha-Gal A). This enzyme deficiency results in the progressive intralysosomal accumulation of substrates in major organs, including the kidneys, cardiovascular system, peripheral nerves, and the gastrointestinal tract.</li> <li>(continued below)</li> </ul>	<ul style="list-style-type: none"> <li>FD includes a spectrum of clinical manifestations and severities with different age of onset and rate of progression dictated by individual genotype and degree of residual enzyme activity.</li> <li>Female FD patients have a less predictable and more variable disease course as compared to male FD patients.</li> <li>(continued below)</li> </ul>

Dimension	Evidence and Uncertainties	Conclusions and Reasons
<b>Analysis of Condition (continued)</b>	<ul style="list-style-type: none"> <li>• Disease manifestations in FD are slowly progressive over many decades. Chronic kidney disease (leading to end-stage renal disease and renal failure), as well as cardiovascular (arrhythmias, myocardial infarction) and cerebrovascular disease (stroke) cause chronic morbidity and early mortality.</li> <li>• Concentration of disease substrates, such as GL-3 and lyso-Gb3, are typically elevated in the lysosomal compartment in affected tissues and in plasma. Males with classic FD typically have the highest levels of disease substrate accumulation both in tissues and in plasma as compared to affected females and to patients with late-onset FD.</li> <li>• Plasma lyso-Gb3 levels are used in clinical practice as an estimation of disease severity. A direct quantitative relationship between plasma lyso-Gb3 levels and clinical outcomes (e.g. renal failure, cardiovascular events), has not been demonstrated.</li> </ul>	<ul style="list-style-type: none"> <li>• Classic FD patients present with symptoms in childhood while those with the late-onset forms typically have manifestations in adult years.</li> <li>• FD is slowly progressive over many decades. Renal and cardiovascular disease cause the main morbidity and mortality in FD patients.</li> <li>• Targeting substrate reduction in the renal tissue, a prespecified goal of the migalastat clinical program, is a very rational goal and is consistent with that of the only previously approved therapy for FD, agalsidase beta, an enzyme replacement therapy approved under Section 601 subpart E of the Public Health Service Act.</li> </ul>
<b>Current Treatment Options</b>	<ul style="list-style-type: none"> <li>• The only approved therapy in the U.S. for FD is Fabrazyme (agalsidase-beta, recombinant human alpha-Gal A enzyme), which is an enzyme replacement therapy administered as an IV infusion every 2 weeks.</li> <li>• Fabrazyme received accelerated approval in 2003 based on histologic clearance of inclusions in kidney cells (KIC) and other tissues. KIC reduction was considered a surrogate endpoint considered to be reasonably likely to predict clinical benefit in FD patients. Of note, the clinical benefit of Fabrazyme has not been verified and described to date.</li> <li>• As an ERT, adverse reactions associated with Fabrazyme include hypersensitivity reactions (including anaphylaxis) and other immune-mediated adverse reactions.</li> </ul>	<ul style="list-style-type: none"> <li>• Despite approval of an ERT for FD, an unmet medical need exists for additional therapies. Confirmation that GL-3 inclusion reduction in kidney vasculature is associated with slower rate of progression of renal disease in FD is still lacking. In addition, as any other ERT, agalsidase beta is associated with immune-mediated adverse reactions and the need for IV infusion of the product every 2 weeks for the duration of the patient’s lifetime.</li> </ul>

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Benefit	<ul style="list-style-type: none"> <li>The main evidence of efficacy for Galafold comes from analyses conducted on data from a 6-month, placebo-controlled, clinical trial (trial AT1001-011) which enrolled treatment-naïve adult (mostly female) patients with Fabry disease with an amenable (initially termed “responsive”) <i>GLA</i> variant.</li> <li>Patients were enrolled in trial AT1001-011 based on a non-GLP assay (“CT HEK-293 assay”) which assessed in vitro responsiveness of mutant alpha-Gal A proteins (generated by transfection of HEK-293 cells with specific <i>GLA</i> variants) after a 5-day incubation with 10 µmol/L migalastat. Specifically, the assay measured and compared the alpha-Gal A enzyme activity of those mutant proteins before and after migalastat treatment of the cells in vitro.</li> <li>A <i>GLA</i> variant was categorized as “responsive” or “amenable” if the resultant mutant alpha-Gal A protein (as measured in the cell lysates) met two criteria: 1) showed a relative increase of ≥ 20% compared to pre-treatment alpha-Gal A activity, and 2) showed an absolute increase of ≥ 3% of the wild-type (normal) alpha-Gal A activity.</li> <li>(continued below)</li> </ul>	<ul style="list-style-type: none"> <li>The treatment difference in the ITT population of all enrolled patients numerically favored Galafold but did not reach statistical significance. However, as this population included patients with non-amenable mutations who would not be expected to show a treatment effect, these results do not appear to be a direct reflection of the Galafold treatment effect in the intended patient population.</li> <li>With respect to tissue deposition of substrates, it is not known how substrate reduction in plasma relates quantitatively to a clinical benefit. With this caveat, substrate reduction in the affected tissues and plasma is a rational goal of any potential disease-modifying therapy in FD.</li> <li>Patients treated with Galafold in the ITT-amenable population, which constitutes the most appropriate target population for the drug, achieved a ≥ 50% reduction in substrate deposition in kidney interstitial capillaries at a higher rate than those treated with placebo over 6 months in trial AT1001-011. This difference was more pronounced in the subgroup of treated patients who had a higher GL-3 inclusion burden (GL-3 inclusions ≥ 0.3) at baseline.</li> <li>(continued below)</li> </ul>

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Benefit (continued)	<ul style="list-style-type: none"> <li>The CT HEK-293 assay used for determination of <i>GLA</i> mutation responsiveness and, thus, patient enrollment in phase 3 trials, underwent GLP validation (which included improved quality control without a change in the criteria used for mutation categorization) during the course of the phase 3 trials. As a consequence, 17 of the 67 enrolled patients with “responsive mutations” (by the CT HEK-293 assay) in trial AT1001-011 were re-categorized as “non-amenable” by the GLP HEK assay. This patient population (“ITT-amenable”) of 50 patients constitutes the main efficacy analysis population proposed by the Applicant based on the mechanism of action of the drug and the use of the validated amenability assay.</li> <li>(continued below)</li> </ul>	<ul style="list-style-type: none"> <li>In concert with decreases in substrate deposition in the renal tissues, reductions from baseline in plasma lyso-Gb3 mirrored those changes in the treated patients and appeared more pronounced in males and in those patients with higher baseline lyso-Gb3 concentration.</li> <li>The efficacy results appear to be driven by the treatment effect in males who had the highest baseline level of substrate deposition. This is expected based on the X-linked nature of the disease and the knowledge that males with FD typically have a more severe disease burden.</li> <li>Most females in the trial started with a low baseline level of substrate deposition in biopsied renal tissues and, as expected, experienced small changes during Galafold treatment. This observation appears to be in line with current knowledge of the pathophysiology and natural history of Fabry disease in females, who typically experience milder disease than affected males.</li> <li>(continued below)</li> </ul>

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Benefit (continued)	<ul style="list-style-type: none"> <li>Pharmacodynamic changes (changes in GL-3 inclusions in renal tissue, plasma lyso-Gb3) were assessed in the initially enrolled patient population (ITT population, N = 67) and in the population of patients with redefined amenable <i>GLA</i> variants (ITT-amenable population, N = 50). However, the analysis population supporting primary efficacy included 45 patients with FD (29 females, 16 males) and amenable <i>GLA</i> variants who had available renal histology data at baseline and at month 6 (ITT-amenable AH population). In trial AT1001-011, patients with Fabry disease, naïve to Galafold and to ERT, were randomized to either Galafold or placebo and treated in a blinded fashion for 6 months (stage 1), which was followed by an open-label treatment phase of an additional 6 months in which all patients were treated with Galafold (stage 2). Substrate deposition (GL-3 inclusions) in KIC in renal biopsy samples was evaluated by light microscopy before and after 6 months and 12 months of treatment. The primary efficacy outcome measure was the proportion (percentage) of patients with ≥ 50% reduction from baseline in the average number of GL-3 inclusions per KIC after 6 months of treatment (placebo-controlled phase).</li> <li>In the ITT population (N = 67), which includes 17 patients with non-amenable <i>GLA</i> variants, the primary efficacy outcome was achieved in 13 of 34 (38%) of Galafold-treated patients as compared to in 9 of 33 (27%) of placebo-treated patients (p = 0.3).</li> <li>(continued below)</li> </ul>	<ul style="list-style-type: none"> <li>Renal function, as assessed by eGFR changes, remained unchanged in both treated and untreated groups during the 6 months of placebo-controlled treatment in trial AT1001-011. Given the slowly progressive nature of renal disease in both males and females with FD the finding that eGFR did not change during the course of treatment in trial AT1001-011 is expected given the short duration of the trial.</li> </ul>

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Benefit (continued)	<ul style="list-style-type: none"> <li>• In FDA’s analysis, of the 45 patients with available renal histology data at baseline and at month 6 in the ITT-amenable population, 29 were female and 16 were male. Of the 16 males, 7 had a baseline alpha-Gal A residual enzyme activity in WBCs of &lt; 1% of the wild-type mean, indicative of classic FD.</li> <li>• In the ITT-amenable population with available histology data (N = 45), 13 of 25 (52%) patients on Galafold achieved the primary efficacy outcome at 6 months as compared to 9 of 20 (45%) patients on placebo.</li> <li>• The median change from baseline to month 6 in the average number of GL-3 inclusions per KIC in trial AT1001-011 was -0.04 (range -1.94, 0.26) in Galafold-treated patients (N = 25) and -0.03 (range -1.00, 1.69) in placebo-treated patients (N = 20).</li> <li>• In the subpopulation of patients with high GL-3 inclusion burden at baseline (GL-3 ≥ 0.3), seven of nine (78%) Galafold-treated patients achieved the primary efficacy outcome at 6 months as compared to two of eight (25%) patients in the placebo group.</li> <li>• Renal function (as measured by eGFR<sub>CKD-EPI</sub> and eGFR<sub>MDRD</sub>) did not change in either treatment group during the 6 months of treatment in trial AT1001-011.</li> </ul>	

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Risk and Risk Management	<ul style="list-style-type: none"> <li>• The safety of Galafold was assessed throughout short-term and long-term treatment in the phase 3 trials including the two randomized controlled trials AT1001-011 and AT1001-012 and the two long-term, open-label extension trials AT1001-041 and AT1001-042.</li> <li>• A total of 127 treatment-naïve FD patients were exposed to Galafold 123 mg every other day for 6 months and 123 patients were exposed for at least one year.</li> <li>• The overall median duration of exposure in the phase 3 trials was 4 years (range 0.1 to 6.8 years). Over 50% of exposed patients in the phase 3 trials had a duration of exposure between 48 and 84 months. In the context of the disease rarity, the submitted safety database for Galafold appears sufficient for assessment of safety.</li> <li>• During phase 3 trials, there were no deaths or serious adverse events associated with Galafold treatment. The most frequently reported adverse events in patients treated with Galafold in trial AT1001-011 over the first 6 months (and with a higher incidence than placebo) included headache, nasopharyngitis, urinary tract infection, nausea, and pyrexia.</li> <li>• No clinically significant laboratory or vital sign changes were observed in Galafold-treated patients in the phase 3 trials.</li> <li>• A thorough QT study of Galafold showed no effects on the QT interval.</li> </ul>	<ul style="list-style-type: none"> <li>• Overall, the safety review did not identify serious safety risks associated with Galafold over a median duration of exposure of 4 years in patients with Fabry disease.</li> <li>• Communication of the identified safety risks can be effectively managed through patient labeling.</li> <li>• Safety monitoring in the postmarketing setting can be continued through routine pharmacovigilance.</li> </ul>

alpha-Gal A = alpha-galactosidase A, CKD-EPI = Chronic Kidney Disease Epidemiology Collaboration equation, eGFR = estimated glomerular filtration rate, ERT = enzyme replacement therapy, FD = Fabry disease, GL-3 = globotriaosylceramide, GLP = good laboratory practice, HEK-293 = human embryonic kidney cell line 293, IV = intravenous, lyso-Gb3 = globotriaosylsphingosine, MDRD = Modification of Diet in Renal Disease Study equation

### 1.3. Patient Experience Data

**Patient Experience Data Relevant to this Application (check all that apply)**

<input type="checkbox"/>	The patient experience data that was submitted as part of the application, include:	Section where discussed, if applicable
	<input type="checkbox"/> Clinical outcome assessment (COA) data, such as	[e.g., Section 6.1 Trial endpoints]
	<input type="checkbox"/> Patient reported outcome (PRO)	
	<input type="checkbox"/> Observer reported outcome (ObsRO)	
	<input type="checkbox"/> Clinician reported outcome (ClinRO)	
	<input type="checkbox"/> Performance outcome (PerFO)	
	<input type="checkbox"/> Qualitative studies (e.g., individual patient/caregiver interviews, focus group interviews, expert interviews, Delphi Panel, etc.)	
	<input type="checkbox"/> Patient-focused drug development or other stakeholder meeting summary reports	[e.g., Section 2.1 Analysis of Condition]
	<input type="checkbox"/> Observational survey studies designed to capture patient experience data	
	<input type="checkbox"/> Natural history studies	
	<input type="checkbox"/> Patient preference studies (e.g., submitted studies or scientific publications)	
	<input checked="" type="checkbox"/> Other: meeting with patients with Fabry disease during NDA review cycle	
<input checked="" type="checkbox"/>	Patient experience data that was not submitted in the application, but was considered in this review: literature review on available therapies and unmet needs in the treatment of Fabry disease	

## 2 Therapeutic Context

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### 2.1. Analysis of Condition

Fabry disease (FD) is an X-linked, slowly progressive, lysosomal storage disease affecting both males and females. With an estimated incidence of 1:40,000- 1: 117,000,<sup>2</sup> it is the second most common lysosomal storage disorder after Gaucher disease. FD is caused by *GLA* mutations (referred to as variants) resulting in complete or partial deficiency of the lysosomal enzyme alpha-galactosidase A (alpha-Gal A) which breaks down glycosphingolipids within the lysosomes. The enzyme deficiency causes progressive intralysosomal accumulation of the substrate glycosphingolipids globotriaosylceramide (Gb3 or GL-3) and globotriaosylsphingosine (lyso-Gb3 or lyso-GL3) in tissues such as vascular endothelial, epithelial, smooth muscle, and ganglion cells.<sup>2,3</sup> Organs primarily affected in FD include the kidneys, cardiovascular system, cerebrovascular system, gastrointestinal (GI) tract, peripheral nerves, and skin.

FD spans a spectrum of disease severity ranging from severe, early-onset disease (classic FD) to later-onset, milder disease (late-onset FD) to even asymptomatic individuals (some heterozygous females). The first clinical manifestations in the classic form of the disease in males typically appear in childhood starting around age 5 years in males and age 9 years in females with development of diarrhea or abdominal pain, neuropathic pain crises, and/or hypo/anhidrosis.<sup>4</sup> Typically, chronic renal insufficiency (initially manifesting as proteinuria, on average appearing in the 20s in classic FD males) slowly progresses to renal failure and end-stage renal disease. Gradual decline in renal function and the development of azotemia typically occur in the third to fifth decades and are managed with hemodialysis and renal transplantation.<sup>2</sup> Males with classic FD with untreated end-stage renal disease (ESRD) typically die in their early 40s.<sup>5</sup> Major causes of mortality in FD include life-threatening cardiovascular and cerebrovascular complications. The cardiovascular manifestations can include hypertension, left ventricular hypertrophy, and ischemic heart disease which can progress to heart failure, myocardial infarction or arrhythmias.<sup>6</sup> Cardiac disease is progressive and is typically present in most males with classic FD by middle age. Certain cardiac phenotypes can develop hypertrophic cardiomyopathy that may lead to cardiovascular events. Cardiac manifestations tend to occur earlier in affected males than in females.<sup>7</sup>

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<sup>2</sup> Germain, DP, 2010, Fabry disease, Orphanet J Rare Dis, 5:30, doi: 10.1186/1750-1172-5-30.

<sup>3</sup> Spada, M, S Pagliardini, M Yasuda, T Tukul, G Thiagarajan, H Sakuraba, A Ponzzone, and RJ Desnick, 2006, High incidence of later-onset fabry disease revealed by newborn screening, Am J Hum Genet, 79(1):31-40.

<sup>4</sup> Mehta A and DA Hughes, 2017, Fabry Disease, In: GeneReviews® [Internet], Adam, MP, HH Ardinger, and RA Pagon, et al., editors. Seattle (WA): University of Washington, Seattle, accessed July 30, 2018, <https://www.ncbi.nlm.nih.gov/books/NBK1292/>

<sup>5</sup> Waldek S and S Feriozzi, 2014, Fabry nephropathy: a review - how can we optimize the management of Fabry nephropathy? BMC Nephrol, 15:72.

<sup>6</sup> Patel, MR, F Cecchi, M Cizmarik, I Kantola, A Linhart, K Nicholls, J Strotmann, J Tallaj, TC Tran, ML West, D Beitner-Johnson, and A Abiose, 2011, Cardiovascular events in patients with fabry disease natural history data from the fabry registry, J Am Coll Cardiol, 57(9):1093-1099.

<sup>7</sup> Linhart, A, C Kampmann, JL Zamorano, G Sunder-Plassmann, M Beck, A Mehta, and PM Elliott, 2007, Cardiac manifestations of Anderson-Fabry disease: results from the international Fabry outcome survey, Eur Heart J, 28(10):1228-1235

The disease course in late-onset FD is highly variable with some patients experiencing severe manifestations and a more rapid rate of disease progression, while others only have mild or slowly progressive symptoms over their lifetime. Typically, affected males experience more severe disease manifestations and a faster rate of disease progression than affected females due to the X-linked nature of the disease. Some affected females may be asymptomatic or only experience few or mild symptoms over their lifetime and have a later disease onset. Other females may develop severe symptoms, although usually at a later age than affected males.<sup>4</sup> Disease manifestations in females with FD depend on the degree of X-inactivation (which is random) of the allele carrying the FD mutation. In females in which the abnormal allele is inactive in relevant tissues, disease manifestations may be milder or appear later in life or not at all.

In this review, in accordance with the current standard nomenclature used for the description and interpretation of DNA sequence changes, the term “variant” is used to signify any change in the *GLA* DNA sequence, more commonly referred to as a “mutation.”<sup>8</sup>

## 2.2. Analysis of Current Treatment Options

There is currently one FDA approved product in the U.S., Fabrazyme, which is an enzyme replacement therapy indicated for the treatment of patients with Fabry disease.

**Fabrazyme** (agalsidase beta) is a recombinant human alpha-Gal A that was approved under subpart E, section 351 of the PHS act in 2003 for the treatment of FD based on histological clearance of the substrate GL-3 inclusions in the kidney interstitial capillary (KIC) endothelial cells. It is given as an IV infusion once every 2 weeks at a dose of 1 mg/kg. Phase 3 trials of Fabrazyme included patients with a diagnosis of FD, plasma alpha-Gal A activity  $\leq$  1.5nmol/hr/ml, and plasma GL-3 level  $\geq$  5 ng/ $\mu$ L. Treatment with Fabrazyme resulted in clearance of GL-3 inclusions in 20 of 29 (69%) treated patients (based on the Genzyme renal histologic methodology<sup>9</sup>), although no differences in symptoms or renal function were observed during the five-month trial. The phase 4 confirmatory trial examined the clinical effects of Fabrazyme on the first occurrence of renal, cardiac or cerebrovascular events and inverse serum creatinine. This postapproval trial did not reach statistical significance in the pre-specified statistical analysis although a point estimate difference relative to placebo was observed and was largely driven by occurrence of renal events. Fabrazyme remains approved under accelerated approval in the U.S. and additional evaluations of renal outcomes over long-term treatment with Fabrazyme is ongoing.

The most common adverse reactions reported in association with Fabrazyme in the phase 3

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<sup>8</sup> Richards, S, N Aziz, S Bale, D Bick, S Das, J Gastier-Foster, WW Grody, M Hegde, E Lyon E, E Spector, K Voelkerding, HL Rehm, and ACMG Laboratory Quality Assurance Committee, 2015, Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology, *Genet Med*, 17(5):405-424.

<sup>9</sup> See appendix 1

trials included infusion reactions. The majority of infusion reactions were related to febrile reactions or pain symptoms. In clinical trials with Fabrazyme, approximately 50 to 55% of patients experienced infusion reactions during Fabrazyme administration. Severe infusion reactions experienced by more than one patient in clinical trials with Fabrazyme included chills, vomiting, hypotension and paresthesia. A few patients in the clinical trials also developed antibodies to Fabrazyme. Ninety-five of 121 (79%) of adult patients in the clinical trials developed IgG antibodies to Fabrazyme.

**Other approved products (outside of the U.S.):**

**Replagal** (agalsidase alpha) is a recombinant human alpha-Gal A enzyme (containing modified mannose residues) approved in multiple countries including in Europe, Australia, Canada, and Japan for long-term treatment of FD.

**Fabagal** (agalsidase-beta) is a recombinant analogue of human alpha-galactosidase A and is produced by recombinant DNA technology using Chinese hamster ovary (CHO) cell culture. Fabagal was approved in South Korea for long term treatment of patients with FD.

**Galafold** was approved in Europe in 2016 and in Japan in 2018.

### 3 Regulatory Background

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#### 3.1. U.S. Regulatory Actions and Marketing History

Migalastat is a new molecular entity (NME) that is not currently marketed in the U.S. The development of migalastat for the treatment of FD was conducted under IND 068456.

#### 3.2. Summary of Presubmission Regulatory Activity

AT1001 (migalastat) was studied under IND 068456 which was opened in the United States in 2004 for the indication of FD.

Table 1 below summarizes key pre-submission regulatory interactions between FDA and the Applicant.

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**Table 1: Key Pre-Submission Regulatory Activity**

Date	Interaction	Topic(s)
February 3, 2004	Pre-IND meeting	AT1001 Development Plan
June 17, 2008	Type B meeting	End of phase 2: The Agency did not agree with the Applicant's proposal on use of the surrogate endpoint, urine GL-3, and advised submission of SPA
October 31, 2008	SPA assessment	Phase 3 trial design (AT1001-011) - no agreement on surrogate endpoint (urine GL-3)
April 13, 2009	SPA Follow Up	Phase 3 trial design (AT1001-011)- agreement on change in GL-3 deposition in IC of kidney as primary endpoint
December 3, 2009	Type B meeting	Phase 3 trial design (AT1001-012) – the Agency had concerns regarding use of agalsidase beta as a comparator. Advised that use of (b) (4) would not likely be an acceptable primary endpoint measure.
June 8, 2010	Type C meeting	CT HEK-293 cell-based assay, bioanalytical validation – the Agency suggested that the amenability assay should be validated prior to starting phase 3 trials.
December 14, 2010	SPA assessment	Mouse carcinogenicity trial design – no agreement on proposed doses
June 24, 2011	Preliminary Comments	Phase 3 trial design (AT1001-011): The Agency did not agree with the Applicant's proposal to change the primary endpoint after initiation of the phase 3 trials as it would no longer be pre-specified. The Agency also did not agree with changing the primary endpoint from GL-3 inclusion reduction in KIC to (b) (4) (b) (4)
July 17, 2012	Type C meeting	Migalastat development plan and planned content for NDA: The Agency agreed with the proposed histological methodology and adjudication processes for analysis of GL-3 inclusions in kidney histology specimens. The Agency did not agree with the responder criterion (b) (4) for the primary analysis. The Agency also required the Applicant to submit all in vitro data for all GLA mutations tested using the CT or GLP? HEK-293 assay and provide detailed justification regarding how the threshold was defined for "amenable to treatment".

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Date	Interaction	Topic(s)
April 19, 2013	OIR meeting	Pre-submission meeting on the potential need for development of a companion diagnostic for patient genotyping to inform amenability to treatment with migalastat. FDA requested rate of genotyping in patients with Fabry disease in the U.S. clinical practice to show that genotyping is part of standard of care which would negate the gene sequencing assay being considered as a companion diagnostic.
May 22, 2013	Type C meeting	Trial AT1001-011 Stage 1 data discussion: primary efficacy endpoint failed to achieve statistical significance. Agency advised conducting a new trial evaluating appropriate clinical endpoints.
July 1, 2015	OIR Interaction	Submission of U.S. survey conducted by (b) (4) (b) (4) which reported that > 90% of patients with FD in the U.S undergo genetic testing as part of standard of care, meeting FDA's request in April 2013.
August 1, 2015	OIR Interaction	FDA acknowledged submission to OIR as being closed with no further action required.
September 9, 2015	Type B meeting	Pre-NDA meeting discussion: Trial AT1001-012 which assessed renal function in non-treatment naïve FD patients was not designed to test a specific statistical hypothesis and would not be adequate to demonstrate efficacy.
July 6, 2017	Information Request	Pre-NDA Advice/Information Request including the agreement to proceed with an NDA submission based on existing data.
October 10, 2017	Type A meeting	Discuss preliminary feedback on proposed program to generate confirmatory evidence of clinical benefit.

Source: Applicant's table with reviewer's edits

FD = Fabry disease, GL-3 = globotriaosylceramide, HEK-293 = human embryonic kidney cell line 293, KIC = Kidney Interstitial Capillary, NDA = new drug application, OIR = Office of In Vitro Diagnostics and Radiological Health, SPA = special protocol assessment

## 4 Significant Issues from Other Review Disciplines Pertinent to Clinical Conclusions on Efficacy and Safety

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### 4.1. Office of Scientific Investigations

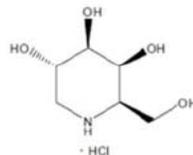
Inspections for this NDA consisted of inspections of three clinical investigator sites and the Applicant, Amicus Therapeutics U.S. Inc. Inspections at one of the clinical sites and the Applicant have received the final classification of No Action Indicated. Two clinical sites have received the preliminary classification of Voluntary Action Indicated. The data generated by these sites and the Applicant are acceptable in support of the application. See separate review by Dr. Susan Leibenhaut (finalized June 7, 2018).

### 4.2. Product Quality

The active pharmaceutical in Galafold capsules is migalastat hydrochloride. It is a white to almost white solid. It is an iminosugar. It is synthesized as a single enantiomer as evidenced by its X-ray crystal structure. The active pharmaceutical ingredient contains only the polymorphic (b) (4). It is a Biopharmaceutical Classification System Class III compound with high solubility across the physiological range and between pH 1.2 to pH 7.5 but with low passive permeability.

Migalastat hydrochloride is manufactured by (b) (4). The active pharmaceutical ingredient manufacturer has provided sufficient information to ensure consistent manufacturing of migalastat hydrochloride with respect to identity, strength, purity and quality for drug product formulation of Migalastat capsules.

**Figure 1: Chemical Structure of Migalastat Hydrochloride**



Migalastat capsule is supplied as size “2” hard gelatin capsule with an opaque blue cap and an opaque white body imprinted with the identifying code “A1001”, containing white to pale brown powder. Each capsule contains 123 mg migalastat equivalent to 150 mg migalastat hydrochloride and the following inactive ingredients: pregelatinized (b) (4) starch and magnesium stearate. The capsules are packed in (b) (4) blister packs with aluminum foil lidding. Each blister pack contains 14 capsules as a 1-month supply.

Migalastat capsules are manufactured by (b) (4). The drug product manufacturing process involves (b) (4).

(b) (4) The drug product manufacturing process was reviewed and was deemed acceptable.

The overall control strategy for the drug product is deemed adequate based on raw material controls and drug product specification including appearance, identity, assay, impurities, uniformity of dosage units, dissolution, and microbial purity. There are no outstanding issues related to the Pharmacology/Toxicology safety qualification of any excipients, impurities, or degradants which preclude the approval of this NDA.

The drug product dissolution methods development, dissolution data and dissolution specification were reviewed and were deemed adequate from biopharmaceutical perspective.

Based on the stability studies of the drug product, a 48-month expiration period is granted when stored at room temperature.

A claim of a categorical exclusion from the requirements of an environmental assessment in accordance with 21 CFR Part 25.31 was deemed acceptable.

The Office of Process and Facilities has made an “Adequate” recommendation for all manufacturing and testing facilities involved in this NDA.

#### **4.3. Devices and Companion Diagnostic Issues**

Based on previous discussions with the Applicant and feedback provided by CDRH/OIR (Office of In Vitro Diagnostics and Radiologic Health), DNA sequencing of the *GLA* gene is part of the standard diagnostic workup in patients with Fabry disease and is performed in commercial molecular genetics laboratories. As such, a companion diagnostic *GLA* genotyping (sequencing) assay to identify *GLA* variants is not required for the marketing of Galafold. For further information, please see review by Dr. Chu dated February 8, 2018.

## 5 Nonclinical Pharmacology/Toxicology

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### 5.1. Executive Summary

The Applicant submitted nonclinical studies of pharmacology (primary and secondary), safety pharmacology, absorption, distribution, metabolism, excretion (ADME)/pharmacokinetics, repeat-dose toxicology (rats, dogs, and monkeys), genetic toxicology, reproductive and developmental toxicology (rats and rabbits), and carcinogenicity (mice and rats). Safety pharmacology studies (central nervous system, cardiovascular, and respiratory) were performed in rats and/or beagle dogs. Pharmacokinetic/ADME studies and general repeat-dose toxicology studies were conducted in mice, rats, beagle dogs, and cynomolgus monkeys. A fertility study was performed in rats, embryo-fetal development studies were performed in rats and rabbits, and a pre- and postnatal development study was performed in rats. In the 14-day oral toxicity study in beagle dogs ((b) (4) 2978), daily doses of 500 mg/kg migalastat HCl (AT1001) (83 times the recommended dose based on the area under the plasma concentration curve (AUC) in Fabry disease patients) caused fecal changes (dark, pale and/or liquid feces) and significant increases in urine chloride (Cl<sup>-</sup>) and potassium (K<sup>+</sup>) in males, without any histopathological changes in the kidney or changes in plasma electrolytes. In the 2-week oral toxicity study in rats ((b) (4) 5850), treatment-related increases in mean urinary Cl<sup>-</sup> levels (in the absence of changes in plasma Cl<sup>-</sup>) were noted in the 1500 mg/kg/day dose-group (32 times the recommended dose based on AUC). However, Cl<sup>-</sup> values returned to the control group levels by the end of recovery period. In the 26-week oral toxicity study in rats (# AA17017), there was minimal to slight increase in lymphoid follicles in the spleen in all treatment-groups (50, 250 and 750 mg/kg twice daily (BID); 4.9, 16, and 40 times the recommended dose based on AUC, respectively). At the end of the recovery period, absolute and relative spleen weights were slightly higher at the 750 mg/kg BID dose-group, with minimal to slight increase in lymphoid follicles in the spleen in 4 of 10 males and 5 of 10 females. Vacuolation of renal tubular cells was observed in the high-dose group. In the 39-week study (# AA17227) in monkeys, there were 10 to 17% increases in systolic blood pressure in females in all treatment groups (25, 100, and 250 mg/kg BID) at Week 39 at 2 hours postdose. Electrocardiograms (ECGs) showed a dose-related increased incidence in changes in isoelectric line in all treatment groups, but no histological changes were observed in the heart. The thymus was identified as a target organ of toxicity, based on atrophy in all treatment groups. Thymus atrophy in monkeys occurred at AUC multiples of approximately 10-fold and higher, relative to the AUC at the recommended dose in Fabry disease patients.

In the reproductive and development toxicology studies conducted, the Applicant has investigated the effects of orally administered AT1001 (migalastat HCl) on fertility, and embryofetal development in rats and rabbits, and pre- and postnatal development in rats. The effects of oral administration of up to 12.5 mg/kg migalastat twice daily on fertility in male rats was assessed (# AA31159). A notable reduction in male fertility was observed in the highest dose group (12.5 mg/kg BID, equivalent to the human dose based on AUC). Consistent

with reduced fertilization potential of males in the high dose group, the mean number of implantations was relatively lower and the pre-implantation loss was markedly higher, compared to the control group. The effects of oral doses of up to 750 mg/kg BID on male fertility were evaluated (# AA26533). All AT1001 treated male groups showed reduction in fertility after 15 weeks of treatment. As a result, the no observed adverse effect level (NOAEL) for migalastat could not be established. The drug-induced infertility in all males was completely reversed at all dose levels after a 4-week treatment-free period. In the fertility study in female rats (# AA93208), the effects of oral administration of up to 500 mg/kg BID given during a 2-week pre-mating period through gestational day 7 (GD 7) was assessed. No treatment-related changes in mating performance, fertility, or embryonic survival was seen. The no observed effect level (NOEL) in the females was 500 mg/kg BID (16 times the human dose based on AUC). In the embryo-fetal development study in pregnant rats (# AA26551), the effects of oral administration of up to 750 mg/kg BID (26 times the human dose based on AUC) given from GD 6 to GD 17 was assessed. There were no treatment-related changes in the caesarean section data, including embryo-fetal survival, fetal weight, or sex ratio. The NOEL for maternal and embryo-fetal toxicity was 750 mg/kg BID. An embryo-fetal development study was performed in pregnant rabbits (# AA26552), where the effects of oral administration of up to 375 mg/kg BID given from GD 6 to GD 19 was studied. Several females (7 of 22) in the 375 mg/kg BID group were euthanized between GD 22 to GD 27 due to treatment-related reductions in food consumption and fecal output following body weight loss. Treatment with 375 mg/kg BID produced reductions in the number of live fetuses and mean fetal body weights, and increased incidence of delayed ossification and postimplantation loss. Delayed ossification was also observed at 150 mg/kg BID (178 times the human dose based on AUC). Dose-dependent maternal toxicity, including a transient decrease in weight gain at 150 mg/kg BID, and reduced food consumption and body weight gain at 375 mg/kg BID, was observed. A pre- and postnatal development study (Segment III) was conducted in pregnant rats (Study No. AA94762). Pregnant females were given oral doses of up to 500 mg/kg BID (16 times the human dose based on AUC) from GD 6 to postnatal day 20 (PND 20). Migalastat levels in milk were similar to the maternal plasma levels at 1 hour postdose and ~2.5-fold higher at 4 hours postdose.

Migalastat was not mutagenic in the bacterial mutagenicity (Ames) assay, in vitro cell mutation assay in L5178Y mouse lymphoma TK<sup>+</sup> cells, and in vivo micronucleus assay in rats. Migalastat produced no increase in tumor incidence in a 2-year carcinogenicity study in rats at doses up to 24 times the recommended dose based on AUC, and in a 26-week carcinogenicity study in Tg.rasH2 mice at doses up to 1000 mg/kg/day in males and 500 mg/kg/day in females. There are no nonclinical issues which preclude the approval of this application.

## 5.2. Referenced NDAs, BLAs, DMFs

None.

### 5.3. Pharmacology

The pharmacology study (# RR1001-01) in human lymphoblast cell lines evaluated the following parameters: the selectivity of AT1001 (migalastat HCl) for alpha-Gal A versus other lysosomal hydrolases/other enzyme targets, AT1001 inhibition of rh-alpha-Gal A activity, and the effect of AT1001 on physical stability of rh-alpha-Gal A. The data suggest that AT1001 weakly inhibited alpha-N-acetylgalactosaminidase and alpha-Gal A with IC<sub>50</sub> values of 6.94 μM and 57.7 nM, respectively. AT1001 has low or no affinity for recombinant human acid alpha-glucosidase (rhGAA) or acid β-glucosidase (rhGCase), although the data were not submitted. Furthermore, alpha-Gal A is stable at low pH (T<sub>m</sub> of 58°C at pH 5.2) and the presence of 10 μM AT1001 (T<sub>m</sub> of 68°C) provided physical stability to alpha-Gal A. Incubation of 1 μM AT1001 with alpha-Gal A prevented temperature-dependent denaturation and inactivation of the enzyme.

The objectives of study # RR1001-02 were to identify AT1001-responsive mutant forms of alpha-Gal A in lymphoblast and fibroblast cell lines derived from male patients with Fabry disease, representing 75 different missense mutations, and to evaluate the potential of AT1001 to increase the levels of mutant GAA or GCase in Pompe or Gaucher patient fibroblast lines, respectively. Overall, 49 of 75 alpha-Gal A missense mutant forms showed a response to AT1001. In addition, responses to AT1001 were seen in lymphoblasts isolated from male patients with Fabry disease with the IVS4+919G→A splice site mutation, and in normal lymphoblasts with the wild-type *GLA* gene. In lymphoblasts from a male Fabry patient with a c.82insG nonsense mutation, baseline level of alpha-Gal A was undetectable, and no response to AT1001 was observed. Across the cell lines, mutant alpha-Gal A baseline levels and responses to AT1001 differed in magnitude and EC<sub>50</sub> value. Half of the missense mutant forms associated with classic (early-onset) Fabry disease and a majority (90%) associated with later onset Fabry disease were responsive. In cultured fibroblasts from males with Fabry disease, the responses to AT1001 were comparable to those seen in lymphoblasts with the same mutation.

The objectives of study # RR1001-003 were to determine whether the increase in alpha-Gal A activity in fibroblast lines derived from Fabry subjects with *GLA* mutations R301Q and L300P leads to greater reduction of accumulated substrate, GL-3. Data show that seven-day incubation with AT1001 followed by a 3-day washout reduced GL-3 levels significantly in R301Q and L300P alpha-Gal A expressing fibroblasts, but not in fibroblasts expressing C52S alpha-Gal A. In contrast, GL-3 reduction was not observed in any Fabry fibroblast cell line after 10 days of constant incubation with AT1001, suggesting that incubation with AT1001 followed by a washout period was critical for efficient reduction of accumulated GL-3 in Fabry cell lines.

The objective of study # RR1001-04 was to measure the alpha-Gal A activity after AT1001 incubation of male Fabry patient lymphoblast cell lines representing 17 different missense mutations as a function of time after AT1001 washout, and to determine the half-life (t<sub>1/2</sub>) of the elevated enzyme. Data show that sustained alpha-Gal A levels were noted after removal of AT1001 from the growth media. The half-lives varied with the specific *GLA* mutation, such that the t<sub>1/2</sub> of increased alpha-Gal A levels was generally longer in normal cells (4 to 5 days) than that in mutant cells (0.5 to 3 days). Half-lives of the alpha-Gal A mutants R112H and R363H were equivalent to that in normal cells (4 to 5 days).

Study # RR1001-05 was conducted to establish a dose-response relationship for AT1001-mediated increase in WT (wild type) alpha-Gal A levels in C57BL/6 mice. Administration of AT1001 (1, 10 or 100 mg/kg/day) in drinking water for 28 days resulted in a dose-dependent increase in WT alpha-Gal A activity in all tissues analyzed (heart, kidney, skin, liver, spleen, and brain). AT1001 was highly selective for alpha-Gal A, as the activities of two other lysosomal hydrolases, GCase and GAA, were not affected. Data also show that WT alpha-Gal A activity in tissues remain elevated for up to 7 days after the last-dose of AT1001, and the half-life of WT alpha-Gal A in tissues was estimated to be 2.7 days in heart, 3.3 days in kidney, and 4.0 days in skin.

The effect of various doses of AT1001 on alpha-Gal A and GL-3 levels was evaluated in the Fabry disease mouse model, hR301Q alpha-Gal A transgenic knock-out (Tg/KO) mice (Study # RR1001-06). AT1001 administered to 8-week old male hR301Q alpha-Gal A Tg/KO mice at doses up to 300 mg/kg/day in drinking water for 2 or 4 weeks caused a dose-dependent increase in alpha-Gal A activity and a concomitant decrease in GL-3 levels in disease-relevant tissues (skin, heart, and kidney). Tissue half-lives of elevated alpha-Gal A and AT1001 were quantified in the hR301Q alpha-Gal A Tg/KO mice after dosing of AT1001 through drinking water for 28 days followed by a 7-day withdrawal phase (Study # RR1001-07). Tissue alpha-Gal A activity was higher on day 1 postwithdrawal, with maximal increases in alpha-Gal A activity in tissues of approximately 6-fold (skin), 22-fold (heart), and 5-fold (kidney). In comparison, tissue levels of AT1001 decreased by ~90 % by day 1 and were further decrease by day 3 in all three tissues. Therefore, the data suggest that the tissue half-life of elevated alpha-Gal A is significantly longer in presence of AT1001.

The effects of daily administration of AT1001 versus less-frequent dosing regimens (4 days dosing/3 days withdrawal) on GL-3 reduction in hR301Q alpha-Gal A Tg/KO mice were evaluated (Study # RR1001-10). Results suggest that the '4 days on/3 days off' regimen caused significantly greater reduction in GL-3 levels in all tissues, compared to daily AT1001 administration.

A four-week study in *GLA* KO mice (not expressing alpha-Gal A) to determine the effects of AT1001 on the levels of alpha-Gal A and GL-3 in disease-relevant tissues was conducted (Study # RR1001-12). Eight-week old male *GLA* KO mice given AT1001 doses (through drinking water) for four weeks did not lead to significant increases in tissue levels of alpha-Gal A or GL-3 levels. Furthermore, the effects of AT1001 dosing were evaluated in young and aged hR301Q alpha-Gal A Tg/KO mice (Study # RR1001-13). Data show that long-term AT1001 administration resulted in a dose-dependent reduction of tissue GL-3 levels in young as well as aged hR301Q alpha-Gal A Tg/KO mice.

The age-dependency of GL-3 accumulation in disease-relevant tissues of hR301Q alpha-Gal A Tg/KO and *GLA* KO mice was investigated (Study # RR1001-14). Data show that the amount of GL-3 increased with age in skin fibroblasts, smooth muscle cells of blood vessel walls of the skin and heart, and distal tubular epithelial cells of renal cortex.

The time-course of GL-3 turnover was studied in normal human fibroblasts after incubation with AT1001 (Study # RR1001-28). The half-life of GL-3 turnover in human wild-type fibroblasts after incubation with AT1001 was 2 to 3 hours. The GL-3 turnover rate was rapid and GL-3

clearance was complete, suggesting that inhibition of cellular alpha-Gal A activity by AT1001 is completely reversed after washout in human fibroblasts.

The potency of AT1001 for inhibition of 26 different mutant forms of alpha-Gal A in 30 different male Fabry patient-derived lymphoblast cell lines were investigated in a traditional biochemical enzyme activity inhibition assay using the artificial substrate, 4-MUG (Study # RR1001-29). Data show that AT1001 has an IC<sub>50</sub> of 91nM for wild-type alpha-Gal A. Similarly, the IC<sub>50</sub> values were below 200nM in 83% (24 out of 29) of the alpha-Gal A mutant forms, whereas IC<sub>50</sub> values between 289 and 441nM were observed in 17% (5 out of 29) of the mutant forms. The 4-MUG substrate K<sub>m</sub> value for wild-type alpha-Gal A was 1.6mM. Similarly, about 76% of the alpha-Gal A mutant forms had K<sub>m</sub> values in the range of 1.6 to 5mM. About 20% of the mutant forms had K<sub>m</sub> values within the range of 5.8 to 9mM, while one exception, E358K, which had a higher K<sub>m</sub> value of 31mM. Data show that AT1001 has a K<sub>i</sub> of 22nM for wild-type alpha-Gal. Similarly, the K<sub>i</sub> values were between 21 and 68nM in 80% (23 out of 29) of the mutant forms. In the remaining 20% of mutant forms, the K<sub>i</sub> values that were between 105 and 254nM.

### **Secondary Pharmacology**

**Table 2: Summary of Secondary Pharmacology Findings**

Study	Study Number	Method	Result
To evaluate, in EnzymeProfilingScreen, the activity of test compound AT1001-HCl (PT# 1080607).	1080607	Potential enzyme targets were pre-incubated/incubated in appropriate buffers and the assay was quantified by using a colorimetric assay.	In the primary screening, AT1001 did not cause ≥ 50% maximum stimulation or inhibition of any of the cellular targets/enzymes tested.
Effects of GR181413A on membrane potential of <i>Xenopus</i> oocytes expressing human SGLT1	2011N125700	hSGLT1 cDNA was injected in <i>Xenopus laevis</i> oocytes. Steady state changes in membrane potential were measured under open circuit conditions, and data was normalized to maximum depolarization induced by alpha-MDG in same oocyte. Inhibition of hSGLT1 by phlorizin was shown by superfusing the oocyte with a single concentration of either alpha-methyl-D-glucopyranoside (alpha-MDG) or GR141813A, followed by the addition of 200µM phlorizin.	alpha-MDG and GR181413A induced dose-dependent membrane depolarization in hSGLT1 expressing oocytes, with EC <sub>50</sub> values of 2.1mM ± 0.09 (n = 2) and 222.8mM ± (n = 2), respectively. Phlorizin (known SGLT1 inhibitor), inhibited both alpha-MDG and GR181413A, and induced depolarization by 100% ± 0.0 and 73.7% ± 9.8, respectively.

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Study	Study Number	Method	Result
Effects of GR181413A on Human Sodium Glucose Cotransporter 1 (SGLT1)	2011N125739	The human SGLT1 sequence (accession NM_000343.3) was inserted in the shuttle plasmid pFastBacMam and transfected in COS-7 cells. Amigal, GR181413A, was dissolved and diluted in assay buffer containing methyl alpha D (U- <sup>14</sup> C) glucopyranoside. The cells were incubated at 37°C for 45 minutes. Maximum activity for the assay was determined by cells treated with assay buffer containing no inhibitor.	GR181413A inhibited glucose uptake in COS-7 cells in dose-dependent manner, estimated IC <sub>50</sub> of 64.7 ± 2.5mM.
Effect of Migalastat HCl on the activity of the three enzymes catalyzing the Leloir pathway of galactose metabolism in human red blood cells	2012N13781	Activities of galactokinase, galactose-1-phosphate uridyltransferase, and UDP-galactose-4-epimerase were assayed in presence and absence of migalastat (3, 10, 30, 100µM) in human hemolysate (from fresh erythrocytes). Using a spectrofluorimeter, the assay measured the amount of NADH or NADPH formed.	Migalastat HCl at all concentrations (3, 10, 30, 100µM) had no effect on the activity of three enzymes in the Leloir pathway, galactokinase, galactose-1-phosphate uridyltransferase, and UDP-galactose-4-epimerase.
Assessment of migalastat HCl (AT1001) as an inhibitor of human SGLT2 mediated transport	OPT-2015-090	Polarized monolayer of Madin-Darby canine kidney (MDCK-II) cells expressing SGLT2 were treated with mixture of substrate and inhibitor and incubated for 5 min. The IC <sub>50</sub> value of migalastat HCl against human SGLT2 mediated transport of probe substrate, methyl-alpha-D-glucopyranoside (M-alpha-DG) was assayed using drug concentrations of 3 - 1000µM.	The experiment was repeated twice. In the 1 <sup>st</sup> experiment, cells transfected with a control-vector (GFP) followed by treatment with 30µM AT1001 showed unusually high percent of inhibition of M-alpha-DG transport. Upon repetition, no significant inhibition was noted. Since the IC <sub>50</sub> could not be determined in the repeat assay, it was concluded that migalastat was not an inhibitor of SGLT2 mediated transport.

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Study	Study Number	Method	Result
Assessment of migalastat HCl (AT1001) as a substrate of human SGLT2 mediated transport	OPT-2015-091	Polarized monolayer of MDCK-II cells expressing SGLT2 were treated with mixture of substrate and/or inhibitor mixture and incubated for 5 min. The transport of probe substrate was assayed by radiometric detection. The transport of migalastat HCl (10, 30, 100 and 300µM) was determined by LC/MS/MS.	SGLT2 expressing cells transported less AT1001 than the corresponding GFP control. However, the difference was statistically significant only at 100µM.
AT1001 is not cytotoxic to normal human fibroblast or human liver HepG2 cultured cells	RR1001-09	Fibroblasts or HepG2 cells were treated with increasing concentrations of AT1001 (51nM to 1mM) and incubated for 24 hours or 5 days (fibroblasts only) in serum free media. After incubation with 3% Alamar blue (dye) for 3 hours at 37°C and 5% or 8% CO <sub>2</sub> , dye fluorescence was measured (excitation 535 nm; emission 580 nm) and expressed as a percent of untreated control cells.	AT1001 (≤ 1mM) was not cytotoxic to normal human fibroblasts or HepG2 cells. Staurosporine (positive control) produced a concentration-dependent decrease in fluorescence [IC <sub>50</sub> = 0.058 ± 0.043µM (fibroblasts; n = 3) and 2.5 ± 0.95µM (HepG2; n = 4)].

alpha-MDG = alpha-methyl-D-glucopyranoside, GFP = green fluorescent protein, LC/MS/MS = liquid chromatography coupled tandem mass spectrometry, M-alpha-DG = methyl-alpha-D-glucopyranoside, MDCK-II = Madin-Darby canine kidney, NADH = nicotinamide adenine dinucleotide, NADPH = nicotinamide adenine dinucleotide phosphate, SGLT1 = sodium-glucose co-transporter 1, SGLT2 = sodium-glucose co-transporter 2, UDP = uridine diphosphate

### **Safety Pharmacology**

#### **Study Title: Effects of AT1001 on hERG K<sup>+</sup>-currents in CHO-K1 Cells (Study # 1748/AMI/03)**

The effects of AT1001 (0.00475, 0.0475, 0.475, 4.75 and 47.5µM) on the human ether-a-go-go related gene (hERG/Kcnh2 gene) K<sup>+</sup>-currents were evaluated in hERG transfected CHO-K1 cells. E-4031 (3µM) was used as the positive control, which produced 98% inhibition of hERG channel current. The IC<sub>50</sub> value for AT1001 was > 47.5µM; 5% inhibition was observed at 47.5µM. Therefore, at the concentrations tested, AT1001 had no significant effect on hERG K<sup>+</sup>-currents.

#### **Study Title: AT1001 - Effects on Arterial Blood Pressure, Heart Rate and Electrocardiogram, Following Oral Administration in the Conscious Beagle Dog Monitored by Telemetry (Study # 247/003)**

The effects of AT1001 on clinical signs, arterial blood pressure, heart rate, ECG, and body temperature following single oral administration of 3, 30, or 100 mg/kg to conscious male beagle dogs (three animals) were evaluated in a randomized, crossover design study. Measurements were carried out over a period of at least 2 hours pre-administration and 24

hours postadministration. A washout period of at least 2 days occurred between treatments. Arterial blood pressure and ECG parameters were monitored through implanted telemetry devices, and respiratory parameters were monitored by external telemetry. There were no mortalities in this study. There were no treatment-related clinical signs or changes in body weights at any dose tested. There were no treatment-related statistically significant changes in mean systolic or diastolic arterial blood pressure, heart rate, PR interval, RR interval, QRS complex duration, or QT<sub>c</sub> interval.

**Study Title: Irwin Profile Test in the Rat After Oral Administration of AT1001 (Study # 247/004)**

The effects of a single oral dose of AT1001 (3, 30, or 100 mg/kg) on a battery of behavioral and physiological parameters (Irwin test), including the major central and peripheral nervous system functions, were evaluated in Sprague-Dawley rats (n = 6 males/group). The animals were evaluated using the Irwin test parameters before administration of test article or vehicle administration and at 0.5, 1, 2, 5, and 24 hours postdosing. There were no treatment-related statistically significant changes in central and peripheral nervous system function parameters.

**Study Title: Respiratory Function Evaluation In Conscious, Freely Moving Rats by Whole Body Plethysmography Method After Oral Administration of AT1001 (Study # 247/005)**

The effects of a single oral dose of AT1001 (3, 30, or 100 mg/kg) on respiratory parameters in Sprague-Dawley rats (n = 8 males/group) were evaluated using barometric plethysmography. After at least 0.5 hr of acclimatization in plethysmography chambers, the respiratory signal was recorded for additional 0.5 hr. After treatment, values were continuously collected and averaged over 5 minutes from 0.5 hr up to 2 hr after administration. There were no treatment-related statistically significant changes in any respiratory parameters measured.

## 5.4. ADME/PK

**Table 3: Summary of ADME/PK findings**

Type of Study	Major Findings
Absorption	
Pharmacokinetics and Tissue Distribution of AT1001 in Wild-Type Mice (Study # RR1001-08)	Following a single oral dose of 10, 30, or 100 mg/kg, the plasma C <sub>max</sub> of AT1001 (T <sub>max</sub> 15-30 minutes) was 6.8 µg/mL (10 mg/kg), 20.2 µg/mL (30 mg/kg), and 27.1 µg/mL (100 mg/kg), equivalent to 42, 124, and 166µM, respectively. Following a single IV dose of 3 mg/kg, the plasma C <sub>max</sub> of AT1001 was 5.8 µg/mL. The mean plasma half-life following oral administration of 100 mg/kg was 3.2 hours. Following the daily oral administration of 100 mg/kg for 7 days, the maximal levels of AT1001 in peripheral tissues were achieved within 0.5 hour of dosing. The peak concentrations were 75µM in skin, 40 ± 3.4µM in heart, and 918 ± 83µM in kidney. The t <sub>½</sub> was 3.8, 3.6, and 2.3 hours in skin, heart, and kidney, respectively. Penetration and clearance from the brain was slower, with concentrations ranging from 2.4 ± 0.2µM to 4.7 ± 0.9µM over the first 8 hours (t <sub>½</sub> > 8 hr).
Distribution	
Tissue Distribution, Metabolism, and Mass Balance Study in Rats with <sup>14</sup> C-labeled AT1001 (Study # (b) (4) 08605)	[ <sup>14</sup> C]AT1001 derived radioactivity concentration was the highest in kidney (33.1 µg Eq/g at 1 hr postdose), followed by liver (11.2 µg Eq/g at 1 hr postdose). Other organs/tissues had lower concentrations at 1 hr (0.4 µg Eq/g in brain to 2.4 µg Eq/g in skin). Other than brain, which had a slightly higher concentration at 4 hr, very low levels of [ <sup>14</sup> C]AT1001-derived radioactivity were detected in kidney and liver, as all other tissues were BLOQ at 24 hr postdose.
Metabolism	
Determination of the Metabolic Stability of AT1001 and AT2101 in Cryopreserved Human, Cynomolgus Monkey, and Sprague-Dawley Rat Hepatocytes (Study # 0332-145-01)	AT1001 and AT2101 were not metabolized by human, cynomolgus monkey, or Sprague-Dawley rat hepatocytes.
Excretion	
Determination of Recovery (0-24 hr) of Dosed Radioactivity in Urine, Feces and as [ <sup>14</sup> C]CO <sub>2</sub> in Expired Air Following a Single Oral Dose of [ <sup>14</sup> C]AT1001 (Study # (b) (4) 07625)	Majority of the drug (radioactivity) was recovered in the feces (50.9% in males and 67.4% in females) and urine (23.8% in males and 17.5% in females). Recovery of radioactivity in the CO <sub>2</sub> traps was 0.10% (male) and 0.14% (female). Total recovery (0-24 hr) was 75% in males and 85% in females.
TK data from general toxicology studies	
A 14-Day Oral Toxicity and Toxicokinetic Study in Beagle Dogs (Study# (b) (4) 2978).	<u>Beagle Dogs (Study# (b) (4) 2978):</u> T <sub>½</sub> : 4.3 to 5.3 hours on day 1 and from 4.5 to 6.2 hours on day 14. <i>Accumulation:</i> No accumulation. <i>Dose proportionality:</i> Mean plasma systemic exposure was greater in females compared to the males over the dose range of 50 to 500 mg/kg/day on days 1 and 14.

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Type of Study	Major Findings
A 2-Week Oral Toxicity Study with a 2-Week Recovery Period in Sprague-Dawley Rats (Study# (b) (4) 5850).	<p>Rats (Study# (b) (4) 5850):  <math>T_{1/2}</math>: 3.0 and 3.2 hours at dose levels of 500 and 1500 mg/kg/day in males on day 1.</p> <p><i>Dose proportionality</i>: The mean plasma exposures (AUC) for both sexes increased in a less than dose proportional manner in the dose-range 100 to 500 mg/kg/day and 1500 mg/kg/day on day 1, and 100 to 1500 mg/kg/day on day 14. Increases in AUC values were dose-proportional between 500 to 1500 mg/kg/day on day 1. The maximum plasma concentration (<math>C_{max}</math>) increased in a less than dose proportional manner over the dose range of 100 to 1500 mg/kg/day on days 1 and 14.</p>
26-week oral (gavage) toxicity study in the Sprague-Dawley rat with an interim kill after 13 weeks, and followed by an 8-week treatment-free period (Study# AA17017).	<p>Rats (Study# AA17017):  Rats (Study# AA17017):  <math>T_{1/2}</math>: Values ranged from 4.6 to 7.1 hours and from 3.8 to 5.1 hours on days 90 and 181, respectively.</p> <p><i>Accumulation</i>: No accumulation was noted over the dose range (50 to 750 mg/kg BID) on days 27 and 90. On day 181, accumulation of ~28.7%, 10.9% and 24.3% was noted at dose levels of 50, 250 and 750 mg/kg BID, respectively.</p> <p><i>Dose proportionality</i>: The mean exposure (AUC) and maximum plasma concentration (<math>C_{max}</math>) on days 0, 27, 90 and 181 was less than dose proportional between 50-750 mg/kg BID. On day 0, systemic exposure was higher in males than females at the 250 mg/kg BID dose. On days 27, 90 and 181, AUC values appeared to be similar in males and females over the dose range studied except at the 50 mg/kg BID dose on day 27 and at 250 and 750 mg/kg BID doses on day 90.</p>
A 39-week oral (gavage) toxicity study in the cynomolgus monkey with an interim sacrifice after 13 and 26 weeks and followed by an 8-week treatment-free period (Study# AA17227).	<p>Monkeys (Study# AA17227):  <math>T_{1/2}</math>: Estimated mean plasma half-life values on day 22 ranged from 3.7 to 4.7 hours and from 3.5 to 4.8 hours in males and females, respectively, whereas, on day 268, the half-life ranged from 3.7 to 4.6 hours.</p> <p><i>Accumulation</i>: On day 268, slight accumulation at dose levels of 25 and 100 mg/kg BID (both sexes) was reported due to high inter-animal variability in plasma concentrations.</p> <p><i>Dose proportionality</i>: The AUC values were dose proportional in male and female monkeys on day 0, 22, 84, 175, and 268. The maximum concentration (<math>C_{max}</math>) was comparable between males and females at dose levels of 100 and 250 mg/kg BID on days 22, 84 and 268, and over the dose range of 25 to 250 mg/kg BID on day 175.</p>
TK data from reproductive toxicology studies AT1001 - Fertility study by the oral route (gavage) in the male rat (Segment I); Study # AA31159	<p><u>Rat (Study# AA31159)</u>  AUC: Increase in AUC was less than dose proportional over the dose range 2.5 to 10 mg/kg/day. Increases in AUC were more than dose proportional between 10 and 25 mg/kg/day.</p>
AT1001 - Embryo toxicity study by the oral route (gavage) in the rabbit (Segment II); Study # AA26552	<p><u>Rabbit (Study # AA26552):</u>  On GD 6, mean exposures increased in a dose proportional manner at 60 to 375 mg/kg BID doses.  On GD 19, mean exposures increased in a more than dose proportional manner between 60- and 150-mg/kg BID doses. Mean exposures were less than dose proportional between 150 to 375 mg/kg BID doses.</p>

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Type of Study	Major Findings
TK data from Carcinogenicity studies AT1001: 104-Week Oral Carcinogenicity Study in Sprague-Dawley Rats (Study# G4970)	In the 2-year rat study, migalastat was not tumorigenic at oral doses of up to 600 mg/kg BID daily (24 times the recommended dose based on AUC).

AUC = area under the curve, BID = twice daily, BLOQ = below the limit of quantification, GD = gestation day, TK = toxicokinetics

## 5.5. Toxicology

### 5.5.1. General Toxicology

**Study title/ number:** (b) (4) 2978/AT1001: A 14-Day Oral Toxicity and Toxicokinetic Study in Beagle Dogs

Key Study Findings [no more than 3 bullets]

- Daily oral dose of 500 mg/kg AT1001 caused fecal changes (dark, pale and/or liquid feces) in male and female dogs. The no observed effect level (NOEL) for AT1001 was 200 mg/kg/day, based on the changes in feces at 500 mg/kg/day.
- Statistically significant increase in urine chloride (Cl<sup>-</sup>) and potassium (K<sup>+</sup>) was noted in the 500 mg/kg/day dose-group males, without corresponding histopathological changes in the kidney.

Conducting laboratory and location



GLP compliance: Yes

**Table 3: Methods of 14-Day Oral Toxicity and Toxicokinetic Study in Beagle Dogs**

Methods	
Dose and frequency of dosing:	50, 200 and 500 mg/kg/day
Route of administration:	Oral
Formulation/Vehicle:	Solution/water
Species/Strain:	Dog/Beagle
Number/Sex/Group:	3 animals/sex/group
Age:	5 to 6 months (males and females, approximately)
Satellite groups/ unique design:	None
Deviation from study protocol affecting interpretation of results:	None affecting the interpretation of study results

**Observations and Results: Changes from Control**

**Table 4: Major findings in the 14-day oral repeated-dose toxicity study of AT1001 in beagle dogs**

Parameters	Major findings
Mortality	There were no mortalities reported during the study.
Clinical Signs	Starting day 1 of treatment to day 14, fecal changes (dark or pale and/or liquid/brown liquid feces) were noted in animals dosed at 500 mg/kg/day. On day 14, test-article related changes in feces (liquid feces along with normal feces) was also noted in one female animal dosed at 200 mg/kg/day.
Body Weights	There were no treatment-related changes in body weights.
Ophthalmoscopy	There were no treatment-related changes in ophthalmoscopy.
ECG	There were no treatment-related changes in ECG (including morphology and ECG intervals, heart rate, or blood pressure (systolic and diastolic).
Hematology	There were no treatment-related changes in hematology parameters.
Clinical Chemistry	There were no treatment-related changes in clinical chemistry parameters.
Urinalysis	On day 15, dose-dependent increases in urinary chloride (Cl <sup>-</sup> ) and potassium (K <sup>+</sup> ) were noted in all treated animals, compared to the control group. Furthermore, significant increases in urinary Cl <sup>-</sup> and K <sup>+</sup> were noted in male animals given 500 mg/kg/day. There were no treatment-related effects on urinary volume or specific gravity.
Gross Pathology	There were no treatment-related changes in gross pathology.
Organ Weights	There were no treatment-related effects on absolute and relative organ weights.
Histopathology Adequate battery: Yes	Histopathology findings noted during the study were considered incidental, part of spontaneous disease processes of laboratory dogs, or associated with some aspect of experimental manipulation, and not related to the test-article.

ECG = electrocardiogram

**Study title/ number:** (b) (4) 5850/AT1001: A 2-Week Oral Toxicity Study with a 2-Week Recovery Period in Sprague-Dawley Rats.

**Key Study Findings**

- Treatment-related increase in mean urinary Cl<sup>-</sup> was noted (males: 91 ± 65 versus 22 ± 12 (control); females: 105 ± 47 versus 47 ± 69 (control)) in the 1500 mg/kg/day dose-group. The chloride values returned to the control levels by the end of recovery period.
- The target organ of toxicity was the stomach. Eosinophil infiltrates within the glandular stomach and edema was noted at the junction of glandular and non-glandular stomach in animals from the 1500 mg/kg/day dose-group. Gastric lesions were reversed after the 14-day recovery period.
- The NOAEL (no adverse effect level) was 500 mg/kg/day.

Conducting laboratory and location: (b) (4)

GLP compliance: Yes

**Table 5: Methods of 14-Day Oral Repeated-Dose Toxicity Study in Sprague-Dawley Rats**

Methods	
Dose and frequency of dosing:	100, 500, and 1500 mg/kg/day
Route of administration:	Oral
Formulation/Vehicle:	Solution/water
Species/Strain:	Sprague-Dawley/Rat
Number/Sex/Group:	10 animals/sex/group
Age:	8 to 11 weeks
Satellite groups/ unique design:	TK analysis: 6 animals/sex/group (only treatment groups)
Deviation from study protocol affecting interpretation of results:	None

TK = toxicokinetic

**Observations and Results: Changes from Control**

**Table 6: Major Findings in the 14-Day Oral Repeated-Dose Toxicity Study in Sprague-Dawley Rats**

Parameters	Major findings
Mortality	None.
Clinical Signs	There were no treatment-related clinical signs.
Body Weights	There were no treatment-related changes in body weights.
Ophthalmoscopy	There were no treatment-related changes in ophthalmoscopy.
Hematology	There were no treatment-related changes in hematology parameters.
Clinical Chemistry	There were no treatment-related changes in clinical chemistry parameters.
Urinalysis	On day 14, a significant increase in mean urinary Cl <sup>-</sup> was noted in 1500 mg/kg/day group (males: 91 ± 65 vs. 22 ± 12 (control); females: 105 ± 47 vs. 47 ± 69 (control)). The Cl <sup>-</sup> values returned to the control levels by the end of recovery period.
Gross Pathology	There were no treatment-related changes in gross pathology.
Organ Weights	There were no treatment-related changes in organ weights.
Histopathology Adequate battery: Yes	Treatment-related histopathology findings include minimal to mild eosinophilic infiltrates within the glandular stomach, along with minimal to mild edema at the junction of glandular and non-glandular stomachs in the 1500 mg/kg/day group. These adverse effects were resolved at the end of the recovery period.

**Study title/ number:** AA17017/AT1001: 26-week oral (gavage) toxicity study in the Sprague-Dawley rat with an interim kill after 13 weeks, and followed by an 8-week treatment-free period.

**Key Study Findings [no more than 3 bullets]**

- At week 13 and 26, minimal to slight increase in lymphoid follicles in spleen was noted in all treatment-groups. At the end of recovery period, absolute and relative spleen weights were slightly higher in the 750 mg/kg BID dose group

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(high-dose) with minimal to slight increase in lymphoid follicles in the spleen in 4 of 10 males and 5 of 10 females.

- At week 26, minimal to slight tubular vacuolation in kidneys was seen at 750 mg/kg BID (4 of 15 males and 9 of 15 females).
- The target organs of toxicity were spleen and kidneys. The maximum tolerated dose (MTD) was 750 mg/kg BID. The NOAEL could not be established due to the increase in lymphoid follicles in spleen at all doses.

Conducting laboratory and location: (b) (4)

Laboratory name was changed to (b) (4) on March 25, 2010.

GLP compliance: Yes

**Table 7: Methods of 26-week Oral (Gavage) Toxicity Study in the Sprague-Dawley Rat**

Methods	
Dose and frequency of dosing:	50, 250 and 750 mg/kg BID
Route of administration:	Oral
Formulation/Vehicle:	Solutions/Water
Species/Strain:	Rats/Sprague-Dawley
Number/Sex/Group:	13-Week sacrifice: 10 animals/sex/group 26-Week sacrifice: 15 animals/sex/group Recovery (week 34): Control and 750 mg/kg BID - 10 animals/sex/group
Age:	6 weeks (approximately)
Satellite groups/ unique design:	Toxicokinetics: Control – 3 animals/sex; 50, 250 and 750 mg/kg BID – 9 animals/sex/group
Deviation from study protocol affecting interpretation of results:	None

BID = twice daily

**Observations and Results: Changes from Control**

**Table 8: Major Findings in the 26-week Oral Toxicity Study in Rats**

Parameters	Major findings
Mortality	No treatment-related mortality was reported. One male and one female in the 750 mg/kg BID group were found dead on day 181, postdose. The deaths were not treatment-related and not considered to be of any toxicological significance.
Clinical Signs	Treatment-related soft feces were seen in the 750 mg/kg BID group from day 42 until the end of the treatment period. This clinical sign was no longer noted during the treatment-free period (except for the males during the first week of recovery).
Body Weights	The mean body weights were not affected by the test article during the treatment period.
Ophthalmoscopy	There were no treatment-related eye lesions. At week 13, a slight linear corneal opacity in the right eye without revascularization was noted in one male in the 750 mg/kg BID group. As the adverse effect to the eye was noted in only one treated-animal, it was not considered to be treatment-related.

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Hematology	At week 13 and week 26, there was no treatment-related difference between control and treatment-groups.
Clinical Chemistry	At week 13, elevated potassium levels were noted in male animals administered 250 mg/kg BID ( $5.0 \pm 0.3$ mg/L) and 750 mg/kg BID ( $4.8 \pm 0.3$ mg/L), compared to the control group ( $4.4 \pm 0.4$ , $P \leq 0.05$ ). Total bilirubin level was lower in female animals administered 750 mg/kg BID ( $1.8 \pm 0.16$ mg/L), compared to the control group ( $2.0 \pm 0.2$ mg/L, $P \leq 0.05$ ). At week 26, sodium levels were lower in male animals in the 250 mg/kg BID group, and males in the 50 and 250 mg/kg BID groups had a lower level of urea ( $P \leq 0.05$ ). All the individual values remained within the normal background control range.
Urinalysis	At week 13 and 26, test-article related lower urinary pH and higher specific gravity were noted in the 750 mg/kg and 250 mg/kg BID group, respectively. These changes were absent at the end of treatment-free period.
Gross Pathology	There were no treatment-related effects in any group.
Organ Weights	<p>At week 13, males in the 750 mg/kg BID group had 39% and 51% increases in absolute and relative spleen weights, respectively (<math>p &lt; 0.05</math> and <math>p &lt; 0.01</math>). Females in the 50 mg/kg BID group had 31% and 30% decreases in absolute and relative thymus weights, respectively (<math>p &lt; 0.05</math>). Significantly decreases in absolute and relative uterus weight was seen in females in the 750 mg/kg BID group (<math>\downarrow 30\%</math> and <math>\downarrow 34\%</math>, respectively, <math>p &lt; 0.01</math>).</p> <p>At week 26, mean absolute (<math>\uparrow 27\%</math>, <math>p &lt; 0.01</math>) and relative (<math>\uparrow 22\%</math>, <math>p &lt; 0.05</math>) spleen weights were higher in females in the 750 mg/kg BID group. Females in the 50 mg/kg BID group had a 21% increase in relative thymus weights (<math>p &lt; 0.05</math>).</p> <p>At the end of recovery phase, the mean absolute and relative spleen weights were slightly higher in males in the 750 mg/kg BID group (without statistical significance). These values were also increased in treated females but only the mean relative weight reached a statistically significant level. There was a 25% increase in absolute thyroid weight in high dose females at the end of recovery period (<math>p &lt; 0.05</math>). At week 13 and 26, a treatment-related increase in spleen weights was noted in the 750 mg/kg BID group. These were correlated histologically with increased lymphoid follicles observed in both sexes. Similar findings were reported for the recovery sacrifice.</p>
Histopathology Adequate battery: Yes	<p>The target organs of toxicity were spleen and kidney.</p> <p>A treatment-related increase in tubular vacuolation in kidneys was seen in the 750 mg/kg BID group (4 of 15 males and 9 of 15 females) at week 26.</p> <p>At the interim kill (week 13), a treatment-related increase in lymphoid follicles in spleen was observed in the 50 mg/kg BID (8 of 10 males and 6 of 10 females), 250 mg/kg BID (9 of 10 males and all females) and 750 mg/kg BID groups (7 of 10 males and 8 of 10 females). At terminal kill (week 26), a treatment-related increase in lymphoid follicles in the spleen was noted in the 50 mg/kg and 250 mg/kg BID groups (7 of 15 males and 5 of 15 females, each) and the 750 mg/kg BID group (12 of 15 males and 11 of 15 females). At recovery kill (week 34), a treatment-related increase in lymphoid follicles in spleen was noted at the 750 mg/kg BID group (4 of 10 males and 5 of 10 females).</p>

BID = twice daily

**Study title/ number:** Study # AA17227/A 39-week oral (gavage) toxicity study in the cynomolgus monkey with an interim sacrifice after 13 and 26 weeks and followed by an 8-week treatment-free period.

#### Key Study Findings

- At week 39, there were 10-17% increase in the systolic blood pressure in females in all dose groups at 2 hr postdose. A dose-related increased incidence in alterations in isoelectric line was seen in animals from all treatment-groups. However, there were no histological findings in the heart.
- At week 39, thickened left ventricle was seen in one of four females in the control group, two of four males and one of four females in the 100 mg/kg BID group (mid-dose), and one of four females in the 250 mg/kg BID group (high dose). This finding does not appear to be drug-related, based on the similar incidence in the control and treatment groups.
- At week 39, AT1001 at 100 and 250 mg/kg BID caused slight to moderate changes in absolute or relative organ weights that included pituitary gland, spleen, adrenal gland, thyroids, and heart. The mean absolute ( $\downarrow$ 22%;  $P \leq 0.01$ ) and relative ( $\downarrow$ 13%;  $P \leq 0.05$ ) liver weights were lower in females in the 25 mg/kg BID group. However, there were no histological changes that correlated with the organ weight changes. The only treatment-related histopathological change was thymus atrophy, which occurred in all drug-treated groups (increased incidence compared to control). Therefore, the target organ of toxicity was thymus. The NOAEL was not established. However, doses up to 250 mg/kg BID were well tolerated.

Conducting laboratory and location (b) (4)

Laboratory name was changed to (b) (4) on March 25, 2010.  
GLP compliance: Yes

**Table 9: Methods of 39-week Oral (Gavage) Toxicity Study in the Cynomolgus Monkey**

Methods	
Dose and frequency of dosing:	0 (water), 25, 100, and 250 mg/kg BID
Route of administration:	Oral (gavage)
Formulation/Vehicle:	Solution/Water
Species/Strain:	Cynomolgus Monkey
Number/Sex/Group:	13-Week Study – 3 animals/sex/group 26-Week Study – 2 animals/sex/group 39-Week Study – 4 animals/sex/group 48-Week Study – 2 animals/sex/group (control and 250 mg/kg BID group only)
Age:	21 to 27 months
Satellite groups/ unique design:	None
Deviation from study protocol affecting interpretation of results:	None

BID = twice daily

**Observations and Results: Changes from Control**

**Table 10: Major Findings in the 39-Week Oral Toxicity Study in Monkeys**

Parameters	Major findings
Mortality	No treatment-related mortality occurred. One female animal in the 100 mg/kg BID group had a swollen right leg due to fracture of the femur with a torn epiphyseal cartilage; the animal was sacrificed on day 26 for ethical reasons.
Clinical Signs	No treatment-related clinical signs or changes in food consumption were noted.
Body Weights	No treatment-related significant changes in body-weights were observed.
Ophthalmoscopy	No treatment-related changes in ophthalmoscopy were noted.
ECG	At week 39 (day 266), mean systolic blood pressure in all test-article treated female-groups increased by 10 to 17% at 2 hours postdose. However, these increases were not dose-related or statistically significant, and were not present during the recovery period. A dose-related increased incidence of changes in isoelectric line in ECG was noted in animals of all treatment groups. No other treatment-related changes in cardiovascular functions were noted.
Hematology	At week 25, high-dose males had slightly higher mean white blood cell count (WBC), compared to the control group (↑31 %, p ≤ 0.05). No other treatment-related changes in hematology parameters were noted.
Clinical Chemistry	Higher mean gamma glutamyl transferase (GGT) activity was noted in males in the 100 and 250 mg/kg BID groups at week 25 (↑22 and ↑32%, respectively) and at week 39 (↑23 and ↑40%, respectively), compared to the control group. Since increases in GGT values were also seen at earlier time-points in control and pretest animals, changes in GGT values in treated animals was considered incidental.

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Parameters	Major findings
Urinalysis	There were no test-article related changes in urine volume or specific gravity. Data for urine electrolytes were not submitted.
Gross Pathology	At week 26, focal and/or multifocal alopecia were observed in 1 of 2 females in the 100 mg/kg BID group and 1 of 2 males and 2 of 2 females in the 250 mg/kg BID group. Microscopically, these changes correlated to multifocal or diffuse empty follicles. At week 39, thickened left ventricle (without any corresponding histological changes in heart muscle fibers) was noted in 1 of 4 females in the control group, 2 of 4 males and 1 of 4 females in the 100 mg/kg BID group, and 1 of 4 females in the 250 mg/kg BID group. No changes were noted at week 47.
Organ Weights	At week 39, increased mean absolute ( $\uparrow$ 60%) and relative ( $\uparrow$ 39%) weights of the pituitary gland were noted in males in the 250 mg/kg BID group ( $P \leq 0.05$ ), when compared to the control group. Significantly increased relative weights of spleen ( $\uparrow$ 31%; $P \leq 0.05$ ) and heart ( $\uparrow$ 19%; $P \leq 0.05$ ) was noted in male animals in the 250 mg/kg BID group and 100 mg/kg BID group, respectively. Decreased mean absolute ( $\downarrow$ 34%, $P \leq 0.01$ ) and relative ( $\downarrow$ 28.8%) adrenal gland weights were seen in females in the 250 mg/kg BID group. Decreased mean absolute ( $\downarrow$ 43%; $P \leq 0.01$ ) and relative ( $\downarrow$ 15%; $P \leq 0.05$ ) liver weights were noted in females in the 50 mg/kg BID group. Increases in the mean absolute and relative thyroids weights of males in the 100 mg/kg BID ( $\uparrow$ 31% and $\uparrow$ 22%, respectively) and 250 mg/kg BID ( $\uparrow$ 51% and $\uparrow$ 24%, respectively) groups and in females in the 250 mg/kg BID ( $\uparrow$ 8% and $\uparrow$ 17%, respectively) were noted. Individual absolute heart weights of males in the 50, 100 and 250 mg/kg BID groups were increased without any change in individual relative heart weights. Furthermore, increase in individual absolute and relative heart weights was also noted in females treated at 250 mg/kg BID.
Histopathology Adequate battery: Yes	Increased incidence of thymus atrophy without any dose-relationship was noted in all treatment groups at week 13, 26 and 39. This change was not observed in animals at week 47. The incidence of thymus atrophy is shown in the table below (taken from the Pharmacology/ Toxicology review of IND 68456 dated June 5, 2007).
Other Evaluations:	Immunological investigations on lymphocyte subsets: Immunological assessment involving the measurement of B-lymphocytes (CD-20 <sup>+</sup> ), T-lymphocytes (CD-3 <sup>+</sup> , CD-4 <sup>+</sup> , and CD-8 <sup>+</sup> ), and Natural Killer cells (CD-16 <sup>+</sup> ) was performed after 12 weeks (on day 84). A statistically significant difference between males treated with 250 mg/kg BID and controls for absolute CD8 <sup>+</sup> /CD16 <sup>+</sup> count ( $0.9 \pm 0.2 \times 10^3$ vs. $0.5 \pm 0.14 \times 10^3$ (control-group)) was considered unrelated to treatment and attributed to the variability between animals. Overall, there were no significant dose-dependent differences on the lymphocyte count or lymphocyte subsets between the test-article treated groups and the control group.

BID = twice daily, ECG = electrocardiogram, GGT = gamma glutamyl transferase, WBC = white blood cell

**Table 11: Thymus Atrophy in 39-Week Oral Toxicity Study in Monkeys**

Time points (week)	Minimal to slight thymus Atrophy							
	Group 1		Group 2		Group 3		Group 4	
	Male	Female	Male	Female	Male	Female	Male	Female
13 (n= 3 or 4)	0	0	1	1	3	0	1	1
26 (n=2)	0	1	1	2	1	2	0	0
39 (n = 4)	0	1	2	3	3	3	2	3
47 (n = 2)	0	0					0	0

**General toxicology; additional studies**

None.

**5.5.2. Genetic Toxicology**

**In Vitro Reverse Mutation Assay in Bacterial Cells (Ames)**

**Study title/ number:** AT-001 - Bacterial reverse mutation test (Plate incorporation and Pre-incubation methods) / Study # 247/001

**Key Study Findings:**

- Toxicity study: AT1001 was not cytotoxic to the five histidine-dependent strains of *Salmonella typhimurium* in the plate incorporation assay or the preincubation assay at the maximum concentration tested (5000 µg/plate). In the plate incorporation method, AT1001 was tested at dose levels of 52, 164, 512, 1600 and 5000 µg/plate (half-log progression). In the preincubation assay, AT1001 was tested at dose levels of 492, 878, 1568, 2800 and 5000 µg/plate (quarter-log progression).
- Mutagenicity test: AT1001 produced statistically significant increases in the number of revertants in the strain TA102 in the absence of metabolic activation at dose levels of 492 µg/plate (1.24-fold,  $p \leq 0.05$ ), 2800 µg/plate (1.31-fold,  $p \leq 0.01$ ) and 5000 µg/plate (1.40-fold,  $p \leq 0.005$ ) in preincubation assay, compared to the negative (vehicle) control. Since the increased number of revertants were below the criteria of a 2-fold increase for indication of a positive result, these findings were not considered biologically relevant. Therefore, AT1001 was not considered mutagenic in the bacterial reverse mutation test, in presence or absence of metabolic activation (S9).

GLP compliance: Yes

Test system: Salmonella (TA98, TA100, TA1535, TA1537, and TA102) up to 5000 µg/plate, in the absence and presence of S9.

Study is valid: Yes

**In Vitro Assays in Mammalian Cells**

**Study title/ number:** AT1001 - In vitro Mammalian Cell Gene Mutation Test on L5178Y Mouse Lymphoma Cells TK<sup>±</sup> (Microwell method) /Study # 247/002

**Key Study Findings:**

Mutagenic potential of AT1001 was tested using long and short treatments in mouse lymphoma cells:

- In the long treatment experiment in the absence of metabolic activation (S9), AT1001 was tested at dose levels of 1.7, 5.4, 17, 52, 164, 512, 1600 and 5000 µg/ml (half-log progression between the dose levels). No precipitate or biologically significant dose-related cytotoxicity or increases in the frequency of mutants were noted up to the dose level of 5000 µg/ml.

- In short treatment experiment in the presence and absence of S9, AT1001 was tested at dose levels of 87, 155, 276, 492, 878, 1568, 2800 and 5000 µg/ml (quarter-log progression between the dose levels). No precipitate or biologically significant dose-related cytotoxicity or increases in the frequency of mutants were noted up to the dose level of 5000 µg/ml.

Thus, under the experimental conditions, AT1001, at up to 5000 µg/ml (maximum dose level), did not induce mutagenic effects in the L5178 mouse lymphoma cells TK<sup>+/-</sup>.

GLP compliance: Yes

Test system: Mouse lymphoma cells (L5178 TK<sup>+/-</sup>); up to 5000 µg/ml; in the absence and presence of S9.

Study is valid: Yes

### **In Vivo Clastogenicity Assay in Rodent (Micronucleus Assay)**

**Study title/ number:** AT1001 - Mammalian Erythrocyte Micronucleus Test in the Rat Bone Marrow/Study # AA18463.

The aim of the study was to evaluate the potential of AT1001 to induce micronuclei in bone marrow erythrocytes in Sprague-Dawley rats.

Key Study Findings:

- A preliminary experiment showed no toxicity/cytotoxicity was at any of the dose levels tested.
- In the main experiment, a limit test at the single dose level of 2000 mg/kg was administered orally for two consecutive days (5 animals/sex). Bone marrow from the drug-treated and control groups were collected at approximately 24-hours posttreatment. The highest individual frequency of micronucleated polychromatic erythrocytes (MNPCEs) obtained in the groups treated with AT1001 was 3.93 per 1000 PCEs, compared to 1.96 in the vehicle control group.
- MNPCE in rats treated with 2000 mg/kg ranged from 0.96 to 3.93 (per 1000 PCEs) in males and 0.00 to 2.82 in females. However, the mean MNPCE values in males and females were within the historical control range (0.54-2.13). Therefore, it is concluded that oral dosing of AT1001 at 2000 mg/kg did not induce micronuclei in rat bone marrow erythrocytes. AT1001 did not produce any clinical signs, however an increase in PCE/NCE (polychromatic erythrocyte/normochromatic erythrocyte) ratio was observed in both sexes (1.15 and 1.11 in 2000 mg/kg males and females, respectively, compared to 0.88 and 0.60 in control males and females, respectively).

GLP compliance: Yes

Test system:

Preliminary test: Two daily oral gavage (10 ml/kg) with 197, 351, 627, 1120 and 2000 mg/kg (five animals/sex/group) for 2 consecutive days; doses given approximately 4 to 6 hours apart.

Main test: 2000 mg/kg AT1001, positive control article, or negative control article was administered orally in five animals/sex/group.

Study is valid: Yes

### **Other Genetic Toxicity Studies**

None.

### **5.5.3. Carcinogenicity**

Full reviews of the carcinogenicity studies are provided in the Nonclinical Memorandum. In a 2-year carcinogenicity study, rats were treated with AT1001 at doses of 25, 100 and 400 or 600 mg/kg twice daily. The high dose of 400 mg/kg BID (800 mg/kg/day) was increased to 600 mg/kg BID (1200 mg/kg/day) during week 36. The study protocol was presented to the Executive Carcinogenicity Assessment Committee (E-CAC) on June 12, 2007. The proposed doses were [REDACTED] <sup>(b) (4)</sup> based on a pharmacokinetic endpoint. However, the E-CAC did not agree with the proposed doses, and stated that there was inadequate data to support dose selection based on exposure (AUC). This assessment was based on the proposal of once daily dosing in the carcinogenicity study protocol, whereas twice daily dosing was used in the 26-week dose-ranging study. The Applicant initiated the study using the proposed doses without further consultation with the E-CAC. However, rats were dosed twice daily 6 hours apart, in contrast to the proposed once daily dosing in the original protocol. At the time of the Applicant's decision to increase in the high dose, the Applicant estimated the rat to human AUC ratios at 800 and 1200 mg/kg/day to be 23 and 39, respectively. These estimated AUC multiples indicated that the weighted-average AUC multiple over the entire study period would be greater than 25-fold, a value which can be used as a PK endpoint for high-dose selection, as recommended in ICH S1C(R2). The weighted-average AUC<sub>0-t</sub> in the 400 or 600 mg/kg BID group is 151,432 ng•hr/mL, and the AUC<sub>0-t</sub> in Fabry disease patients is 12,371 ng•hr/mL at the proposed dose. However, the calculation of the rat to human AUC ratio should account for the proposed dosing frequency in humans, with migalastat taken once every other day. Therefore, the weighted-average AUC multiple is 24.5 for the high-dose group (151,432 ng•hr/mL ÷ [12,371 ng•hr/mL ÷ 2]).

Based on the Agency's statistical analysis, treatment with AT1001 increased the incidence of the cortical cell adenoma in adrenal glands in male rats at the high dose. The trend and pairwise tests were significant ( $p = 0.043 < 0.05$  for rare tumors, pairwise test for adenoma;  $p = 0.0190 < 0.025$  for rare tumors, trend test for adenoma). Although there was no incidence of cortical cell adenomas in the male control groups (0 of 99 males), cortical cell adenomas are generally considered as common tumors in male and female SD rats. It is noteworthy that cortical cell adenomas occurred in 6 of 100 control females. Neither the pairwise comparison nor the trend test show statistical significance when the common tumor significance criteria are used (pairwise comparison,  $p = 0.0430 > 0.01$  for common tumors; trend test,  $p = 0.0190 > 0.005$  for common tumors). Similarly, the combination of cortical cell adenoma and carcinoma in adrenal glands failed to show significance in the pairwise comparison and trend

test based on the common tumor significance criteria (pairwise comparison,  $p = 0.0430 > 0.01$  for common tumors; trend test,  $p = 0.0388 > 0.005$  for common tumors). The E-CAC concluded that there was no drug-related increase in tumors, and the study was considered as adequate.

In a 26-week carcinogenicity study, Tg.rasH2 mice were treated with AT1001 at doses of 100, 300, and 1000 mg/kg/day for males, and 50, 150, and 500 mg/kg/day for females. These doses were recommended by the E-CAC. Treatment with AT1001 did not increase tumor incidence. The positive control article, urethane, significantly increased the incidence of hemangiosarcoma, Harderian gland adenoma, bronchiolo-alveolar adenoma, and carcinoma. The E-CAC concluded that there were no drug-related neoplasms in either males or females in this study. The study was considered as adequate.

#### 5.5.4. Reproductive and Developmental Toxicology

##### **Fertility and Early Embryonic Development**

**Study title/ number:** AT1001 - Fertility study by the oral route (gavage) in the rat (Segment I) /Study # AA26533.

##### Key Study Findings

- All AT1001 treated male groups showed treatment-related reduction in fertility. Thus, the NOAEL for AT1001 was not established in the study.
- The drug-induced infertility in males was completely reversed after a 4-week treatment-free period following 15 weeks of treatment.

Conducting laboratory and location:

 (b) (4)

GLP compliance:

Yes

**Table 12: Methods of Oral Gavage Fertility Study in the Rat**

Methods	
Dose and frequency of dosing:	Animals were given oral BID doses (approximately 6 hours $\pm$ 30 minutes apart) of 0 (control), 50, 250, or 750 mg/kg AT1001.
Route of administration:	Oral
Formulation/Vehicle:	Solution/Water
Species/Strain:	Rat/Sprague-Dawley
Number/Sex/Group:	F1: 20 animals/sex/group F2: 20 animals/sex/group F3: 10 animals/sex/group
Satellite groups:	None
Study design:	In F1 rats, there were 20 animals/sex/group; males and females were pretreated with AT1001 doses for 4- and 2-weeks, respectively. The mated females were then dosed up to presumed gestational day 7 (GD 7). In F2 rats, treatment of 20 males/group continued throughout a second mating period for an additional 10 weeks (total of 13 weeks treatment) before mating with 20 untreated females/group until the day of necropsy of 10 males/group, post caesarean section (GD 13). In F3 rats, the remaining 10 males/group were treated for 15 weeks followed by a 4-week treatment-free period. Subsequently, 10 males/group were paired with an additional 10 untreated females/group, to evaluate the regression of treatment-related infertility. Females from each mating period were sacrificed on GD 13.
Deviation from study protocol affecting interpretation of results:	No

BID = twice daily, GD = gestation day

**Observations and Results**

**Table 13: Major Findings of the Effects of AT1001 on Male Fertility in Sprague-Dawley Rats**

Generation	Major Findings
First mating	Treatment-related reduction in male fertility was noted in all AT1001 groups, compared to the control group. Pregnancy was observed in 4 of 20 females mated with 50 mg/kg/BID treated males, 1 of 20 females mated with 250 mg/kg/BID treated males, and 1 of 20 females (mated with 750 mg/kg/BID treated males), compared to 19 of 19 females in the control group
Second Mating	Treatment-related reduction in male fertility was noted in all AT1001 groups. Pregnancy was observed in 9 of 20 females mated with 50 mg/kg/BID treated males, 5 of 20 females mated with 250 mg/kg/BID treated males, and 2 of 20 females mated with 750 mg/kg/BID treated males, compared to 19 of 20 females in the control group. A statistically significant decrease in the mean terminal body weight was noted in all untreated females mated with treated males ( $p < 0.01$ ). The mean absolute ovary weight was decreased in untreated females mated with males given 500 mg/kg/day ( $p < 0.05$ ).
Third Mating (treated males after recovery period)	There were no treatment-related changes in male fertility after a 4-week treatment-free period. Pregnancy was observed in 10 of 10 females mated with 50 mg/kg/BID treated males, 10 of 10 females mated with 250 mg/kg/BID treated males and 9 of 10 females mated with 750 mg/kg/BID treated males, compared to 8 of 9 females in the control group. No statistically significant difference in the mean terminal body weight or in the mean absolute or relative ovary weight was noted in untreated females mated with treated males after the treatment-free period.

BID = twice daily

**Study title/ number:** AT1001 - Fertility study by the oral route (gavage) in the male rat (Segment I)/Study # AA31159.

**Key Study Findings**

- A treatment-related reduction in male fertility was observed in the high-dose group (12.5 mg/kg BID). Consistent with reduced fertilization potential of males in the high dose group, the mean number of implantations was lower and the pre-implantation loss was markedly higher, compared to the control group.

Conducting laboratory and location:



GLP compliance:

Yes

**Table 14: Methods of Oral Gavage Fertility Study in the Male Rat**

Methods	
Dose and frequency of dosing:	0 (vehicle), 1.25, 5, or 12.5 mg/kg BID AT1001 (doses given approximately 6 hours ± 30 minutes apart)
Route of administration:	Oral
Formulation/Vehicle:	Solution/Water for Injection
Species/Strain:	Rat/Sprague-Dawley
Number/Sex/Group:	20 males/group
Satellite groups:	None
Study design:	The males were treated during a 4-week pre-mating period and treatment continued throughout a 3-week mating period (with groups of untreated females) and through the day before necropsy (total of at least 9 weeks of treatment). The males were sacrificed after 9 weeks of treatment (after completion of the caesarean sections) and submitted to a necropsy examination. The untreated females were sacrificed on or close to gestational day 13 (GD 13).
Deviation from study protocol affecting interpretation of results:	None

GD = gestation day

### **Observations and Results**

**Table 15: Major Findings of the Effects of AT1001 on Male Fertility in Sprague-Dawley Rats**

Generation	Major Findings
Male Fertility	Pregnancy was observed in 15 of 20 females mated with 1.25 mg/kg/BID treated males, 17 of 20 females mated with 5 mg/kg/BID treated males, and 11 of 20 females mated with 12.5 mg/kg BID treated males, compared to 19 of 20 females in the control group. In addition, the mean number of corpora lutea and implantations were relatively lower ( $9.0 \pm 6.0$ and $4.1 \pm 4.0$ , respectively) in the 12.5 mg/kg BID group, compared to the control group ( $17.3 \pm 2.0$ and $15.7 \pm 3.0$ , respectively). Pre-implantation loss was markedly higher in the 12.5 mg/kg BID group ( $53.1 \pm 30\%$ ), compared to the control group ( $9.2 \pm 12\%$ ). The totality of the data indicates that AT1001 produced a decrease in male fertility in the 12.5 mg/kg BID group. The decreased number of pregnant females in the 1.25 and 5 mg/kg BID groups was not clearly indicative of impaired male fertility.

BID = twice daily

**Study title/ number:** AT1001 - Fertility toxicity study by the oral route (gavage) in the female rat (Segment I)/Study # AA93208.

#### Key Study Findings

- There were no treatment-related clinical changes in mating performance, fertility or embryo survival.
- The no observed effect level (NOEL) in female Sprague-Dawley rats was 500 mg/kg BID.

Conducting laboratory and location:

(b) (4)

(b) (4)

GLP compliance: Yes

**Table 16: Methods of Oral Gavage Fertility Toxicity Study in the Female Rat**

Methods	
Dose and frequency of dosing:	0 (vehicle), 5, 50, or 500 mg/kg BID AT1001 (doses given approximately 6 hours ± 30 minutes apart)
Route of administration:	Oral
Formulation/Vehicle:	Solution/Water for Injection
Species/Strain:	Rat/Sprague-Dawley
Number/Sex/Group:	20 females/group
Satellite groups:	None
Study design:	The females were treated during a 2-week pre-mating period and treatment continued to gestational day 7 (GD 7). The females were sacrificed on or close to GD 13.
Deviation from study protocol affecting interpretation of results:	None

GD = gestation day

### **Observations and Results**

**Table 17: Major Findings of the Effects of AT1001 on Female Fertility in Sprague-Dawley Rats**

Generation	Major Findings
Female Fertility	No treatment-related clinical changes in mating performance, fertility, or embryo survival were observed. All females in each group mated and became pregnant. The NOAEL in the female rats was > 500 mg/kg BID.

GD = gestation day, NOAEL = no observed adverse effect level

### **Embryo-Fetal Development**

**Study title/ number:** AT1001 - Embryo toxicity study by the oral route (gavage) in the rat (Segment II)/Study # AA26551.

#### Key Study Findings

- There were no treatment-related changes in embryo-fetal survival, fetal weight or sex ratio.
- The NOEL for maternal and embryo-fetal toxicity was > 750 mg/kg BID.

Conducting laboratory and location:

(b) (4)

GLP compliance: Yes.

**Table 18: Methods of Oral Gavage Embryo Toxicity Study in the Rat**

Methods	
Dose and frequency of dosing:	0 (vehicle), 50, 250, or 750 mg/kg BID AT1001 (doses given approximately 6 hours ± 30 minutes apart)
Route of administration:	Oral
Formulation/Vehicle:	Solution/Water for Injection
Species/Strain:	Rat/Sprague-Dawley
Number/Sex/Group:	25 females/group
Satellite groups:	None
Study design:	Females were treated from gestation day 6 (GD 6) to GD 17. Caesarean section was conducted on GD 20.
Deviation from study protocol affecting interpretation of results:	None

GD = gestation day

### **Observations and Results**

**Table 19: Major Findings of the Maternal and Embryo-Fetal Effects of AT-10001 in Sprague-Dawley Rats**

Generation	Major Findings
F0 and F1	No treatment-related effects were noted in the caesarean section data, including embryo-fetal survival, fetal weight, and sex ratio. The incidence of malformed fetuses was 1 of 25, 1 of 25, 3 of 25, and 0 of 25 in the control, 50, 250, and 750 mg/kg BID groups, respectively. The malformations appeared to be spontaneous in origin, and not related to the test-article. No signs of maternal toxicity were observed. Therefore, the NOEL for maternal and embryo-fetal toxicity was 750 mg/kg BID.

BID = twice daily, NOEL = no observed effect level

**Study title/ number:** AT1001 - Embryo toxicity study by the oral route (gavage) in the rabbit (Segment II)/Study # AA26552.

#### Key Study Findings

- Signs of maternal toxicity were noted at 150 mg/kg BID (transient decrease in weight gain) and 375 mg/kg BID (reduced food consumption and body weight gain). Several females (7 of 22) in the 375 mg/kg BID group were euthanized during gestation days 22 (GD 22) through 27 due to treatment-related reductions in food consumption and fecal output, associated with body weight loss.
- Administration of 375 mg/kg BID produced reductions in live fetuses and mean fetal body weights, and increased incidence of postimplantation loss and delayed ossification in fetuses. The increase in delayed ossification was also noted in the 150 mg/kg BID group.

Conducting laboratory and location:

(b) (4)

GLP compliance:

Yes.

**Table 20: Methods of Oral Gavage Embryo Toxicity Study in the Rabbit**

Methods	
Dose and frequency of dosing:	0 (vehicle), 60, 150, or 375 mg/kg BID AT1001 (doses given approximately 6 hours ± 30 minutes apart); dose selection was based on the results of a dose-range finding study in female rabbits (Study # AA 26552)
Route of administration:	Oral
Formulation/Vehicle:	Solution/Water for Injection
Species/Strain:	Rabbit/New Zealand White
Number/Sex/Group:	22 females/group
Satellite groups:	Toxicokinetics - Four satellite groups of 3 mated animals each were sampled on GD 6 and GD 19
Study design:	Females were treated from GD 6 to GD 19. Caesarean section was conducted in surviving females on GD 29.
Deviation from study protocol affecting interpretation of results:	None

BID = twice daily, GD = gestation day

### Observations and Results

**Table 21: Major Findings of the Maternal and Embryo-Fetal Effects of AT1001 in New Zealand White Rabbits**

Generation	Major Findings
F0 and F1	7 of 22 females in the 375 mg/kg BID group were euthanized during GD 22 to 27 due to treatment-related reductions in food consumption and fecal output, which resulted in body weight loss. One female died due to a dosing error on GD 11. Treatment with 375 mg/kg BID produced reductions in live fetuses and mean fetal body weights, and increased the incidence of postimplantation loss and delayed ossification in fetuses. An increased incidence of delayed ossification was also observed at 150 mg/kg BID. These effects were associated with signs of maternal toxicity, including a transient decrease in weight gain at 150 mg/kg BID, and reduced food consumption and body weight gain at 375 mg/kg BID.

BID = twice daily, GD = gestation day

### Prenatal and Postnatal Development

**Study title/ number:** AT1001 - Pre- and postnatal development study by the oral route (gavage) in the rat/Study # AA94762.

#### Key Study Findings

- Migalastat was detected in plasma and milk at 1 and 4 hours postdose in all treated females. The migalastat concentration in milk was similar to the maternal plasma levels at 1 hour postdose, and was ~2.5-fold higher than plasma levels at 4 hours postdose.
- A higher percentage of postimplantation loss was noted at 100 and 500 mg/kg BID, but no dose-relationship was evident.

Conducting laboratory and location:

(b) (4)

(b) (4)

GLP compliance: Yes.

**Table 22: Methods of Oral Gavage Pre- and Postnatal Development Study in the Rat**

Methods	
Dose and frequency of dosing:	0 (vehicle), 25, 100, or 500 mg/kg BID AT1001 (doses given approximately 6 hours ± 30 minutes apart)
Route of administration:	Oral
Formulation/Vehicle:	Solution/Water for Injection
Species/Strain:	Rat/Sprague-Dawley
Number/Sex/Group:	F0 generation: 25 females/group F1 generation: 25 animals/group
Satellite groups:	F0 generation: 12 females/group
Study design:	Pregnant females were treated from gestation day 6 (GD 6) to postnatal day 20 (PND 20). The pregnant females were allowed to deliver their off-spring, and the pre-weaning viability, growth, and development of the offspring (F1) were evaluated. F1 litter sizes were standardized to four male and four female pups by culling on PND 4. F0 females and unselected F1 offspring were necropsied for macroscopic examination at weaning. Blood samples for measurement of plasma AT1001 levels were taken from satellite dams on GD 6, from satellite dams and fetuses on GD 20, and from satellite dams on day 15 of lactation. Drug concentrations in milk were measured in these females on day 15 of lactation. The F1 generation (25/sex/group) was selected from the F1 offspring and were maintained for evaluation of postweaning development, behavioral tests, and mating and fertility. Body weights of the F1 females were monitored during the pre-mating period and during gestation. Body weights of the F1 males were monitored from selection up to necropsy. The study was terminated by sacrifice and necropsy of the F1 males after completion of the caesarean sections in F1 females, on or close to day 13 <i>post coitum</i> . All F1 animals were necropsied and examined macroscopically. The pregnancy status, number of corpora lutea, and numbers and types of uterine implantations were determined in the females.
Deviation from study protocol affecting interpretation of results:	None

BID = twice daily, GD = gestation day, PND = postnatal day

### Observations and Results

**Table 23: Major findings of the Pre- and Postnatal Developmental Effects of AT1001 in Sprague-Dawley Rats**

Generation	Major Findings
F0 and F1	Migalastat was present in plasma and milk in all drug-treated animals. The concentration in milk following oral administration of up to 500 mg/kg twice daily was approximately ~2.5 times higher than levels in maternal plasma at four hours postdose. The concentration of migalastat in plasma from pups was ~11 times lower than the maternal plasma concentration at 1 hour postdose. Although a higher percentage of postimplantation loss was noted at 100 mg/kg BID and higher, there was no dose-relationship for this change.

BID = twice daily

### 5.5.5. Other Toxicology Studies

None.

## 6 Clinical Pharmacology

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### 6.1 Executive Summary

Galafold (123 mg migalastat) is for treatment of adults with Fabry disease with amenable *GLA* variants at the proposed dose of 123 mg orally every other day (QOD). Migalastat is a new molecular entity and pharmacological chaperone designed to bind selectively and reversibly to the active site of the enzyme alpha-Gal A and to stabilize certain mutant forms of alpha-Gal A in the endoplasmic reticulum, facilitating their proper trafficking into lysosomes, their site of action. Once in the lysosomes, at a lower pH and a higher concentration of endogenous substrates, migalastat dissociates from alpha-Gal A, allowing it to hydrolyze its natural substrates, globotriaosylceramide (GL-3 or GB-3) and globotriaosylsphingosine (lyso-Gb3 or lyso-GL3) which accumulate within lysosomes in patients with Fabry disease.

The identification of amenable *GLA* variants that produce mutant misfolded alpha-Gal A proteins, to which migalastat binds and stabilizes, is based on an in vitro assay using human embryonic kidney-293 (HEK) cells transfected with cDNA containing specific *GLA* variants (confirmed via DNA sequencing). This in vitro HEK-293 assay was used to determine which *GLA* variants were “responsive” (per the CT HEK-293 assay) or “amenable” (per the GLP HEK-293 assay) to migalastat treatment based on pre-specified biochemical criteria (increases in alpha-Gal A enzyme activity) after incubation with migalastat. The in vitro identification of mutation “responsiveness” or “amenability” was used for selection of the intended patient population and trial enrollment. The GLP HEK-293 in vitro assay, used to determine *GLA* variant amenability, is deemed acceptable from an analytical validity standpoint. However, certain methodological issues associated with the submitted sequencing data of the *GLA* cDNA constructs used in the HEK-293 assay (see sections 6.2 and 6.5.3) were identified; these sequencing methodological issues will be further evaluated postapproval via the agreed-upon post-marketing commitment as described in section 6.3. From a clinical validity standpoint, in relation to the Galafold primary efficacy outcome measure ( $\geq 50\%$  reduction from baseline to month 6 in KIC GL-3 inclusions), the GLP HEK-293 assay shows a modest positive predictive value: PPV 30% in females and PPV 47% in males in trial AT1001-011. Furthermore, in relation to all outcome measures assessed in the Galafold trials as a whole (KIC GL-3 inclusions, plasma lyso-Gb3, residual WBC alpha-Gal A activity) and using the review team’s definitions for specific biomarker response thresholds ( $\geq 4\%$  increase in residual WBC alpha-Gal A activity,  $\geq 10\%$

reduction in plasma lyso-Gb3), about 50% of Galafold-treated patients with amenable *GLA* variants in trial AT1001-011 showed a corresponding biomarker response in either of the 3 outcomes. All in all, the Office of Clinical Pharmacology (OCP) finds the patient selection strategy based on the HEK-293 in vitro assay to be overall acceptable based on the described limitations of the phase 3 trials (see section 8.1.6) and given that biomarker responses may be assessed clinically during treatment to monitor the patient's pharmacodynamic responses. The assay may continue to be used under the Applicant's specified procedures for purposes of future addition of newly tested amenable *GLA* variants.

Dose exploration was limited and, as such, dosing for the general FD patient population could have been optimized. However, the chosen dose of migalastat for clinical trials and for marketing – 123 mg orally once every other day – is acceptable based on the benefit-risk profile of the drug, and the purported mechanism of action. In addition, Galafold is to be administered on an empty stomach (2 hours before or 2 hours after meals). The impact of this proposed administration schedule on migalastat pharmacokinetics was not directly evaluated in the food effect study. However, because this administration schedule is consistent with dosing instructions in the phase 3 trials supporting the efficacy and safety of migalastat, the OCP finds the proposed administration schedule regarding food intake acceptable.

Dosing for patients with renal impairment, which occurs as part of the natural progression of Fabry disease, is based on results of a dedicated renal impairment PK study. No dose adjustment is recommended in patients with mild or moderate renal impairment as renal function had only a minor impact on migalastat pharmacokinetics in this subgroup of patients. Migalastat is not recommended for the treatment of patients with FD who have severe renal impairment (eGFR < 30 mL/min/1.73m<sup>2</sup>) since the migalastat exposure was more than four times higher in subjects with severe renal impairment compared to subjects with normal renal function. The safety and efficacy of migalastat was not adequately assessed in patients with severe renal impairment because such patients were not included in the completed phase 3 trials. Because patients with Fabry disease can develop progressive renal impairment as part of natural disease progression, the OCP recommends that the Applicant further study or who are on kidney dialysis as part of a postmarketing study (PMC 3412-3, see section 6.3). In addition, the OCP recommends that patients with various degrees of renal impairment, including severe renal impairment, should be included in the postmarketing confirmatory trial (PMR-3412-1) to obtain additional safety and efficacy data.

Dosage adjustment for hepatic impairment or drug-drug interactions are not likely to be necessary based on available knowledge of migalastat disposition (from the completed mass balance and in vitro studies); of note, no formal human studies were conducted to assess the potential effects of hepatic impairment on the drug's PK and in vitro liabilities were not identified.

Throughout this review, Galafold is also referred by its code name AT1001 which was used throughout the development program. An OCP briefing on the clinical pharmacology review of the Galafold NDA was held on June 11, 2018.

## 6.2. Recommendations

The OCP found the NDA submission acceptable from a clinical pharmacology standpoint and recommends it for accelerated approval. The OCP found that the observed biomarker responses (plasma lyso-Gb3 reduction, residual WBC alpha-Gal A activity increases in males), both indicative of the biologic and pharmacodynamic activity of Galafold, in combination with the observed reductions in the surrogate endpoint, KIC GL-3 inclusions in renal biopsy samples, support the accelerated approval of Galafold in adults with Fabry disease and amenable *GLA* variants.

**Table 24: Recommendations and Comments on Review Issues**

Review Issue	Recommendations and Comments
Pivotal or supportive evidence of effectiveness	<p>The primary evidence of effectiveness for migalastat was the reduction from baseline in GL3 inclusion in kidney interstitial capillary (KIC GL-3) observed after 6 months of treatment with 123 mg migalastat once every other day in a placebo-controlled phase 3 trial in patients with Fabry disease who have amenable mutations. Notably, migalastat reduced KIC GL-3 inclusions, mainly in male patients, and in those with baseline GL-3 inclusion <math>\geq 0.3</math> per KIC; the treatment effect was not as evident in female patients and in those patients with baseline KIC GL-3 <math>&lt; 0.3</math>. See Section 8 for more details on the clinical efficacy and sex-dependent treatment responses.</p> <p>Migalastat had an effect on relevant pharmacodynamic biomarkers (i.e., decrease in plasma lyso-Gb3, and increase in WBC alpha-Gal A activity) in male patients with Fabry disease. The mean change in plasma lyso-Gb3, and WBC alpha-Gal A activity supported the mechanism of action of migalastat in vivo and was consistent with the observed decreases in KIC GL-3. Exposure-response relationships for KIC GL-3 or PD biomarkers were not observed in the phase 3 trial in the treated patients with Fabry disease.</p> <p>While GL-3 deposition in the kidney vasculature underlies the pathophysiology of the renal dysfunction in Fabry disease, it remains to be seen whether the reduction in KIC GL-3 inclusions will result in clinical benefits in this population. As such, the clinical benefit of Galafold needs to be further evaluated via a confirmatory clinical trial (as required for accelerated approval).</p>
General dosing instructions	<p>The proposed dose of 123 mg migalastat administered once every other day (QOD) in patients with Fabry disease who have an amenable <i>GLA</i> variant is acceptable.</p> <p>The Applicant proposed an indication for Galafold which would include patients with FD (b) (4) (b) (4)</p>

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Review Issue	Recommendations and Comments
Dosing in patient subgroups (intrinsic and extrinsic factors)	<p><b>Renal Impairment</b> No dose adjustment is recommended in patients with mild or moderate renal impairment. Migalastat is not recommended in patients with severe renal impairment (eGFR &lt; 30 mL/min/1.73m<sup>2</sup>). In subjects with severe renal impairment, the migalastat systemic exposure (AUC) was 4.3-fold higher than in subjects with normal renal function and patients with severe renal impairment were excluded from clinical trials. As such, the OCP recommends that additional studies be performed to determine a safe and effective dosing regimen in patients with FD who have severe renal impairment as patients with Fabry disease develop progressive renal impairment as part of the natural disease progression. See Section 6.2.</p> <p><b>Food intake</b> Migalastat should be administered at least 2 hours before or 2 hours after food ingestion. Food, when consumed 1 hour before or after migalastat administration, significantly decreased the systemic exposure to migalastat while the effect of food consumed 2 hours before or 2 hours after the migalastat administration on migalastat PK was not evaluated; however, the recommended dosing instructions in relation to food intake is consistent with the dosing schedule studied in the phase 3 trials and is acceptable.</p>
Labeling	<p>The OCP recommends that Section 12 of the labeling include a detailed description of how the amenability of <i>GLA</i> variants was determined based on the HEK-293 cell assay.</p> <p>The OCP recommends that Section 12 include the results of the thorough QT study.</p>
Bridge between the to-be-marketed and clinical trial formulations	<p>The to-be-marketed formulation (TBM) differs from the phase 3 clinical formulation (b) (4). No in vivo study (e.g., relative BA/BE study) was deemed necessary to bridge the TBM formulation and the clinical formulation to account for these minor changes.</p>
Patient Selection	<p>The Applicant used an in vitro assay (HEK-293 assay) to determine which <i>GLA</i> variants were “amenable” (GLP HEK assay) to migalastat as a criterion for trial enrollment and subsequent treatment following approval.</p> <p>The design of the assay is appropriate from a methodologic standpoint. Except for sequence analysis in the DNA qualification step (see Section 6.2), the assay was properly validated and is reproducible.</p> <p>A total of 753 <i>GLA</i> variants were tested for amenability with the GLP HEK-293 assay, of which 348 (46%) were classified as amenable based on the GLP HEK assay. Of note, only a fraction of the in vitro tested, amenable <i>GLA</i> variants were represented in the patients dosed in trial AT1001-011 (50 patients in the trial had 31 unique amenable <i>GLA</i> variants or genotypes). Also, while the amenable genotypes classified by the GLP HEK-293 assay support the patient selection for migalastat treatment, they do not ensure a clinical or pharmacodynamic response in vivo based on the observed positive predictive value of the assay in relation to PD changes in the phase 3 trial (as outlined above). Treated patients with amenable <i>GLA</i> variants should be clinically monitored for a therapeutic response throughout treatment with Galafold.</p> <p>Overall, the assay is acceptable from a methodological standpoint and may continue to be used under the Applicant’s specified procedures for future testing and addition of amenable <i>GLA</i> variants.</p>

alpha-Gal = alpha-galactosidase A, Gb3 = globotriaosylceramide, GL-3 = globotriaosylceramide, HEK-293 = human embryonic kidney cell line 293, KIC = Kidney Interstitial Capillary, lyso-Gb3 = globotriaosylsphingosine, OCP = Office of Clinical Pharmacology, PD = pharmacodynamic, QOD = every other day, TBM = to-be-marketed, WBC = white blood cell

### 6.3. Postmarketing Requirements and Commitments

**Table 25: Postmarketing Commitments**

Studies	Rationale	Design
<p><b>PMC 3412-4:</b> PK/PD trial in patients with Fabry disease and amenable <i>GLA</i> variants who have severe renal impairment (eGFR &lt; 30mL/min/1.73m<sup>2</sup>/year) or who are on kidney dialysis.</p>	<p>Use of Galafold in patients with severe renal impairment (eGFR &lt; 30 mL/min/1.73m<sup>2</sup>) will not be recommended in the product label based on the 4.3-fold higher systemic exposure of migalastat in subjects with severe renal impairment (without Fabry disease) than in subjects with normal renal function. Given that patients with Fabry disease can develop renal impairment as part of natural disease progression, prolonged high plasma concentrations to migalastat in Fabry patients with severe renal impairment may adversely affect the residual alpha-Gal activity and result in enzyme inhibition. Therefore, the Applicant should evaluate and identify an appropriate dosing regimen for patients with Fabry disease and severe renal impairment.</p> <p>In addition, the OCP recommends that patients with various degrees of renal impairment, including severe renal impairment, should be included in the postmarketing confirmatory trial (PMR-3412-1) to obtain additional safety and efficacy data.</p>	<p>The study should evaluate PK and appropriate PD markers (b) (4) in (b) (4) adults with Fabry disease and severe renal impairment (eGFR &lt; 30 mL/min/1.73m<sup>2</sup>) or who are on kidney dialysis to determine an appropriate dosing regimen.</p>

Studies	Rationale	Design
<p><b>PMC 3412-5:</b> Additional sequencing analysis of all <i>GLA</i> cDNA constructs used in the GLP HEK-293 assay (amenable and non-amenable) to provide the necessary sequence information that is currently missing from the full length forward and reverse cDNA single strands.</p>	<p>As a standard practice, both research and clinical laboratories use bidirectional sequencing for confirmation of mutation and DNA context of a full-length DNA target to minimize potential for false positive or false negative findings. For the GLP HEK-293 assay system, the Applicant confirmed more than 65% of individual <i>GLA</i> gene mutations from recombinant constructs using data from only one primer or single DNA strand, which is not the standard approach. This sequencing approach creates potential for error when the entire length and context of the DNA target is not covered and confirmed via bidirectional sequencing.</p> <p>As such, the Applicant should complete bidirectional sequencing (or sequencing of both strands) of the full length <i>GLA</i> cDNA in all recombinant constructs (including wild-type, 348 amenable and 405 nonamenable mutations) using internal <i>GLA</i> primers to confirm DNA context and the presence of each <i>GLA</i> mutation tested in the GLP HEK-293 assay.</p> <p>We recommend that the Applicant sequence all 181 amenable and 265 nonamenable constructs that currently have complete unidirectional data for the forward strand and incomplete data for the BGHR primer strand with an internal reverse <i>GLA</i> primer.</p> <p>Further, we recommend that they sequence all remaining mutant-<i>GLA</i> constructs (and wild-type <i>GLA</i>) that currently have incomplete bidirectional data (from T7 and BGHR primers) with internal forward and reverse <i>GLA</i> primers.</p>	<p>Perform further sequence analysis using internal bidirectional <i>GLA</i> primers. The newly generated data should be appropriately matched with previous data to complete bidirectional analysis and produce a consensus <i>GLA</i> cDNA sequence for each construct. To confirm the presence of a mutation and the sequence context of the cDNA, the consensus should be compared to the NCBI CCDS reference.</p>

alpha-Gal A = alpha-galactosidase A, bp = base pairs, CCDS = Consensus Coding Sequence, eGFR = estimated glomerular filtration rate, Gb3 = globotriaosylceramide, GL-3 = globotriaosylceramide, HEK-293 = human embryonic kidney cell line 293, NCBI = National Center for Biotechnology Information, OCP = Office of Clinical Pharmacology, WT = wild type, BGHR = bovine growth hormone reverse

## 6.4. Summary of Clinical Pharmacology Assessment

The Applicant presented the dose of migalastat based on migalastat HCl in the submission: 150 mg migalastat HCl is equivalent to 123 mg migalastat.

### 6.4.1. Pharmacology and Clinical Pharmacokinetics

#### Pharmacology:

Migalastat is a pharmacological chaperone designed to bind selectively and reversibly to the active site of alpha-galactosidase A (alpha-Gal A). The binding of migalastat stabilizes variant forms of alpha-Gal A in the endoplasmic reticulum, facilitating their proper trafficking to lysosomes. Because migalastat binds to the active site of alpha-Gal A, migalastat competitively inhibits alpha-Gal A in a concentration-dependent manner. Once in the lysosomes, at a lower pH and in the presence of a high concentration of endogenous substrates, migalastat dissociates from alpha-Gal A allowing it to bind to and break down its natural substrates, globotriaosylceramide (GL-3, also known as Gb3) and globotriaosylsphingosine (lyso-Gb3, also

known as lyso-GL3), which accumulate within lysosomes in Fabry disease. As migalastat binds to the active site of alpha-Gal A, it should work only on the variant forms of alpha-Gal A with the active site that allows migalastat binding as well as wild-type alpha-Gal A.

#### **Clinical Pharmacokinetics:**

Following oral administration of 123 mg migalastat (equivalent to 150 mg migalastat HCl), the peak plasma concentrations for migalastat were achieved 2 to 3.5 hours postdose with mean peak plasma concentration ( $C_{max}$ ) of approximately 1630 ng/mL. The absolute bioavailability of oral migalastat capsule was 75%. In healthy subjects, the systemic exposure of migalastat increased in a dose-proportional manner between 75 mg to 1250 mg migalastat HCl after a single oral dose administration and between 50 mg to 250 mg migalastat HCl after multiple doses under every other day dosing. Migalastat did not accumulate following 150 mg migalastat HCl dosing once every other day (QOD) for 12 weeks.

Migalastat does not bind to plasma proteins to any significant extent, and protein binding was not dose-dependent. In a mass balance study with oral administration of 123 mg [ $^{14}C$ ] migalastat, approximately 77.2% of the [ $^{14}C$ ]-radioactivity was recovered in urine and 20.4% was recovered in feces with overall total recovery of 97.6% within 96 hours postdose. In urine, unchanged migalastat accounted for 80% of the radioactivity which equates to 62% of the administered dose. In feces, unchanged migalastat was the only drug-related component. Migalastat appears to undergo minor metabolism. While the metabolic pathways and responsible metabolic enzymes have not been clearly elucidated, glucuronidation of metabolites was observed. Dehydrogenated O-glucuronide conjugated metabolites (M1, M2 and M3) accounted for 5%, 2% and 6% of the plasma radioactivity in the mass balance study. The apparent half-life of migalastat following oral administration of 123 mg migalastat capsule was 3.9-7.3 hours.

#### **Clinical Pharmacodynamics**

After treatment with 123 mg QOD migalastat for 6 months in patients with Fabry disease (study AT1001-011), plasma lyso-Gb3 was reduced from the baseline in Fabry patients with an amenable genotype. In a subgroup analysis, more noticeable reductions in plasma lyso-Gb3 and increases in WBC alpha-Gal A activity were observed in male patients compared to female patients despite similar migalastat plasma exposures between male versus female patients.

#### **6.4.2. General Dosing and Therapeutic Individualization**

##### **General Dosing**

The recommended dose for Galafold is one capsule (123 mg migalastat) administered once every other day (QOD) in patients with Fabry disease with an amenable variant form of alpha-Gal A enzyme is acceptable. Galafold is recommended to be administered at least 2 hours before and 2 hours after meal intake.

### **Therapeutic Individualization**

#### **Renal Impairment:**

Galafold is not recommended in patients with severe renal impairment (eGFR < 30 mL/min/1.73m<sup>2</sup>). No dosage adjustment in patients with mild, moderate and severe renal impairment is recommended.

#### **Genotype-based Patient Selection:**

Migalastat is recommended only for those patients who carry an amenable *GLA* genotype. The amenable *GLA* genotypes, which were identified by the in vitro GLP HEK-293 assay, with corresponding predicted amino acid changes, will be listed in Section 12.1 of the label.

As a pharmacological chaperone, migalastat binds to the active site of wild-type alpha-Gal A enzyme and variant alpha-Gal A enzymes with the active site formed and conformationally permissible for migalastat binding. The *GLA* variants (mutations) encoding these mutant proteins are termed amenable. As such, migalastat is recommended only for patients who have amenable *GLA* genotypes that will produce a variant alpha-Gal A with an active site with conformation that permits migalastat binding.

Because the relationship between genotype and molecular phenotype of alpha-Gal A is not completely understood and there are over 1000 individual *GLA* variants, the amenable *GLA* genotypes were identified using an in vitro assay (herein referred to as the HEK-293 assay). The assay used a conventional transient transfection method to produce each mutant alpha-galactosidase A (alpha-Gal A) enzyme in HEK-293 cells. Following treatment with migalastat (AT1001), residual enzyme activity was measured in extracted lysates and activity was normalized to total protein content. The in vitro assay did not evaluate the trafficking of the variant alpha-Gal A proteins into the lysosome or the dissociation of migalastat from the variant alpha-Gal A proteins within the lysosome.

Of note, patient selection for enrollment in the phase 3 trials AT1001-011 and AT1001-012 was based on variant categorization according to the CT (clinical trial) HEK-293 in vitro assay, which categorized *GLA* variants as either “responsive” to migalastat or “non-responsive.” The criteria for this categorization remained unchanged when the HEK-293 assay underwent GLP validation; however, additional quality control measures were implemented as part of the validation process, which resulted in the re-classification of a proportion of “responsive” *GLA* variants (per the CT HEK-293 assay) to “non-amenable” (per the GLP HEK-293 assay) and vice versa (~10% of variants).

In the HEK-293 assay (both CT and GLP assays), a variant was categorized as amenable (“responsive” for CT assay) if, after a 5-day incubation with 10 µmol/L migalastat, residual mutant alpha-Gal A enzyme (as measured in the total protein lysates) showed: 1) an absolute enzyme activity increase of at least 3% relative to the wild-type (normal) alpha-Gal A activity

and 2) at least a 1.2-fold relative increase in activity compared to untreated baseline. Also see Appendix 5 for more details with regards to the two HEK-293 assays used in the clinical development program.

Overall, a total of 348 variants were classified as amenable of the 753 screened. Of note, only a fraction of amenable *GLA* genotypes were studied in clinical trials (47 FD patients had 30 unique amenable *GLA* variants or genotypes unique amenable genotypes in trial AT1001-011).

Also, while the amenable genotypes classified by GLP HEK-293 assay support the patient selection for migalastat treatment, it does not ensure a clinical or pharmacodynamic response in vivo. The Applicant used cutoff values that did not consider the analytical performance of the assay for clinical biomarker response. Using adjusted cutoff values, the analysis showed that the correlation of the HEK assay with clinical biomarker response (including KIC GL-3, residual alpha-Gal A activity in white blood cells, and plasma lyso-Gb3) is only moderate at best. Overall, a mutation classified as amenable by the HEK assay had only around a 50% chance of showing a response for any one of the three clinical biomarkers evaluated. Because the assay does not have high positive predictive value, a therapeutic trial period with measurement of WBC alpha-Gal A and plasma Lyso-Gb3 should be considered in patients. See Section 6.4.1 and Appendix 5 for more details.

### **Outstanding Issues**

The Applicant reported that 498 of 753 individual *GLA* mutation or mutant constructs tested in the HEK-293 assay were confirmed by sequencing only one strand of the double-stranded *GLA* cDNA insert. This is not in accordance with the Applicant's stated criteria or in accordance with the standard or best practice of bidirectional sequencing. Other independent sequence data generated by the Applicant neither alone nor in combination provides the required bidirectional analysis because the reads are not long enough to cover the full length of each cDNA strand or sequencing was completed in full for only one strand (unidirectional).

The OCP considers that these data provide sufficient support that the specific *GLA* mutation and remaining sequence context of each amenable construct presently confirmed using single strand data only (albeit, replicated single strand data) to allow inclusion in the label.

Nevertheless, sequencing with only one primer or one single DNA strand creates the potential for error when the entire length and context of the double-stranded DNA target is not covered and confirmed via bidirectional sequencing. Therefore, additional and complete sequencing of both cDNA strands is needed to confirm mutation and DNA context of a full-length DNA target (which will be addressed through the planned postapproval study, see section 6.3).

## 6.5. Comprehensive Clinical Pharmacology Review

### 6.5.1. General Pharmacology and Pharmacokinetic Characteristics

#### General Pharmacology

##### Mechanism of Action:

Migalastat is a pharmacological chaperone that reversibly binds to the active site of the alpha-Gal A protein. Specific variants of the gene encoding alpha-galactosidase A (*GLA*) can result in the production of abnormally folded and less stable mutant forms of the enzyme which, however, retain enzymatic activity. Migalastat can stabilize these alpha-Gal A mutant proteins thereby increasing their cellular and intralysosomal levels and, thus, restoring their intralysosomal enzyme activity.

##### In vitro binding to recombinant human alpha-Gal A:

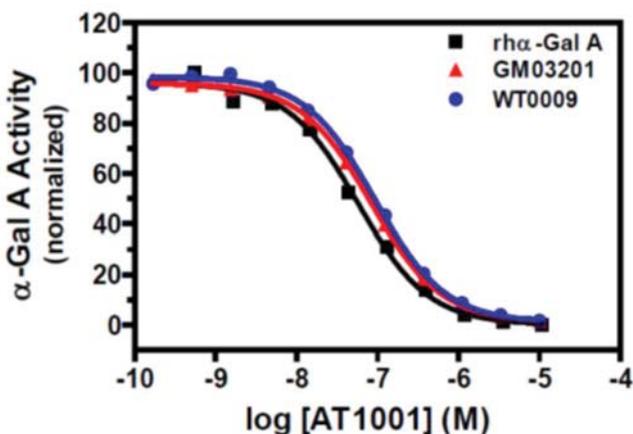
In in vitro studies using an enzyme inhibition assay, migalastat binds to recombinant human alpha-Gal A (ralpha-Gal A, agalsidase beta) and agalsidase alfa at both neutral pH (as in the endoplasmic reticulum) and acidic pH (as in lysosomes) with dissociation constants ( $K_i$  values) in the range of 10 to 29nM (Table 26). Migalastat also binds endogenous alpha-Gal A similarly to exogenous alpha-Gal A (Figure 2). The binding of migalastat was selective for alpha-Gal A relative to other lysosomal hydrolases and galactose-pathway enzymes.

**Table 26:  $K_i$  Values for Migalastat Binding to Agalsidase Beta and Agalsidase Alfa**

pH	Agalsidase Beta		Agalsidase Alfa	
	n	$K_i$ (nM)	n	$K_i$ (nM)
4.6	8	28.6	Not determined	
5.2	3	10.5	2	10.9
7.0	4	16.4	3	16.1

Source: 2.6.2 Pharmacology Written Summary, table 1, page 11

**Figure 2: Migalastat Binds and Inhibits Agalsidase Beta and Endogenous alpha-Gal A**

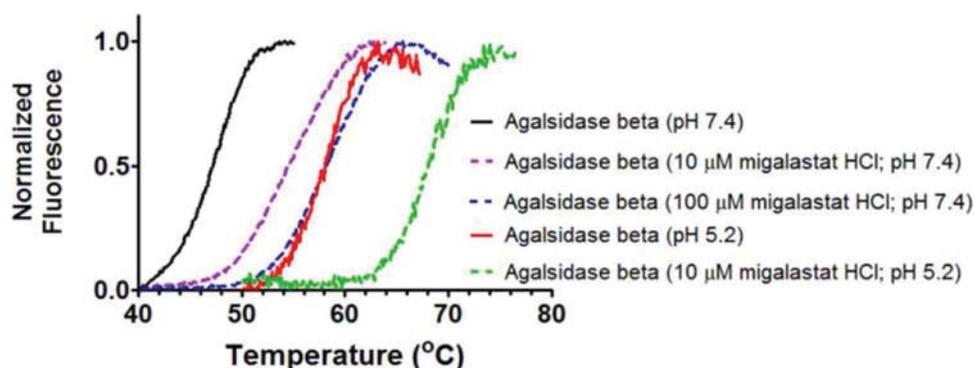


Migalastat (AT1001) inhibits the activity of recombinant human alpha-galactosidase A (ralpha-Gal A or agalsidase beta) and the activity of alpha-Gal A in lysates prepared from 2 different normal human lymphoblast cell lines (GM03201 and WT0009). In the experiments shown, the half maximal inhibitory concentration (IC<sub>50</sub>) values for inhibition of alpha-Gal A activity at pH 4.6 were 58nM, 83nM, and 93nM for ralpha-Gal A, GM03201 lysates, and WT0009 lysates, respectively. Each point represents the mean of duplicate determinations. The ralpha-Gal A inhibition curve is representative of five independent experiments; the GM03201 and WT0009 inhibition curves are representative of four and five independent experiments, respectively. Data were normalized to the alpha-Gal A activity in the absence (100% value) and presence of 10μM migalastat HCl (0% value). Source: 2.6.2 Pharmacology Written Summary, Figure 2, page 12

#### **In vitro Stabilization of agalsidase:**

Binding of migalastat increased the physical stability of agalsidase beta and agalsidase alfa in a concentration-dependent manner and attenuated time-, temperature-, and pH-dependent denaturation (Figure 3, Figure 4, Figure 5), and inactivation in human whole blood ex vivo (Figure 6). As expected for a lysosomal enzyme, agalsidase beta and alpha were more stable at lower pH 5.2 compared to higher pH of 7.4 (Figure 3 and Figure 4).

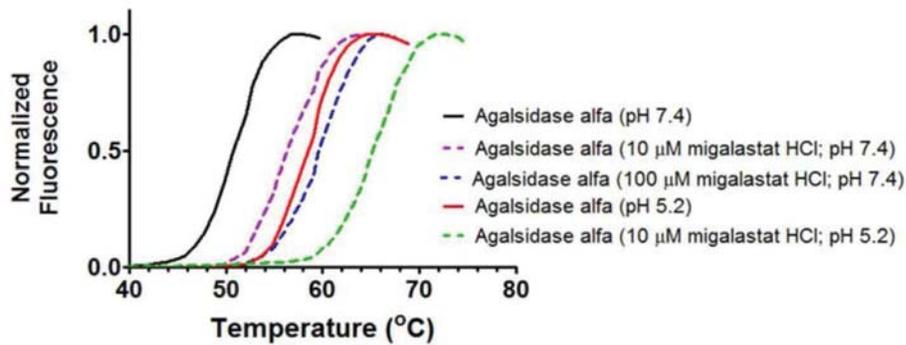
**Figure 3: Migalastat Stabilizes Agalsidase Beta In Vitro**



Representative thermal stability scans of agalsidase beta in the absence and presence of varying concentrations of migalastat HCl. Thermal stability scans were performed at neutral and acidic pH values of 7.4 and 5.2, respectively. Denaturation and unfolding of agalsidase beta was monitored using SYPRO Orange fluorescence changes (y-axis) as a function of increasing temperature (x-axis). The data shown are representative of 3 independent experiments.

Source: 2.6.2 Pharmacology Written Summary, Figure 4, page 14

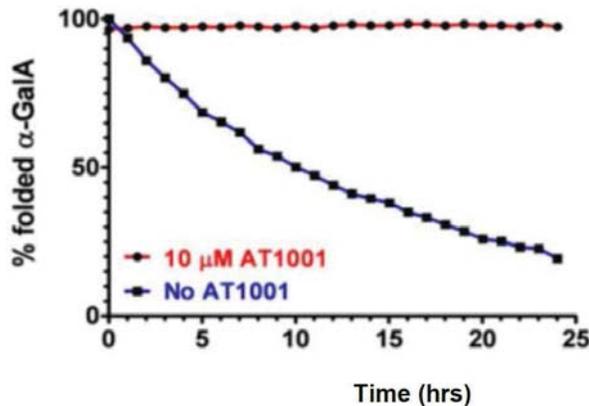
**Figure 4: Migalastat Stabilizes Agalsidase Alfa In Vitro**



Representative thermal stability scans of agalsidase alfa in the absence and presence of varying concentrations of migalastat HCl. Thermal stability scans were performed at neutral and acidic pH values of 7.4 and 5.2, respectively. Denaturation and unfolding of agalsidase alfa was monitored using SYPRO Orange fluorescence changes (y-axis) as a function of increasing temperature (x-axis).

Source: 2.6.2 Pharmacology Written Summary, Figure 5, page 14

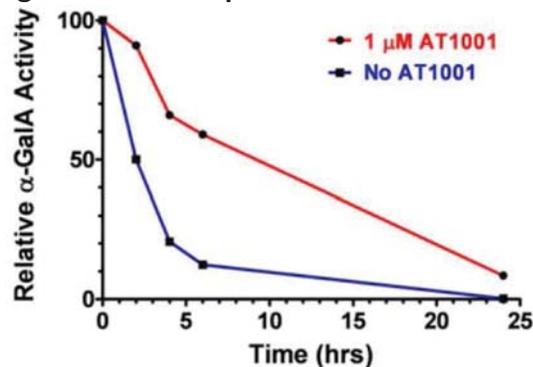
**Figure 5: Time-dependent Denaturation of Agalsidase Beta in Buffer at Neutral pH**



Time course of recombinant human alpha-galactosidase A (ralpha-Gal A or agalsidase beta) unfolding in neutral buffer (pH 7.4) at 37°C in the absence and presence of 10μM migalastat HCl (AT1001). Unfolding of ralpha-Gal A was monitored by changes in the fluorescence of SYPRO Orange as a function of time.

Source: 2.6.2 Pharmacology Written Summary, Figure 6, page 15

**Figure 6: Time-dependent Inactivation of Agalsidase Beta in Human Whole Blood**



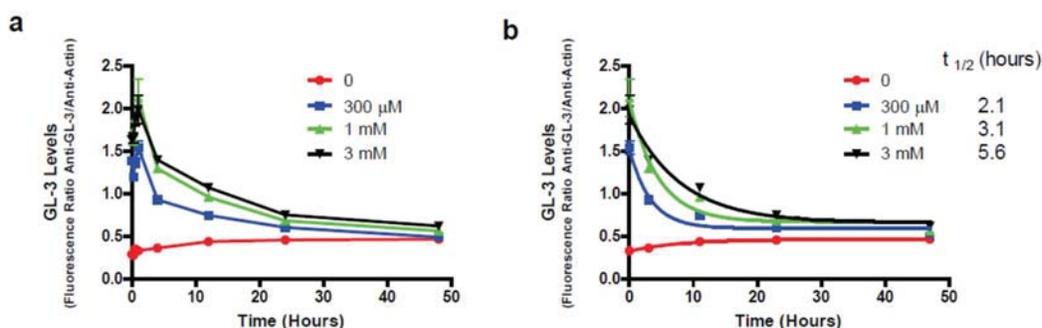
Time course of recombinant human alpha-galactosidase A (ralpha-Gal A or agalsidase beta) inactivation in human whole blood at 37°C ex vivo in the absence and presence of 1μM migalastat HCl (AT1001). Ralpha-Gal A activity was determined at the indicated time points using 4-methylumbelliferyl-alpha-D-galactopyranoside. To obtain relative enzyme activity levels, measurements at the various time points were normalized to the activity at time zero.

Source: 2.6.2 Pharmacology Written Summary, Figure 7, page 16

### **Reversibility of Inhibition of alpha-Gal A by Migalastat In Vitro**

The reversibility of migalastat inhibition of alpha-Gal A is important when migalastat binding is no longer needed once the alpha-Gal A has been trafficked to the lysosome, where free alpha-Gal A is needed to metabolize its natural substrates, e.g., GL-3. In an in-vitro assay, GL-3 levels were increased in normal human fibroblasts in the presence of a high concentrations of migalastat HCl (300 $\mu$ M, 1mM, and 3mM) after incubation with migalastat for 7 days. However, when migalastat was removed from media after 7 days of incubation, GL-3 concentrations returned to baseline levels within 48 hours, suggesting that migalastat inhibition of cellular alpha-Gal A activity is reversible. The cells incubated with the highest concentration (3mM) of migalastat HCl took the longest to reduce GL-3 back to baseline levels.

**Figure 7: GL-3 Levels in Human Wild-type Fibroblasts over Time After Removing Migalastat from Media**



(a) The measured GL-3 levels at every time point. (b) One-phase exponential decay analysis after adjusting for the initial increase in GL-3 levels in the first 60 minutes. Each point represents the mean  $\pm$  standard error of three data points.

Source: 2.6.2 Pharmacology Written Summary, Figure 10, page 21

### **Assessment for QT Prolongation:**

In a dedicated TQT (thorough QT) study of migalastat, no significant QTc prolongation was observed after a single dose of 150 mg migalastat HCl or after a suprathreshold dose of 1025 mg of migalastat HCl. At the 1250-mg dose (8.3-fold higher than 150 mg), the mean  $C_{max}$  and  $AUC_{0-inf}$  were 7.8-fold and 7.0-fold higher, respectively, compared to those parameters after 150 mg migalastat HCl.

**Clinical Pharmacokinetics:**

**Table 27: Summary of Clinical Pharmacokinetics:**

General Information	
Bioanalysis	The drug product migalastat was measured with a validated LC/MS/MS method in plasma.
Healthy subjects vs. patients	Following a single oral dose of 150 mg migalastat HCl, healthy subjects (phase 1 trials) and patients with Fabry disease (AT1001-013 and study FAB-CL-204) have similar PK parameters. Population PK analysis indicated a 16% reduction in clearance for patients with Fabry disease and a 28% reduction in volume of distribution that was not explained by renal function or body weight as covariates on population PK. See Appendix 7 for further details regarding sensitivity analysis without the effect of disease status in the model.
Drug exposure at steady state following the therapeutic dosing regimen	Following 150 mg migalastat HCl QOD dosing, the geometric mean (CV%) $C_{max}$ was approximately 2029 (40%) ng/mL and AUC <sub>0-12</sub> was 10638 (35.6%) ng.hr/mL at the steady state in patients with Fabry disease (study FAB-CL-204).
Maximally tolerated dose or exposure	<u>Patients with Fabry disease:</u> Migalastat HCl is well-tolerated following single doses up to 450 mg (Study AT1001-013), multiple doses up to 250 mg BID for up to 2 weeks (FAB-CL-201), and multiple doses at 250 mg QOD for up to 48 weeks (study FAB-CL-204). <u>Healthy subjects:</u> Migalastat HCl was well-tolerated following single doses up to 2000 mg (oral solution; study FAB-CL-104) and up to 150 mg BID for 7 days (Study FAB-CL-102).
Dose Proportionality	<u>Fabry Patients:</u> Systemic exposure of migalastat increased in an approximately dose-proportional manner between the 50 mg to 250 mg migalastat HCl dosages following single dose administration on day 1 and following multiple QOD dose administration on day 14 and day 84 (study FAB-CL-204). Systemic exposure of migalastat also increased in a dose-proportional manner from 25 mg BID to 250 mg BID migalastat HCl (Study FAB-CL-201). <u>Healthy Subjects:</u> <u>Oral Solution:</u> Following a single oral dose administration of migalastat HCl as oral solution, migalastat exposure (both $C_{max}$ and AUC) increased in dose-proportional manner from 75 mg to 675 mg (Study FAC-CL-101) and from 500 mg to 1250 mg (Study FAB-CL-104). However, from 25 mg to 75 mg, AUC increased in more than dose-proportional manner, while $C_{max}$ increases in a dose-proportional manner (Study FAB-CL-101). In addition, the exposure appears to plateau after 1250-mg dose as the PK profiles and exposure of 2000-mg dose is similar to that of 1250-mg dose (Study FAB-CL-104). <u>Capsule:</u> $C_{max}$ and AUC increased in a dose-proportional manner from 50 mg to 450 mg migalastat HCl in healthy Japanese subjects (study MGM115806). <u>IV:</u> Following IV infusion over 2 hours, migalastat exposure (both $C_{max}$ and AUC) increased in approximately dose-proportional manner from 0.3 mg/kg to 10 mg/kg (Study AT1001-018). <u>Multiple dose:</u> Following BID dosing for 7 days, AUC and $C_{max}$ increased in a dose-proportional manner between 50 mg and 150 mg migalastat HCl on day 7 (n = 6) (Study FAB-CL-102).

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Accumulation	<p><b>Fabry Patients:</b> Migalastat does not accumulate following 50 mg, 150 mg and 250 mg QOD migalastat HCl dosing for 12 weeks in patients with Fabry disease (study FAB-CL-204).</p> <p><b>Healthy Subjects:</b> Following 150 mg BID migalastat HCl dosing in healthy subjects, migalastat does not accumulate significantly as the systemic exposure (AUC and C<sub>max</sub>) of migalastat on day 7 was similar to that of day 1 (Study FAB-CL-102).</p>
Variability	Coefficient of variation (%CV) for migalastat PK parameters (AUC and C <sub>max</sub> ) ranged from 25% to 37%. %CV was similar in healthy subjects and patients with Fabry disease.
Absorption	
Bioavailability	Following a single-dose oral administration of 150 mg migalastat HCl capsule (phase 3 formulation), the absolute bioavailability was 75% compared to 150 mg IV infusion (Study AT1001-018).
T <sub>max</sub>	Following 150 mg migalastat HCl oral capsule, T <sub>max</sub> reached between 2.5-3 hr (1.5-4 hr) postdose administration in both patients with Fabry disease and healthy subjects.
Distribution	
Volume of Distribution	Population PK analysis of data from 260 subjects estimated the total volume of distribution at steady state to be 89 L. The central volume of distribution was 63.6 L while the peripheral volume of distribution was estimated to be 25.7 L. The relative standard error for these estimates were 5 and 11% respectively. Between subject variability as indicated by CV% was 12% for V <sub>2</sub> and not estimated for V <sub>3</sub> . Shrinkage on V <sub>2</sub> did not exceed 8%.
Plasma Protein Binding	Migalastat, in concentration range of 1 to 100µM, does not bind to human plasma protein to any significant amount and protein binding was not dose-dependent.
Substrate transporter systems [in vitro]	Migalastat does not appear to be a substrate for P-gp, BCRP, MATE1, MATE2, OAT1, OAT3 and OCT2 at concentrations up to 100µM.
Elimination	
Terminal Elimination half-life	Following a single oral administration of 150 mg migalastat HCl capsule, the apparent elimination half-life ranges from 3.7 to 7.2 hours.
Metabolism	Metabolism is a minor elimination pathway for migalastat. However, the metabolic pathway and responsible metabolic enzyme have not been clearly elucidated. Dehydrogenated O-glucuronide conjugated metabolites (M1, M2 and M3) accounted for 5%, 2% and 6% of the plasma radioactivity in the mass balance study.

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Excretion	<p>In a mass balance study, following oral administration of 123 mg [<sup>14</sup>C] Migalastat, 77.2% of the [<sup>14</sup>C]-radioactivity was recovered in urine and 20.4% was recovered in feces with overall total recovery of 97.6% within 96 hours postdose. In urine, unchanged migalastat accounted for 80% of the radioactivity, which equates to 62% of the administered dose. In feces, unchanged migalastat was the only drug-related component. In plasma, unchanged migalastat accounted for approximately 77% of the plasma radioactivity and 3 dehydrogenated O-glucuronide conjugated metabolites, M1 to M3, together accounted for approximately 13% of the plasma radioactivity, none of which comprised more than 6% of the radio-labeled dose. Approximately 9% of the total radioactivity in plasma was unassigned. (Study AT1001-014).</p> <p>Following single oral dose administration, the proportion of the dose recovered in the urine as unchanged drug over 24 hours was between 32% to 49% (Study FAC-CL-101).</p> <p>Following single oral dose administration (50 mg to 450 mg) of migalastat HCl in healthy Japanese subjects, the proportion of the dose recovered in the urine as unchanged drug was between 45% to 50% after 24 hours of dose administration (study MGM115806).</p> <p>Following a single IV dose administration (0.3-10 mg/kg), the percentage of migalastat recovered in the urine as unchanged drug was 69% to 85% over 24 hours suggesting that renal excretion is a major elimination pathway for migalastat after IV administration (AT1001-018).</p>
In vitro interaction liability (Drug as perpetrator)	
Inhibition/Induction of metabolism	<p>Migalastat does not induce CYP1A2 and CYP3A4 at concentrations up to 1000µM, or CYP2B6 at concentrations up to 500µM.</p> <p>Migalastat did not cause direct, time-dependent or metabolism-dependent inhibition of CYP enzymes, namely CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4/5 at concentrations up to 500µM.</p>
Inhibition/Induction of transporter systems	<p>AT1001 at concentrations up to 500µM does not inhibit BCRP, P-gp (MDR1), BSEP, OATP1B1, OATP1B3, OAT1, OAT3, OCT1, OCT2, MATE1, and MATE2-K human transporters.</p>

The pharmacokinetic parameters of migalastat following a single-dose 150 mg of migalastat HCl (capsule; phase 3 formulation) are summarized in Table 28.

**Table 28: Geometric Mean (CV%) PK Parameters for Following Single Oral Dose Administration of 150 mg Migalastat HCl Capsule (Phase 3 Formulation) under fasted condition**

Studies	Subjects	n	C <sub>max</sub> (ng/ml)	t <sub>max</sub> <sup>a</sup> (hr)	AUC <sub>0-t</sub> (hr*ng/ml)	AUC <sub>0-inf</sub> (hr*ng/ml)	t <sub>1/2</sub> <sup>b</sup> (hr)	CL/F (L/hr)	Vd/F (L)
AT1001-016 Food effect	Healthy	19	1561 (33.8%)	3.0 (1.5- 6.0)	9696 (27.1%) t = 24 hr	9805 (26.8%)	3.9 (0.45)	12.5 (26.8%)	70.2 (32.7%)
AT1001-018 Absolute BA	Healthy	10	1786 (25.9%)	2.75 (1.5-4)	9777 (25.7%) t = 48hr	9881 (25.6%)	7.28 (4.3)	n.d.	n.d.
AT1001-015 Renal impairment	Healthy	8	2100 (26%)	2.5 (1.5-3 hr)	12306 (27.9%) t = 120 hr	12397 (27.7%)	6.42 (1.93)	12.1 (27.7%)	107 (38%)
AT1001-013 DDI study <sup>c</sup>	Fabry Patients	12	1630 (32.25%)	3.00 (2-4)	12371.21 (36.19%) t = 24hr	12858.27 (37.15%)	5.08 (0.874)	n.d.	n.d.

<sup>a</sup> For t<sub>max</sub> median and (minimum - maximum) are presented.

<sup>b</sup> For t<sub>1/2</sub>, arithmetic mean (SD) are presented

<sup>c</sup> Fasted for two hours before and after migalastat administration

n.d. :not determined/not reported

## 6.5.2. Clinical Pharmacology Questions

### 6.5.3. To what extent does the available clinical pharmacology information provide pivotal or supportive evidence of effectiveness?

*The primary evidence of effectiveness for Galafold was based on the subgroup analysis of placebo controlled phase 3 trial, AT1001-011, where reduction from baseline in GL-3 inclusions in kidney interstitial capillaries (KIC GL-3), a surrogate endpoint was observed after 6-month treatment with 150 mg QOD migalastat HCl in patients with Fabry disease with amenable GLA variants, particularly in male patients and in patients with high baseline GL-3 inclusions per KIC (GL-3 ≥ 0.3). Please refer to sections 7 and 8 for a detailed discussion of the efficacy findings. While the accumulation of KIC GL-3 is observed in patients with Fabry disease, it is not known whether reduction in KIC GL-3 will result in clinical benefits.*

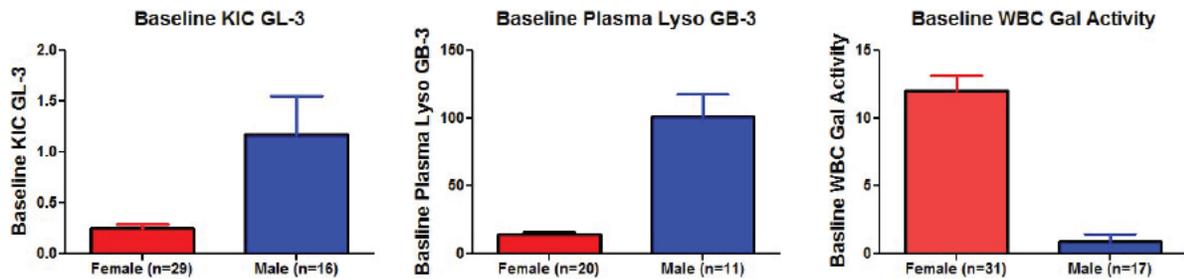
*The observed changes in disease-specific pharmacodynamic biomarkers (plasma lyso-Gb3 and WBC alpha-Gal A activity) in phase 3 trials, particularly in male patients, and in vitro studies supporting migalastat's mechanism of action (refer to section 6.5.1) provide evidence that migalastat is pharmacodynamically active at the doses studied.*

*Dose-response or exposure-response relationships were not observed in phase 2 and 3 trials in patients with Fabry disease to support the evidence of effectiveness (see section 6.5.4 for detailed discussion of dose-response and exposure-response analysis).*

**PD Biomarkers:**

In Study AT1001-011, notable differences in baseline KIC GL-3, plasma lyso-Gb3, and WBC alpha Gal-A activity were observed between male and female patients with Fabry disease (Figure 8). Furthermore, for a given genotype, male patients had lower WBC alpha Gal A activity and higher plasma lyso-Gb3 and KIC GL-3 at baseline compared to female patients (Figure 9).

**Figure 8: Baseline Biomarker Values in Fabry Patients With Amenable GLA Variants by Sex (Study AT1001-011):**

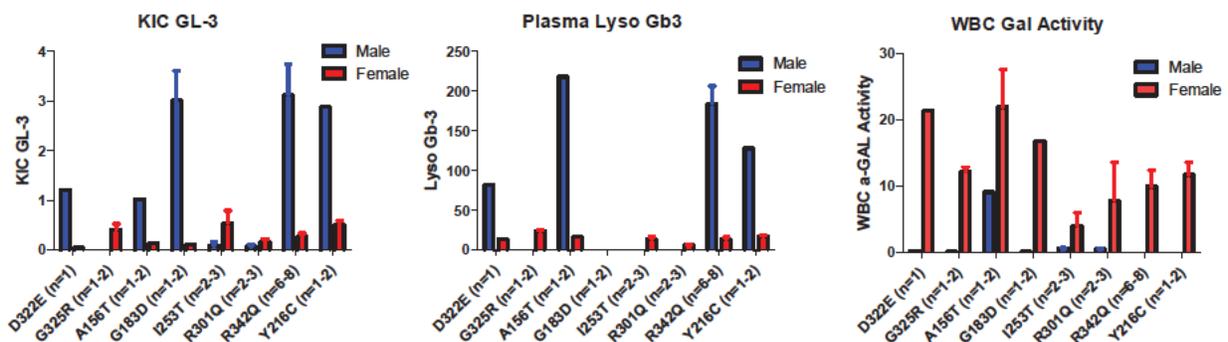


Source: Reviewer’s analysis based on amenable patients with Fabry disease in study AT1001-011

Gal = galactosidase, GL-3 = globotriaosylceramide, KIC = Kidney Interstitial Capillary, lyso-GB3 = globotriaosylsphingosine, WBC = white blood cell

Units: KIC GL-3 (Inclusion per capillary), Lyso Gb-3 (nmol/L), WBC Gal A activity (nmol/hr/mg protein)

**Figure 9: Baseline Biomarker Values in Fabry Patients by Genotype and Sex (Study AT1001-011):**



Source: Reviewer’s analysis based on patients with Fabry disease in study AT1001-011.

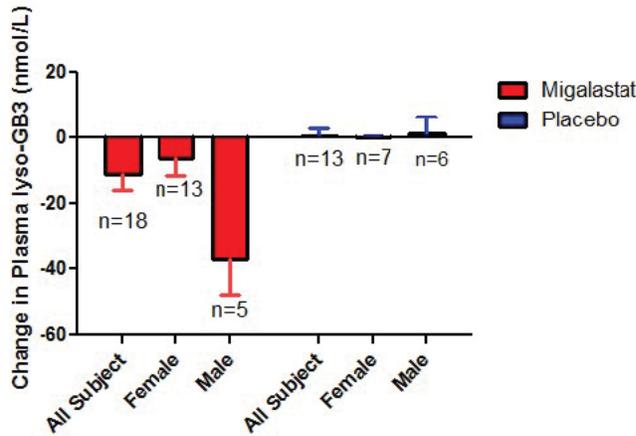
Gal = galactosidase, GL-3 = globotriaosylceramide, KIC = Kidney Interstitial Capillary, lyso-Gb3 = globotriaosylsphingosine, WBC = white blood cell

Note: All mutations were amenable except for R342Q (non-amenable)

Units: KIC GL-3 (Inclusion per capillary), Lyso Gb-3 (nmol/L), WBC Gal A activity (nmol/hr/mg protein)

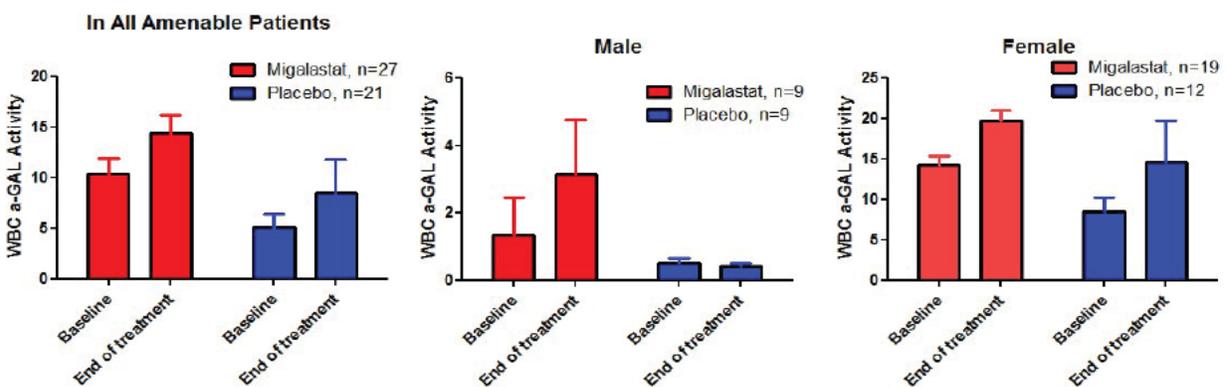
In study AT1001-011, after treatment with 150 mg migalastat HCl QOD for 6 months, plasma lyso-Gb3 was reduced from the baseline in all amenable patients with Fabry disease (Figure 10). More noticeable reductions in plasma lyso-Gb3 (Figure 10) and increases in WBC alpha-Gal A activity (Figure 11) were observed in male patients compared to female patients despite the similar migalastat plasma exposure between male versus female patients (Figure 12).

**Figure 10: Mean ( $\pm$  SE) Change from Baseline to Month 6 in Plasma Lyso-Gb3 (nmol/L) in Patients With Amenable *GLA* Variants with Available Data (Study AT1001-011)**



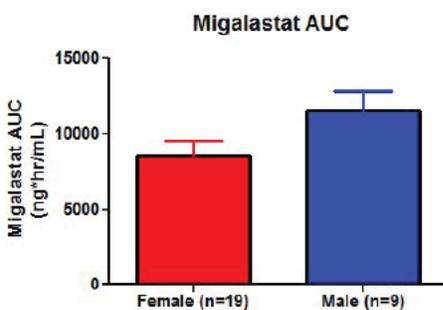
Source: Reviewer's analysis based on patients with amenable variants in study AT1001-011  
lyso-GB3 = globotriaosylsphingosine

**Figure 11: Mean ( $\pm$  SE) WBC alpha-Gal A Activity (nmol/hr/mg protein) at Baseline and At Month 6 in Patients With Amenable *GLA* Variants (Study AT1001-011)**



Source: Reviewer's analysis based on patients with amenable variants in study AT1001-011  
alpha-Gal A = alpha-galactosidase A, SE = standard error, WBC = white blood cell

**Figure 12: Mean ( $\pm$  SE) Migalastat AUC (ng\*hr/mL) in Patients With Amenable *GLA* Variants by Sex (Study AT1001-011)**



AUC = area under the curve, SE = standard error

### **Sequencing of *GLA* cDNA Constructs and in vitro HEK-293 Assay for Amenability**

#### **Determination:**

The Applicant has proposed to use an in vitro assay to select patients who are eligible for treatment and allow extension of the approval beyond mutations for which clinical trial data exist. In this case, the Applicant has developed a transient transfection system coupled with a fluorescent enzyme activity assay. The system has been developed through three generations and the most recent, herein referred to as the GLP HEK assay, has been formally validated (see Appendix 5). In brief, a specific point mutation known to be associated with FD is created in the wild-type galactosidase alpha (*GLA*) cDNA insert of an expression vector/construct. This mutated-*GLA* cDNA construct is then used to transiently transfect HEK-293 cells which are cultured for 5 days in the presence or absence of migalastat to allow expression of the specific alpha-Gal A protein form. Then, the cells are lysed and mutant alpha-Gal A activity is measured along with wild-type alpha-Gal A activity using a standard enzyme activity assay with a fluorogenic substrate. Appropriate controls are included; please see Appendix 5 for a detailed summary of the assay's steps and a more complete evaluation.<sup>10</sup>

Among the 753 total *GLA* variants tested, 348 (46.2%) were classified as “amenable” based on the pre-defined biochemical criteria for amenability. The Office of Study Integrity and Surveillance inspected the analytical site for the HEK-293 cell assay and recommended that the review team accept the data from study RR1001-26.

The overall design of the assay and the availability of relevant data are acceptable and met the six criteria suggested in a previous publication.<sup>11</sup> Data quality and calculations were checked at several steps in the assay. In step 1, the plasmid DNA quality and the digestion were of acceptable quality. However, the OCP identified an issue where the sequence confirmation of WT- and mutant-*GLA* cDNA sequences was not performed using bidirectional sequencing and according to the Applicant's 3<sup>rd</sup> criterion, as follows:

- The Quality Score (QS) should be higher than 30.
- The Continuous Read Length (CRL) score should be higher than 500.
- Sequencing data from both reverse and forward primers are needed and should match.
- There should be at least a 50 base pair (bp) overlap of readable sequence between the sequence read in the forward and reverse directions.

In actual practice, the Applicant used a single primer for each forward and reverse strand located ~120 bp inside the vector sequence and flanking the cDNA insert. The *GLA* cDNA insert is 1290 bp, so a read length of ~1500 nucleotides would be needed to ensure full coverage of

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<sup>10</sup> Memorandum to file dated May 21, 2018 in DARRTS (Reference ID: 4265929)

<sup>11</sup> Durmowicz, AG, R Lim, H Rogers, CJ Rosebraugh, and BA Chowdhury, 2018, The U.S. Food and Drug Administration's Experience with Ivacaftor in Cystic Fibrosis. Establishing Efficacy Using In Vitro Data in Lieu of a Clinical Trial, *Ann Am Thorac Soc*, 15(1):1-2.

each of the forward and reverse strands. Among sequence data for 35 mutations examined, the read lengths averaged about 950 bp. Thus, for mutations located in the extreme 5' and 3' regions of the *GLA* cDNA sequence, there is only information from a single strand available.

Further, the Applicant confirmed that 498 of 753 individual mutant-*GLA* cDNA constructs were confirmed using data from only one single strand. While the Applicant submitted additional independent sequence data for all 753 mutant-*GLA* constructs used in the GLP HEK-293 assay, these data do not provide complete bidirectional sequencing of the *GLA* cDNA which is the standard approach. However, collectively these independent data provide sufficient interim data and confidence of mutation confirmation and DNA context/integrity for mutant-*GLA* cDNA constructs pending completion of bidirectional analysis. See Section 6.2 for postmarketing commitment.

Data recreation for step 4 (total cell lysate protein) and for step 5 (enzyme activity) were completed after several additional data requests. The recreation was successful and the numbers for individual values agreed with the Applicant's results. Recalculation of final calculations for amenable versus nonamenable were successful and agreed with the calls made by the Applicant. Data for the step 7's transfection quality control were obtained and reevaluated. Linearity and efficiency were acceptable and within the standards appropriate to this assay. To evaluate the reproducibility of the assay, the OCP requested individual well data for the assays used to classify 20 mutants. The positive control (R301Q) was selected due to the data from *in vitro* and from transgenic mouse models. The absolute increase for the positive control (R301Q) was  $47.9\% \pm 2.16\%$ . The relative increase was  $8.04 \pm 0.43$  fold. Evaluation of the data from the 20 individual mutants suggests that the absolute increase of 3% has an error of around 0.2% and that the relative increase of 1.2-fold has an error in the range of 0.1. These results, combined with the data in the assay validation report, demonstrate that the GLP HEK-293 assay can be performed in a reproducible manner.

The next stage in evaluation was to determine whether the results of the GLP-HEK-293 assay correlate with the results of clinical biomarker responses. The three biomarkers examined included: residual alpha-Gal A enzyme activity in white blood cells (WBC), kidney capillary GL-3 inclusions and plasma lyso-Gb3.

#### **Correlation of biomarker responses with *in vitro* *GLA* variant amenability**

To define responder vs. nonresponder changes in the biomarker levels *in vivo*, the Applicant selected cutoff values, where the reproducibility of the respective biomarker assay/method was not considered. For WBC alpha-Gal A enzyme activity, the Applicant selected a cutoff of at least 2% increase in enzyme activity from baseline. For the kidney GL-3 inclusion counts and plasma lyso-Gb3, subjects with any reduction (any negative value) from baseline were considered responders. Prior to evaluating the correlation between the *in vitro* amenability data and biomarker response, the OCP evaluated the reproducibility of the respective biomarker assay/method, including counts of KIC GL3 inclusions. Based on sensitivity analyses of available data (see Appendix 5) together with information submitted by the Applicant on the methods

used (e.g., precision metrics in Study AA42942-05 and RR1001-57), the review team selected adjusted cut-offs that account for the analytical variation and the interobserver variability (pathologist reads of KIC GL3 inclusion number). For example, for plasma lyso-Gb3 measurements, the inter-assay precision across four studies ranged from 2.3% to 9.9%. Therefore, an adjusted cut-off value of at least a 10% decrease from baseline was used to define a responder for plasma lyso-Gb3. Thus, based on this data processes, the following adjusted cut-off values for each biomarker were selected by the review team and used to define a responder: at least a 50% decrease in kidney GL-3 inclusions from baseline; at least a 10% decrease in plasma lyso-Gb3 from baseline; and at least a 4% increase in residual WBC alpha-Gal A enzyme activity. See Appendix 5 for additional details.

The OCP then evaluated the correlation between the GLP HEK-293 assay results and each independent clinical biomarker by sex using the review team’s adjusted cut-offs for a responder as described above (See Appendix 5 for details). Consolidated biomarker correlation results by sex are shown in Table 29 below.

**Table 29: Correlation of biomarker responses with GLA variant amenability per the GLP HEK-293 assay.**

	GLP HEK vs Biomarker		adjusted cutoffs				
	Patients By Sex						
	WBC Gal A		Kidney GL3		Plasma Lyso GB3		
	Male	Female	Male	Female	Male	Female	Average
Sensitivity	0.89	0.73	1.00	0.80	1.00	1.00	0.90
Specificity	0.31	0.22	0.43	0.24	0.38	0.40	0.33
PPV	0.47	0.76	0.47	0.30	0.53	0.44	0.49
NPV	0.80	0.20	1.00	0.75	1.00	1.00	0.79

Adjusted cut-offs: Response was defined for each clinical biomarker using the following adjusted cut-offs (accounting for variation in the biomarker method): a  $\geq$  4% increase in residual WBC alpha-Gal A activity from baseline; a  $\geq$  50% decrease in kidney GL-3 inclusions from baseline; and a  $\geq$  10% decrease in plasma lyso-Gb3 from baseline.

alpha-Gal A = alpha-galactosidase A, GL-3 = globotriaosylceramide, GLP = good laboratory practice, HEK-293 = human embryonic kidney cell line 293, lyso-GB3 = globotriaosylsphingosine, NPV = negative predictive value, PPV = positive predictive value, WBC = white blood cell

Overall the results show that sex had a clear effect on the correlation between in vitro amenability and clinical biomarker responses as expected based on knowledge of the X-linked nature of FD. The sensitivity of in vitro response is acceptable but specificity is poor. Overall, the positive predictive value is approximately 0.5 across all three biomarkers, while the negative predictive value is approximately 0.8.

When examining for potential correlations between the absolute increase in enzyme activity, as measured in the HEK-293 assay, and changes in the relevant biomarkers, there was no apparent correlation between the two as shown in Figures 5-7 of Appendix 5.

In summary, the overall design and performance metrics of the GLP HEK-293 assay appears acceptable from a methodological assessment standpoint. However, based on the available PD

data from trial AT1001-011, the correlation between in vitro assay amenability and clinical biomarker changes in trial AT1001-011 stage 1 appears to be moderate. Overall, a variant classified as amenable by the HEK assay had around a 50% chance of showing a biomarker response for any one of the three PD biomarkers evaluated in the clinical trial (with the biomarker responders as defined by the review team above).

**6.5.4. Is the proposed dosing regimen appropriate for the general patient population for which the indication is being sought?**

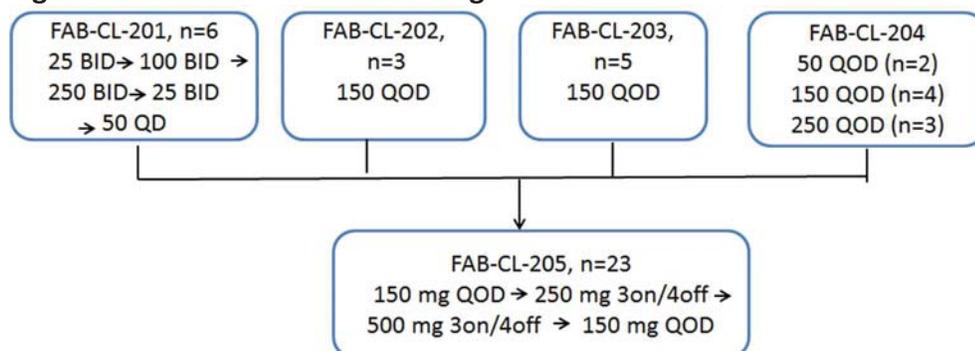
Yes. The proposed dose of Galafold 123 mg once every other day is appropriate. The proposed QOD dosing regimen was studied in the phase 3 trial and resulted in a decrease in KIC GL-3, particularly in male patients with Fabry disease. The Applicant's proposed rationale for the intermittent dosing is to facilitate the dissociation of migalastat from alpha-Gal A in the presence of the natural substrates in lysosomes by allowing time for migalastat to be cleared. The dose exploration was limited and the longer dosing interval, (b) (4) was not studied and available clinical data do not allow a reliable comparison between every day dosing and every other day dosing. As such dosing for the general Fabry patient population may be further optimized.

Dose and Dose Frequency Selection Rationale for Phase 3 Trials:

The selection of dose and dose-frequency for phase 3 trials was based on the findings of animal disease model data and the results of phase 2 trials. In an animal disease mouse model (hR301Q alpha-Gal A transgenic/knockout mice), 30 mg/kg dose showed a significant increase in alpha-Gal A activity and GL-3 substrate reduction in all evaluated tissues, and 30 mg/kg dose in the mouse model gave similar systemic exposure to that of approximately 150 mg in humans.

The Applicant had conducted five phase 2 trials (Figure 13) and none of them were robust dose-ranging trials to provide a meaningful assessment of dose-response because of the small number of patients and trial design limitations, e.g., within subject dose or dose-frequency changes (FAB-CL-201, and -205) or only evaluating one dose level (FAB-CL-202, and-203). Out of five phase 2 trials, only one phase 2 trial, FAB-CL-204, had multiple dose levels studied in parallel; this trial assessed the effect of every other day dosing of migalastat HCl at 50 mg (n = 2), 150 mg (n = 4) and 250 mg (n = 3) on efficacy/PD biomarkers. No clear dose-response relationship was observed for alpha-Gal A activity and GL-3 in this dose-ranging phase 2 trial. Although PK samples were collected in study FAB-CL-204, no exposure-response analysis was conducted. It would have been more informative if the Applicant had conducted a trial with a larger sample size instead of multiple trials with two to six patients despite the sample size constraints given that Fabry is rare.

**Figure 13: Overview of Phase 2 Program**



BID = twice daily, QD = daily, QOD = every other day

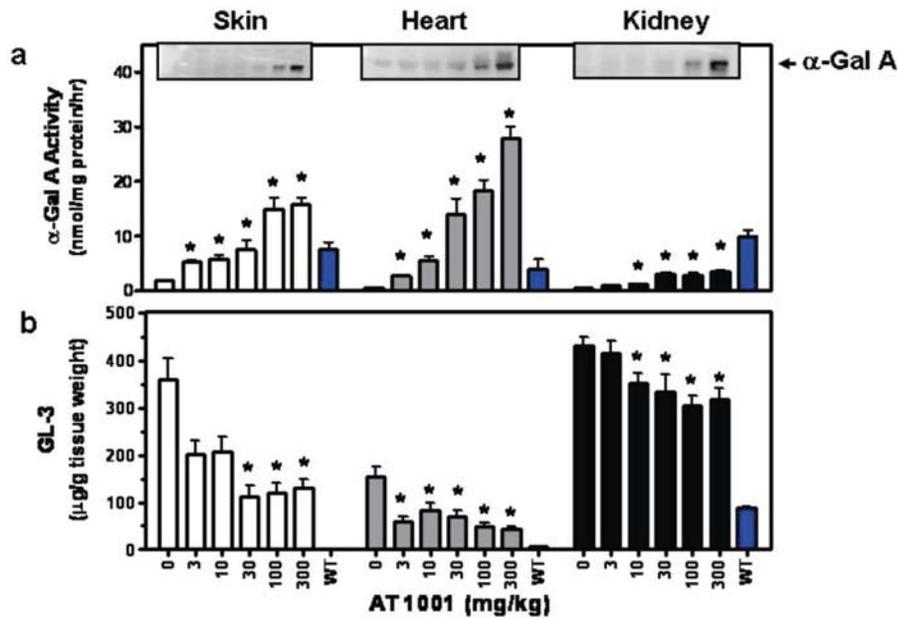
In the phase 3 trial, only one dosing regimen, i.e., 150 mg QOD migalastat HCl, was evaluated. Dosing regimens with potentially better adherence, e.g., 150 QD or 75 mg QD were not explored. Nonetheless, the dosing frequency of QOD appears to be reasonable based on the mechanism of action because migalastat needs to be released from the alpha-Gal A in the lysosome for alpha-Gal A to hydrolyze its natural substrate GL-3.

#### **Non-clinical Data:**

In hR301Q alpha-Gal A transgenic/knockout mice disease model, 30 mg/kg dose showed significant increase in alpha-Gal A activity and GL-3 substrate reduction in all tissues and no further improvements were observed at higher doses (Figure 14). Additionally, the effects on GL-3 level were greater with less-frequent dosing regimens, in a decreasing order of a repeated cycles of 4 days with drug followed by 3 days without (4 on/3 off) > every other day dosing > once daily (Figure 15). The Applicant chose the 150-mg dose for humans to achieve a similar systemic exposure to that at 30 mg/kg dose in the transgenic mouse model (AUC of 18400 ng·h/ml).

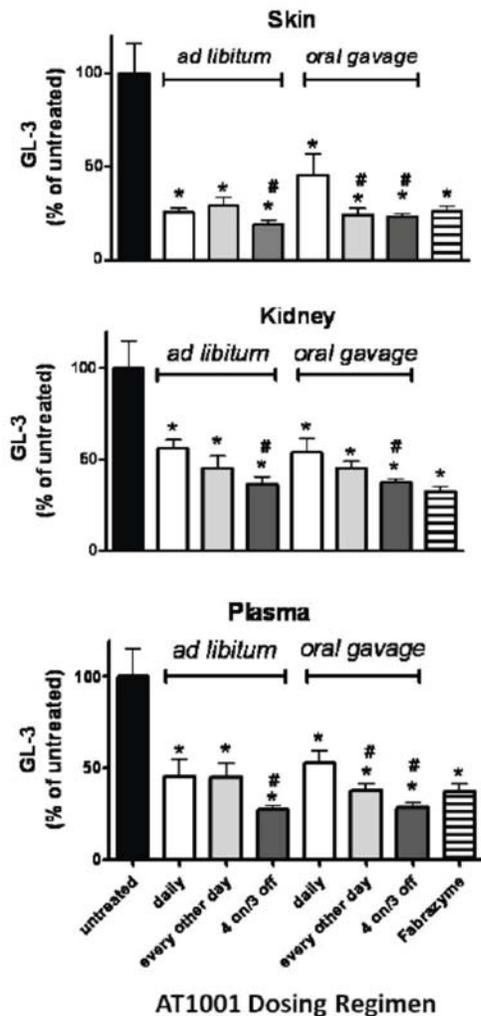
Of note, there were five patients with Fabry disease who also had R301Q mutation (amenable) in trial AT1001-011, the same genotype as for the transgenic mouse disease model, of whom three patients were treated with migalastat and two patients were treated with placebo. However, there were too few subjects and no consistent observed trends to make a meaningful interpretation to compared the mouse disease model with human data.

Figure 14: Migalastat HCl Administration via Drinking Water Increases alpha-Gal A Activity and Reduces GL-3 in hr301Q alpha-Gal A Tg/KO Mice



Source: 2.6.2 Pharmacology Written Summary, Figure 15, page 29  
alpha-Gal A = alpha-galactosidase A, GL-3 = globotriaosylceramide, Tg/KO = transgenic knock-out

Figure 15: Effects of Dosing Frequency on Tissue GL-3 in hr301Q alpha-Gal A Tg/KO Mice



Eight-week old male hr301Q alpha-Gal A Tg/KO mice were orally administered migalastat HCl (AT1001) (300 mg/kg/day free base) *ad libitum* in drinking water or by gavage for 4 weeks. Migalastat HCl was administered either daily or less-frequently using four cycles of a 4 days on/3 days off regimen or every other day regimen. A separate group of mice was given a once per week tail vein injection of 1 mg/kg agalsidase beta for 4 weeks and GL-3 was measured. Less-frequent migalastat HCl administration was as effective as agalsidase beta in reducing GL-3 in tissues and plasma in these mice.

Source: 2.6.2 Pharmacology Written Summary, Figure 19, page 35

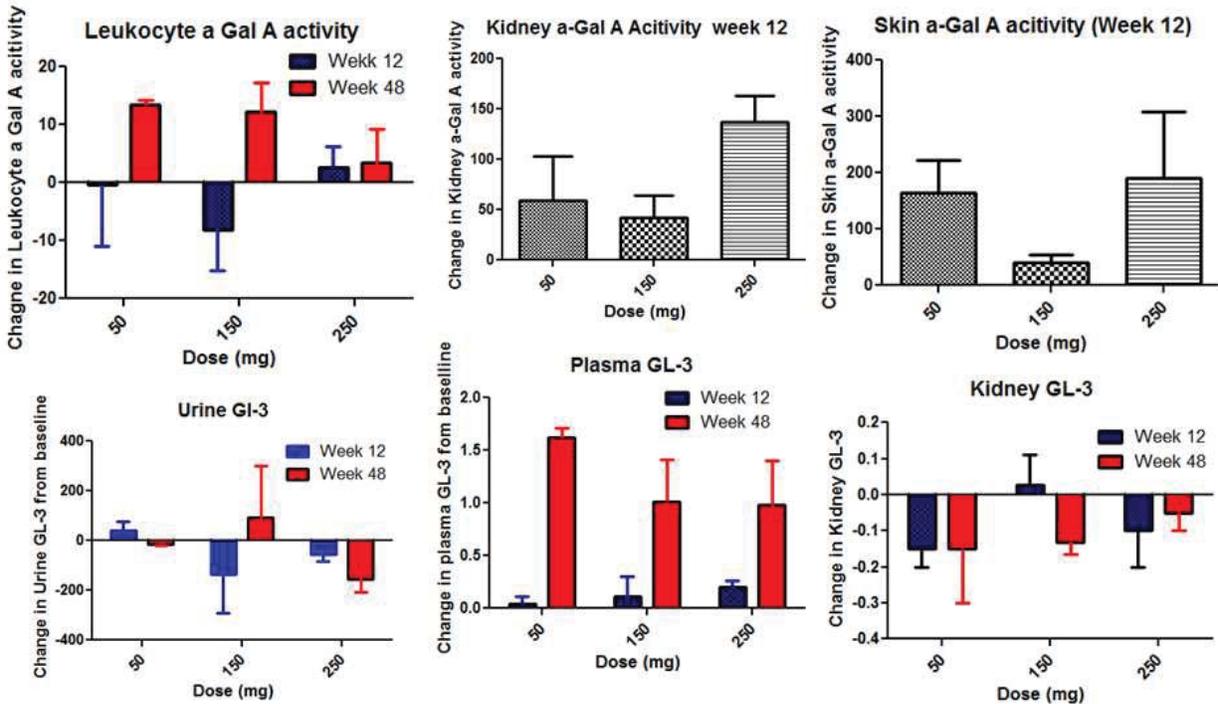
alpha-Gal A = alpha-galactosidase A, GL-3 = globotriaosylceramide, Tg/KO = transgenic knock-out

### Exploration of Dose-Response Relationship:

FAB-CL-204 was an open-label trial in female patients with Fabry disease evaluating 50 mg QOD (n = 2), 150 mg QOD (n = 4) and 250 mg QOD (n = 3) migalastat dose levels over a 12-week treatment period (and 36 weeks of optional extension). The PD markers that were evaluated in this dose-ranging trial included alpha-Gal A activity in leukocyte, kidney, skin and GL-3 in urine, plasma, kidney. No clear dose-response was observed for alpha-Gal A activity and GL-3 in any of the matrices. This trial served as a proof-of-concept study and showed alpha-Gal A activity in leukocyte increased from baseline at week 48 and in kidney and skin at week 12. However, combination of the limited number of patients in each dose cohort (n = two to four per dose

cohort) and high variability in alpha-Gal A activity and GL-3 levels prevents a reliable interpretation of dose-response relationship.

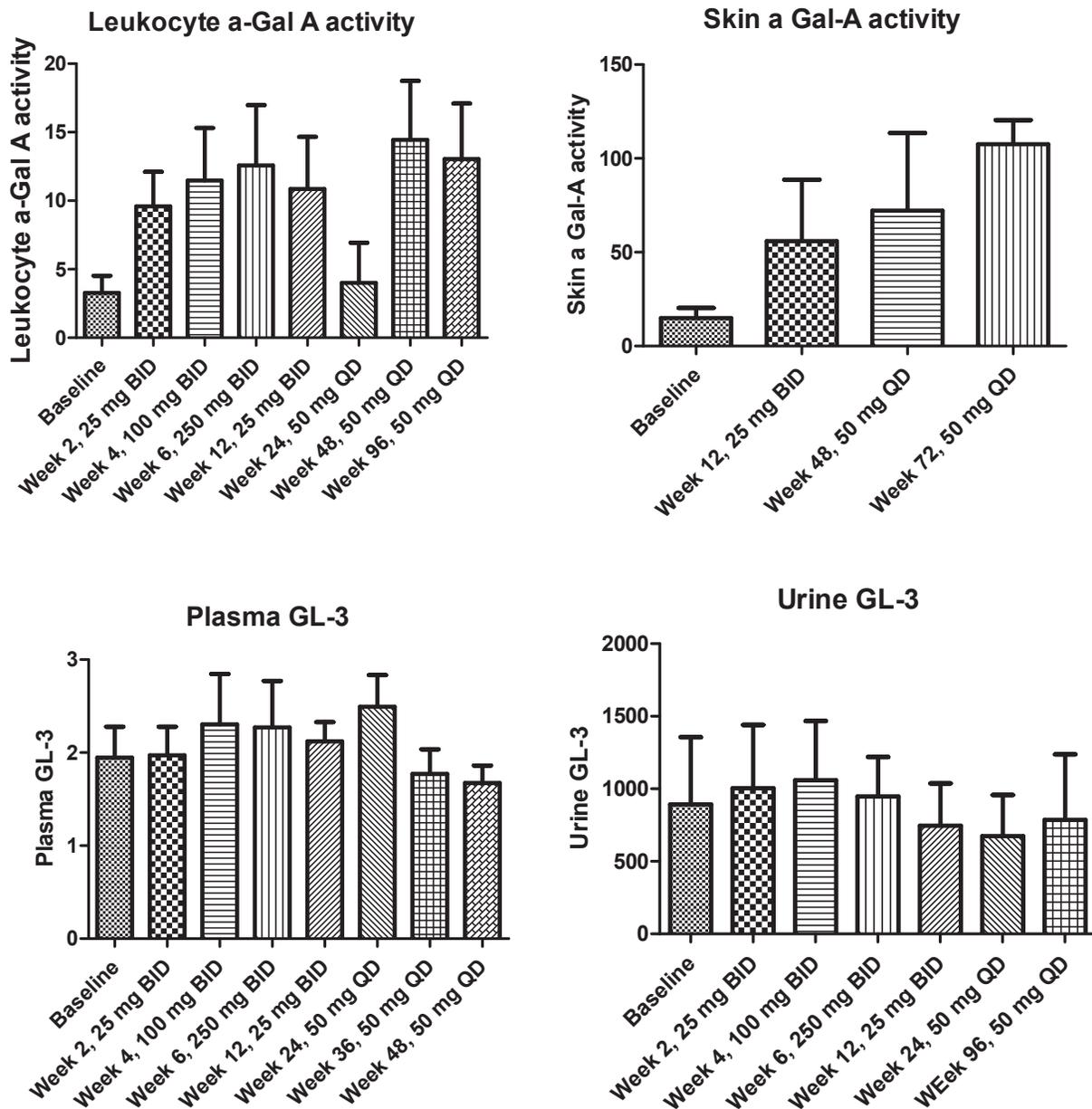
**Figure 16: Dose-Response Relationship in Dose-Ranging Phase 2 Trial in Mean Change from Baseline in alpha-Gal A Activity and GL-3 (study FAB-CL-204)**



Source: Reviewer's analysis based on data from CSR FAB-CL-204; 50 mg QOD (n = 2), 150 mg QOD (n = 4) and 250 mg QOD (n = 3)  
alpha-Gal A = alpha-galactosidase A, GL-3 = globotriaosylceramide, QOD = every other day  
Units: Gal A activity (nmol/hr/mg protein); Urine GL-3 (pmol/nmol phosphatidylcholine); Plasma GL-3 (ug/mL); Kidney GL3 (average GL3 inclusion/capillary)

Study FAB-CL-201 was an open-label trial in nine male patients with Fabry disease where doses were escalated within subjects from 25 mg BID to 100 mg BID to 250 mg BID, each for 2 weeks, then deescalated to 25 mg BID for 6 weeks (i.e., week 6 through week 12). Following 12 weeks of treatment, patients could participate in an optional extension period where subjects were treated with 50 mg QD dose onward. As shown in Figure 17, while alpha-Gal A activity in leukocytes have increased, the plasma and urine GL-3 level did not change as a function of dose or duration of treatment. It is not clear whether the increase in alpha-Gal A activity leukocytes was due to the increase in dose or due to the increase in duration of treatment within each subject.

**Figure 17: Mean WBC/Skin alpha-Gal A Activity and Plasma/Urine GL-3 by Dose and Duration of Treatment (Study FAB-CL-201)**



Source: Reviewer's analysis based on data from CSR FAB-CL-201

alpha-Gal A = alpha-galactosidase A, BID = twice daily, GL-3 = globotriaosylceramide, QD = daily

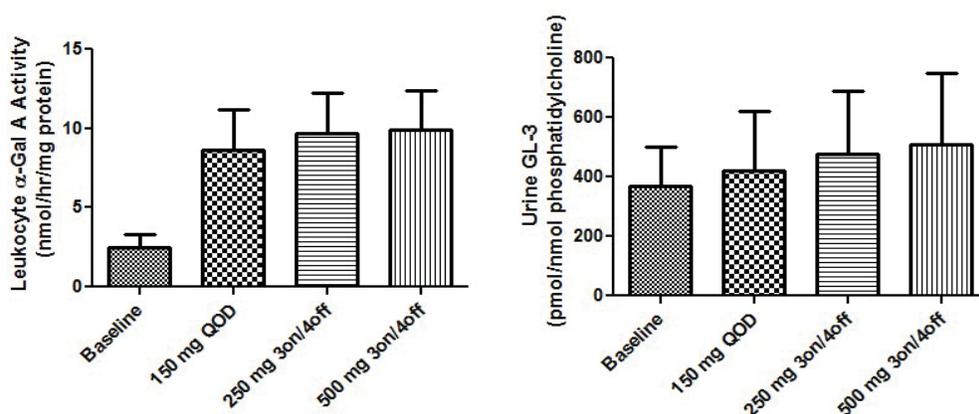
Units:  $\alpha$ -Gal A activity (nmol/hr/mg protein); Urine GL-3 (pmol/nmol phosphatidylcholine); Plasma GL-3 ( $\mu$ g/mL)

**Exploration of Dosing Frequency:**

In a phase 2, open-label, non-comparative, long-term extension trial (Study FAB-CL-205) in 23 male and female subjects with Fabry disease who had completed the treatment period of one of the four prior phase 2 clinical trials (Study FAB-CL-201, 202, 203, or 204), the Applicant had explored higher doses with different frequencies. Doses were escalated within subjects from

150 mg QOD migalastat HCl dosing to 250 mg 3 days on, 4 days off dosing regimen for 2 months and further escalated to 500 mg 3 days on, 4 days off dosing regimen. Out of 23 patients with Fabry disease, 12 subjects had amenable variants (nine males and three females). No noticeable changes were observed in both alpha-Gal A activity and urine GL-3 when dose was escalated from 150 mg QOD to higher dose with longer interval between treatment (250 and 500 mg, 3 days on, 4 days off) in Fabry patients with amenable variants. A similar trend among dosing regimens (no apparent differences among regimens) was observed between males and females while female patients tend to have higher WBC alpha-Gal A activity and lower urine GL-3 compared to male patients. The 150-mg dose with longer dosing interval was not evaluated. It is unknown if dose-response would be more evident if the doses were compared in a parallel study in treatment naïve patients.

**Figure 18: Mean WBC alpha-Gal A Activity and Urinary GL-3 by Different Dosing Regimens in Male Fabry Patients with Amenable GLA Variants\* (study FAB-CL-205, N = 9)**



Source: Reviewer's analysis based on data (Tables 12 and 14) from CSR FAB-CL-205 (Dose in migalastat HCl)

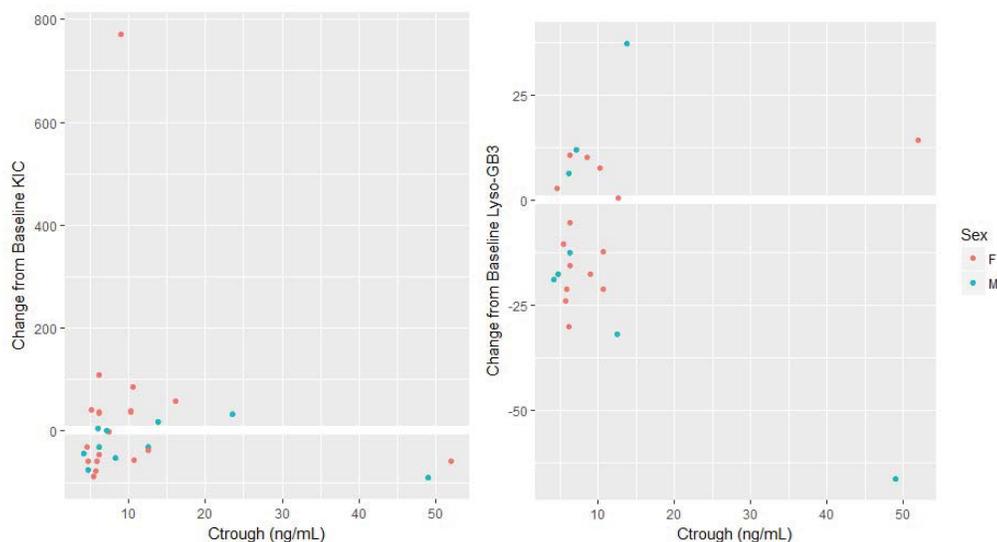
alpha-Gal A = alpha-galactosidase A, GL-3 = globotriaosylceramide, QOD = once every other day

\*Doses were escalated within subjects from 150 mg QOD migalastat dosing to 250 mg 3 days on, 4 days off dosing regimen for 2 months and further escalated to 500 mg 3 days on, 4 days off dosing regimen for 10 months. The baseline values were from the feeder studies and patients were on 150 mg QOD regimen for 8-120 weeks prior to dose escalation. Alpha-Gal A activity and urinary GL-3 were measured approximately 8 weeks after dose escalation to 250 mg or 500 mg regimens.

### Exposure-Response:

The relationships between exposure (i.e.,  $C_{trough}$ ,  $C_{max}$  and  $C_{avg}$ ) and response (change in KIC GL-3 and lyso-Gb3 from baseline) were explored based on data from the phase 3 trial (study AT1001-011). Results for  $C_{trough}$  are shown below in Figure 19. No exposure-response relationship was observed in this analysis. This could be because the phase 3 trial had evaluated only a single dose level, i.e., 150 mg QOD migalastat dosage. Nonetheless, in the majority of patients with amenable variants, KIC GL-3 was reduced after treatment with migalastat. See Figure 28 in Section 8.1.2.

**Figure 19: No Evidence of Exposure-Response Relationship for KIC GL-3 and Lyso-Gb3 in Trial AT1001-011**



Source: Reviewer's Analysis, Appendix 7  
Units: KIC GL3 (Inclusion per capillary), Lyso Gb-3 (nmol/L),

#### 6.5.5. Is an alternative dosing regimen or management strategy required for subpopulations based on intrinsic patient factors?

*Yes. Galafold is not recommended for patients with severe renal impairment (eGFR < 30 mL/min/1.73m<sup>2</sup>). No dosage adjustment is recommended for patients with mild or moderate renal impairment.*

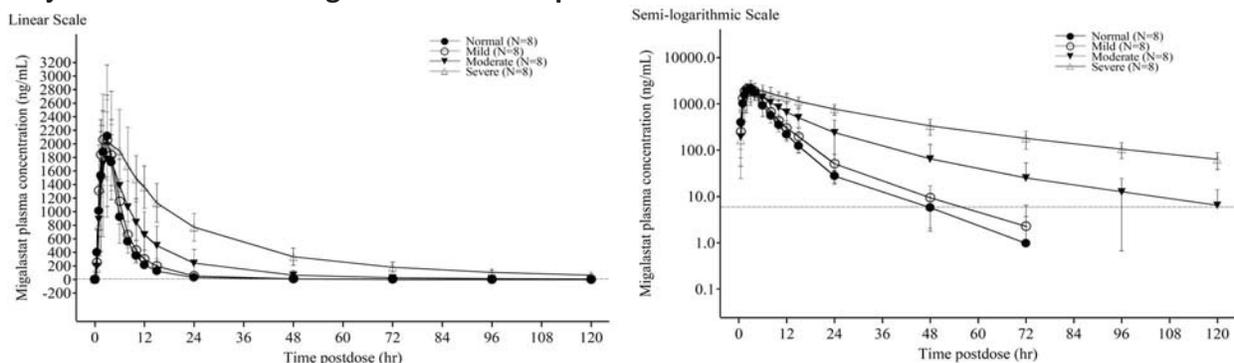
Following a single oral dose administration of 123 mg migalastat under fasting condition (study AT1001-015), the exposure (AUC) of migalastat increases and oral clearance (CL/F) decreases with the severity of renal impairment. Subjects with mild, moderate and severe renal impairment had about 1.2-, 1.8- and 4.3-fold higher AUC compared to subjects with normal renal function, respectively, while the C<sub>max</sub> was not significantly altered by renal impairment. Subjects with mild, moderate and severe renal impairment had about 1.6, 11.3 and 58.6-fold higher mean plasma concentrations at 48 hours (C<sub>48</sub>) than subjects with normal renal function, respectively. Additionally, mean half-life also increased to 7.7 h, 22.2 h, and 32 h in subjects with mild, moderate and severe renal impairment, respectively from 6.4 h in healthy subjects (Table 30). There was no notable difference in AE profiles across all different renal function subgroups in this single dose PK study; although, safety assessment is limited in this single dose study. Additional safety data may be gleaned from patients with Fabry disease and moderate and mild renal impairment in Trial AT1001-011. There were nine patients with moderate renal impairment in Study 011. These data were included in the population PK analysis and support no dose adjustment for mild or moderate renal impairment (see Appendix 7 and Figure 20 for further details).

The Applicant's proposal for patients with mild to moderate renal impairment is acceptable based on the following:

1. No major accumulation of migalastat is expected from QOD dosing in patients with moderate renal impairment based on multiple dose simulation (approximately 18%) and estimated exposure by a population PK approach in patients with moderate renal impairment in phase 3 trial AT1001-011 (Table 32 and Table 33).
2. Migalastat was well tolerated following single doses up to 450 mg (trial AT1001-013), multiple doses up to 250 mg BID dose for up to 2 weeks (FAB-CL-201), and multiple doses at 250 mg QOD dose for up to 48 weeks (study FAB-CL-204) in patients with Fabry disease.
3. From an efficacy standpoint, in phase 3 trial AT1001- 011, patients with Fabry disease and moderate renal impairment ( $30 \leq \text{eGFR} < 60 \text{ mL/min/1.73 m}^2$ ) had similar efficacy responses to 150 mg QOD migalastat (e.g., reduction in KIC GL-3 ( $n = 4$ ) and plasma lyso-Gb3 ( $n = 2$ )) as patients who had  $\text{eGFR} > 60 \text{ mL/min/1.73 m}^2$  to indicate that higher systemic exposure in patients with moderate renal impairment did not adversely affect PD response (Table 32 and Table 33).

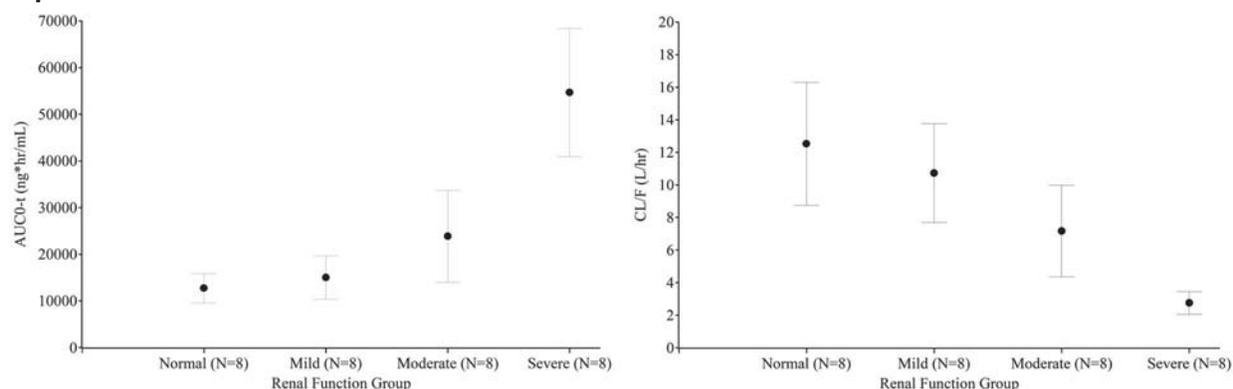
The Applicant's proposal to not recommend use of Galafold in FD patients with severe renal impairment is also acceptable as migalastat was not studied in patients with Fabry disease and severe renal impairment ( $\text{eGFR} < 30 \text{ mL/min/1.73m}^2$ ) in this development program. In both phase 3 trials (AT1001-011 and AT1001-012), patients with severe renal impairment ( $\text{eGFR} < 30 \text{ mL/min/1.73m}^2$ ) were excluded.

**Figure 20: Arithmetic Mean ( $\pm$  SD) Plasma Concentration-Time Profiles for Migalastat in Subjects with Various Degrees of Renal Impairment**



Source: CSR AT1001-015, Figure 11.1, page 49  
SD = standard deviation

**Figure 21: Arithmetic Mean ( $\pm$  SD) AUC<sub>0-t</sub> and Oral Clearance of Migalastat Versus Renal Impairment**



Source: CSR AT1001-015, Figure 11.2, page 50 and Figure 14.2.2-1b, page 91  
AUC = area under the curve, CL/F = oral clearance, SD = standard deviation

**Table 30: Geometric Mean (geo CV%) Migalastat Plasma PK Parameters in Subjects with Various Degrees of Renal Impairment**

PK Parameter	Units	Renal Function Group			
		Normal (N=8)	Mild (N=8)	Moderate (N=8)	Severe (N=8)
AUC <sub>0-t</sub>	(ng*hr/mL)	12306 (27.9)	14389 (31.1)	22126 (42.8)	53070 (27.0)
AUC <sub>0-∞</sub>	(ng*hr/mL)	12397 (27.7)	14536 (30.7)	22460 (42.2)	56154 (24.9)
C <sub>max</sub>	(ng/mL)	2100 (26.0)	2191 (28.8)	1868 (32.1)	2078 (45.5)
t <sub>max</sub> <sup>a</sup>	(hr)	2.50 (1.50, 3.00)	2.50 (1.50, 4.00)	3.00 (1.50, 4.00)	4.27 (3.00, 8.00)
t <sub>1/2</sub> <sup>b</sup>	(hr)	6.42 (1.93)	7.66 (3.02)	22.2 (14.2)	32.3 (7.35)
λ <sub>r</sub>	(1/hr)	0.113 (32.9)	0.0965 (40.1)	0.0386 (84.6)	0.0219 (21.2)
CL/F	(L/hr)	12.1 (27.7)	10.3 (30.7)	6.68 (42.2)	2.67 (24.9)
Vd/F	(L)	107 (38.0)	107 (37.7)	173 (102.3)	122 (43.7)
C <sub>48</sub> <sup>b</sup>	(ng/mL)	5.70 (3.63)	9.34 (7.57)	64.5 (68.1)	334 (126)

<sup>a</sup> Median (Min, Max) presented for t<sub>max</sub>.

<sup>b</sup> Arithmetic Mean (SD) is presented

Note, below the limit of quantification (BLQ) values were treated as 0 at predose and treated as missing after the last quantifiable concentration in a profile in PK analysis.

Source: CSR AT1001-015, Table 11-2, page 52

AUC = area under the curve, CL/F = oral clearance, CV = coefficient of variation, PK = pharmacokinetic, SD = standard deviation, Vd/F = apparent volume of distribution

**Table 31: Statistical Analysis of Migalastat Plasma PK Parameters in Subjects with Various Renal Impairment**

Parameter (unit)	Renal Function Group	N <sup>a</sup>	Geometric Least Squares Means	Ratio of Geometric Least Squares Means Renally-impaired/Normal <sup>b</sup>	90% CI for the Ratio <sup>b</sup> (Lower, Upper)	Between Subject CV%
AUC <sub>0-∞</sub> (hr·ng/mL)	Mild	8	14536	1.17	(0.90, 1.53)	31.9
	Moderate	8	22460	1.81	(1.39, 2.36)	
	Severe	8	56154	4.53	(3.48, 5.90)	
	Normal	8	12397			
AUC <sub>0-t</sub> (hr·ng/mL)	Mild	8	14389	1.17	(0.89, 1.53)	32.6
	Moderate	8	22126	1.80	(1.37, 2.36)	
	Severe	8	53070	4.31	(3.29, 5.65)	
	Normal	8	12306			
C <sub>max</sub> (ng/mL)	Mild	8	2191	1.04	(0.79, 1.38)	33.7
	Moderate	8	1868	0.89	(0.67, 1.18)	
	Severe	8	2078	0.99	(0.75, 1.31)	
	Normal	8	2100			

<sup>a</sup> N = number of subjects.

<sup>b</sup> The ratio and corresponding CI are back-transformed from the difference and confidence interval calculated on the log scale to the linear scale.

Source: CSR AT1001-015, Table 11-3, page 54

AUC = area under the curve, CI = confidence interval, CV = coefficient of variation, PK = pharmacokinetic

**Table 32: AT1001-011 Exposure vs. KIC GL-3 by Renal Function Category at Baseline<sup>a</sup>**

Renal Function Category at Baseline	Statistic	AUC <sub>0-τ</sub> <sup>b,c</sup> ng·h/mL	C <sub>48h</sub> <sup>b</sup> ng/mL	Change from Baseline at Month 12 <sup>d</sup>		
				Baseline KIC GL-3 inclusions per capillary	KIC at Month 12 <sup>d</sup> GL-3 inclusions per capillary	GL-3 inclusions per capillary
< 60 mL/min/1.73m <sup>2</sup> eGFR <sub>MDRD</sub>	N <sup>e</sup>	4	4	4	4	4
	Mean	17885	38.57	0.36	0.10	-0.26
	CV%	26.1	44.1	159.1	68.2	-221.2
	Min	13549	24.17	0.03	0.04	-1.10
	Median	17191	35.74	0.10	0.09	-0.02
	Max	23611	58.63	1.22	0.20	0.12
≥ 60 mL/min/1.73m <sup>2</sup> eGFR <sub>MDRD</sub>	N <sup>f</sup>	37	37	37	37	37
	Mean	8870	7.65	0.69	0.39	-0.30
	CV%	32.4	68.8	175.3	199.2	-190.4
	Min	3518	1.07	0.01	0.03	-2.28
	Median	8357	6.29	0.22	0.12	-0.08
	Max	18505	28.49	5.69	3.77	0.14

<sup>a</sup> Of 50 patients who had baseline eGFR<sub>MDRD</sub>, 41 had matching PK and KIC data

<sup>b</sup> PK parameters were estimated by population PK analysis using NONMEM version 7.3, ADVAN4 subroutine and first-order conditional estimation with interaction (FOCE-I) method

<sup>c</sup> AUC was estimated for the dosing interval, τ, from time 0 to 48 hours post-dose

<sup>d</sup> 12 month data provides sufficient duration for migalastat effect on KIC

<sup>e</sup> Three patients were randomized to AT1001/AT1001 and 1 patient was randomized to Placebo/AT1001

<sup>f</sup> Twenty-one patients were randomized to AT1001/AT1001 and 16 patients were randomized to Placebo/AT1001

Source: Submission dated July 21, 2018 in a response to an IR, page 3

AUC = area under the curve, eGFR = estimated glomerular filtration rate, GL-3 = globotriaosylceramide, KIC = Kidney Interstitial Capillary, MDRD = based on Modification of Diet in Renal Disease Study equation

**Table 33: AT1001-011 Exposure vs. Plasma Lyso-Gb3 by Renal Function Category at Baseline<sup>a</sup>**

Renal Function Category at Baseline	Statistic	AUC <sub>0-τ</sub> <sup>b,c</sup>		Baseline Plasma Lyso-GB3	Plasma Lyso-GB3 at Month 12 <sup>d</sup>	Change from Baseline at Month 12 <sup>d</sup>
		ng•h/mL	ng/mL	nmol/L	nmol/L	nmol/L
< 60 mL/min/1.73m <sup>2</sup>	N <sup>e</sup>	2	2	2	2	2
eGFR <sub>MDRD</sub>	Mean	21655	52.7	41.43	12.9	-28.53
	CV%	12.8	15.9	137.4	127.6	-141.8
	Min	19700	46.77	1.19	1.26	-57.13
	Median	21655	52.7	41.43	12.9	-28.53
	Max	23611	58.63	81.67	24.53	0.08
≥ 60 mL/min/1.73m <sup>2</sup>	N <sup>f</sup>	29	29	29	29	29
eGFR <sub>MDRD</sub>	Mean	8778	7.67	45.5	34.31	-11.19
	CV%	34.7	73.9	121	119.9	-164.6
	Min	3518	1.07	5.65	4.69	-61.13
	Median	8357	6.29	16.8	15.67	-2.59
	Max	18505	28.49	218.33	164	0.87

<sup>a</sup>Of 50 patients who had baseline eGFRMDRD, 31 had matching PK and plasma lyso-GB3 data

<sup>b</sup>PK parameters were estimated by population PK analysis using NONMEM version 7.3, ADVAN4 subroutine and first-order conditional estimation with interaction (FOCE-I) method

<sup>c</sup>AUC was estimated for the dosing interval, τ, from time 0 to 48 hours post-dose

<sup>d</sup>12 month data provides sufficient duration for migalastat effect on plasma lyso-GB3

<sup>e</sup>Two patients were randomized to AT1001/AT1001 and 0 patients were randomized to Placebo/AT1001

<sup>f</sup>Sixteen patients were randomized to AT1001/AT1001 and 13 patients were randomized to Placebo/AT1001

Source: Submission dated July 21, 2018 in a response to an IR, page 3

AUC = area under the curve, eGFR = estimated glomerular filtration rate, Gb3 = globotriaosylceramide, KIC = Kidney Interstitial Capillary, lyso-GB3 = globotriaosylsphingosine, MDRD = based on Modification of Diet in Renal Disease Study equation, PK = pharmacokinetic

### 6.5.6. Are there clinically relevant food-drug or drug-drug interactions and what is the appropriate management strategy?

**Food-drug:** *Yes. Galafold is recommended to be administered at least 2 hours before and after meal intake. Concomitant food intake significantly decreased the systemic exposure to migalastat. The timing of the food intake, i.e., one hour before or one hour after migalastat did not diminish the food effect. The different types of food, i.e., high fat meal vs. light fat meal showed similar food effect.*

**DDI:** *No clinically relevant drug-drug interactions that need a management strategy are identified.*

#### **Food-Drug Interaction:**

*The effect of food administered 2 hours before or after the migalastat administration on migalastat PK was not evaluated; however, in phase 3 trials (Studies AT1001-011 and AT1001-012), patients were instructed to fast 2 hours before and 2 hours after taking migalastat.*

The effect of food and timing of food administration on 150 mg migalastat HCl (to-be-marketed formulation) PK was evaluated in an open-label, randomized, single-dose, five-period cross-over study in 20 healthy subjects (Study AT1001-016). In each treatment period, subjects were randomly assigned to receive a single dose of migalastat HCl as shown in Table 34.

**Table 34: Dosage Regimens in Study AT1001-016**

Regimen	Description
A	150-mg migalastat HCl in the fasting state (reference treatment)
B	150-mg migalastat HCl with simultaneous consumption of a glucose drink
C	150-mg migalastat HCl 1 hour before consumption of a high-fat meal
D	150-mg migalastat HCl 1 hour before consumption of a light meal
E	150-mg migalastat HCl 1 hour after consumption of a light meal

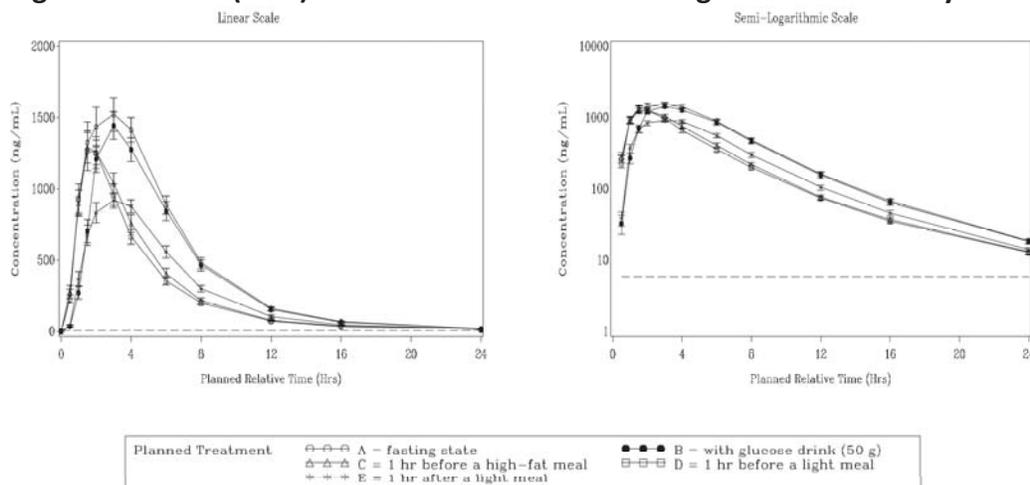
In all treatment regimens, the subjects underwent an overnight fast of at least 8 hours before the dosing. Under fasting condition, the subjects continued to fast 4 additional hours after the administration of a single dose of 150 mg migalastat HCl. Subjects receiving treatment regimen B (with simultaneous consumption of a glucose drink) were allowed to eat a light snack 3 hours before dosing.

**Table 35: Composition of Fed Treatments**

Treatment Condition	Composition
Glucose drink	Trutol Glucose Tolerance Test Beverage composed of: water, 50%; liquid dextrose, 20%; sodium benzoate, < 0.5%; citric acid, < 0.5%; and orange flavoring, < 0.5%
High-fat meal	High-fat breakfast composed of: 850 total calories, 477 calories from fat, 29 g protein, 64 g carbohydrates, 53 g fat, and 457 mg cholesterol
Light meal	Light breakfast composed of: 507 total calories, 150 calories from fat, 13 g protein, 80 g carbohydrates, 17 g fat, and 40 mg cholesterol

The total calories and high fat calories in the high-fat meal in this study appears to be consistent with the suggested test meal in the FDA guidance “Food Effect Bioavailability and Fed Bioequivalence Studies” where recommended the standard high-fat breakfast contained approximately 800 to 1000 total calories, with 50% of calories being derived from fat content.

**Figure 22: Mean (± SD) Plasma Concentrations of Migalastat vs. Time by Treatment**



Source: CSR AT1001-016, Figure 1, page 37  
SD = standard deviation

**Table 36: Geometric Mean (geometric CV) Plasma Pharmacokinetic Parameters of Migalastat**

Parameter (unit)	Treatment				
	A Fasting n = 19	B Glucose n = 19	C 1 hour before high-fat meal n = 19	D 1 hour before a light meal n = 19	E 1 hour after a light meal n = 19
AUC(0-t) (h•ng/mL)	9696 (27.1)	8342 (29.2)	6021 (27.6)	5573 (32.0)	5801 (27.0)
AUC(0-inf) (h•ng/mL)	9805 (26.8)	8451 (28.8)	6132 (26.8)	5668 (31.4)	5890 (26.5)
C <sub>max</sub> (ng/mL)	1561 (33.8)	1408 (29.6)	1323 (28.3)	1278 (39.6)	945 (28.3)
t <sub>max</sub> (h) <sup>1</sup>	3.00 (1.50, 6.00)	3.00 (2.00, 4.00)	1.50 (1.00, 3.00)	2.00 (1.50, 3.08)	3.00 (1.52, 6.00)
t <sub>1/2</sub> (h)	3.9 (11.3)	4.0 (11.8)	4.9 (20.5)	4.9 (13.4)	4.3 (10.9)

<sup>1</sup> Median (minimum, maximum)

Source: CSR AT1001-016, Table 10, page 38

AUC = area under the curve, CV = coefficient of variation

- Compared to the fasted state, administration of 150 mg migalastat HCl
  - at 1 hour before a high-fat meal reduced the AUC and C<sub>max</sub> by 37% and 15%, respectively, and shortened the t<sub>max</sub> by 1.5 hours.
  - at 1 hour before a light meal still reduced AUC and C<sub>max</sub> by 42% and 18%, respectively, and shortened the t<sub>max</sub> by 1.0 hours. Therefore, the type of meal (high-fat versus light) did not change the effect of meal on migalastat PK.
  - at 1 hour after a light meal still reduced both AUC and C<sub>max</sub> by 40%, and while t<sub>max</sub> remained unchanged. Therefore, change in the timing of meal from 1 hour before to 1 hour after did not eliminate the food effect.
  - with high concentration of glucose drink (50 g) reduced the AUC and C<sub>max</sub> only by 14% and 10%, respectively, and did not change t<sub>max</sub>.

**Table 37: Statistical Analysis of Migalastat Plasma PK Parameters**

Parameter (unit)	Treatment	n	Geometric LS Means	Treatment Comparison	Ratio of Geometric LS Means	90% CI of the Ratio
AUC(0-inf) (h•ng/mL)	A: Fasting	19	9835.95	–	–	–
	B: Glucose	19	8497.48	B/A	0.864	0.771-0.968
	C: 1 h before High fat meal	19	6165.57	C/A	0.627	0.559-0.702
	D: 1 h before light meal	19	5703.58	D/A	0.580	0.518-0.650
	E: 1 h after High fat meal	19	5924.40	E/A	0.602	0.538-0.675
C <sub>max</sub> (ng/mL)	A: Fasting	19	1579.14	–	–	–
	B: Glucose	19	1426.64	B/A	0.903	0.799-1.021
	C: 1 h before High fat meal	19	1340.30	C/A	0.849	0.751-0.959
	D: 1 h before light meal	19	1295.79	D/A	0.821	0.726-0.927
	E: 1 h after High fat meal	19	956.36	E/A	0.606	0.536-0.684

Source: CSR AT1001-016, Table 11, page 38

AUC = area under the curve, CI = confidence interval, LS = least squares, PK = pharmacokinetic

**Drug Interaction with Agalsidase (recombinant human alpha-Gal A):**

*Co-administration of migalastat with agalsidase beta (the approved enzyme replacement therapy for Fabry disease in the US, Fabrazyme) or with Agalsidase alpha (Replagal, approved only outside the US) in male patients (n = 3 to 5) did not significantly affect the plasma exposure of migalastat in patients with Fabry disease compared to when migalastat was administered alone. Single dose migalastat did not affect PK of agalsidase (measured as protein by Western blot) while appeared to increase the activity of agalsidase in plasma (measured by a fluorogenic substrate, 4-methylumbelliferone glucuronide [4-MUG]). Effect of migalastat on the efficacy of concomitant agalsidase was not studied, and whether concomitant migalastat may interfere with agalsidase activity in tissues is unknown.*

**Migalastat -Agalsidase Interaction:**

The drug-drug interaction between migalastat HCl and recombinant agalsidase beta (Fabrazyme) and recombinant agalsidase alfa (Replagal®) was evaluated in an open-label, single-dose, non-randomized study (Study AT1001-013). Migalastat HCl (150 mg or 450 mg) was administered 2 hours prior to infusion of recombinant agalsidase. Subjects fasted for at least 2 hours before and 2 hours after migalastat HCl administration. Single dose agalsidase alfa at 0.2 mg/kg was administered as a 40-minute intravenous infusion, and agalsidase beta at 0.5 mg/kg or 1 mg/kg was administered as a 2-hour intravenous infusion.

**Effect of Agalsidase on Migalastat:**

Coadministration of either agalsidase did not significantly affect the plasma exposure of migalastat in patients with Fabry disease. The PK of migalastat observed in these Fabry subjects following a single dose administration were similar to those observed in healthy subjects. Although the sample size for each agalsidase dose was limited (four to six patients) to draw a definitive conclusion, no significant effects of agalsidase on migalastat PK seem to be consistent with known mechanisms of migalastat elimination (primarily via urinary excretion).

**Table 38: Summary of Plasma Migalastat PK Parameters by Treatment\***

Treatment Group	C <sub>max</sub> <sup>a</sup> (ng/mL)	t <sub>max</sub> <sup>b</sup> (hr)	AUC <sub>0-t</sub> <sup>a</sup> (hr*ng/mL)	AUC <sub>infinity</sub> <sup>a</sup> (hr*ng/mL)	AUC F <sub>rel</sub>	t <sub>1/2</sub> <sup>c</sup> (hr)
150 mg migalastat HCl + Agalsidase (N = 12)	1625.99 (35.78)	3.04 (2.0;4.4)	13104.50 (40.00)	13520.99 (41.23)	1.06 (31.70) <sup>d</sup>	5.11 (0.845)
150 mg migalastat HCl Alone (N = 12)	1630.11 (32.25)	3.00 (2.0;4.0)	12371.21 (36.19)	12858.27 (37.15)	-	5.08 (0.874)
450 mg migalastat HCl + Agalsidase (N = 11)	3935.34 (30.82)	4.00 (2.0;6.0)	31846.98 (34.50)	33101.26 (35.07)	2.57 <sup>e</sup>	4.85 (1.244)

<sup>a</sup> Geometric mean (% Coefficient of variation of geometric mean)

<sup>b</sup> Median (Range)

<sup>c</sup> Arithmetic Mean (Standard deviation)

<sup>d</sup> Geometric mean AUC<sub>0-t</sub> for 150 mg migalastat HCl + agalsidase/ geometric mean AUC<sub>0-t</sub> for 150 mg migalastat HCl alone

<sup>e</sup> Geometric mean AUC<sub>0-t</sub> for 450 mg migalastat HCl + agalsidase/ geometric mean AUC<sub>0-t</sub> for 150 mg migalastat HCl alone

\*Pooled analysis: Agalsidase was given at 3 different doses (0.5 mg/kg, 1 mg/kg for agalsidase beta, and 0.2 mg/kg for agalsidase alpha)

AUC F<sub>rel</sub> = ratio of relative exposure (eg, AUC<sub>0-t</sub> following combination therapy to AUC<sub>0-t</sub> following migalastat HCl alone), PK = pharmacokinetic  
Source: CSR AT1001-013, Table 18, page 79

### **Effect of Migalastat on Agalsidase:**

Co-administration of a single dose migalastat with a single dose agalsidase increased the AUC<sub>0-inf</sub> alpha-Gal A activity (measured using 4-MUG) by 2.0- to 4.15-fold relative to when agalsidase (alfa or beta) was administered alone. The relative increase in active alpha-Gal A AUC<sub>0-inf</sub> by concomitant migalastat was 2-to 2.8- fold for agalsidase beta and 3.14-to 4.15-fold for agalsidase alpha. The magnitude of the increase in alpha-Gal A activity in plasma was not correlated with migalastat HCl dose (150 mg versus 450 mg).

**Table 39: Summary of Plasma PK Parameters for Active alpha-Gal A by Treatment**

Treatment Group	C <sub>max</sub> <sup>a</sup> (nmol/hr/mL)	t <sub>max</sub> <sup>b</sup> (hr)	AUC <sub>infinity</sub> <sup>a</sup> (hr*nmol/hr/mL)	t <sub>1/2</sub> <sup>c</sup> (hr)	AUC Ratio <sup>a</sup>
0.2 mg/kg agalsidase alfa alone (N = 4)	299.52 (29.08)	0.66 (0.7;1.0)	381.48 (21.56)	4.48 (3.132)	
0.2 mg/kg agalsidase alfa + 150 mg migalastat HCl (N = 4)	511.09 (14.88)	0.66 (0.7;1.0)	1583.90 (28.01)	4.27 (1.536)	4.15 (20.15)
0.2 mg/kg agalsidase alfa alone (N = 4)	358.10 (31.37)	0.66 (0.7;1.0)	672.01 (79.59)	5.15 (3.645)	
0.2 mg/kg agalsidase alfa + 450 mg migalastat HCl (N = 4)	605.20 (25.81)	0.66 (0.7;1.0)	2108.57 (46.69)	5.31 (2.462)	3.14 (38.24)
0.5 mg/kg agalsidase beta alone (N = 5)	508.55 (16.52)	2.00 (2.0;2.3)	1128.90 (19.56)	3.91 (2.054)	
0.5 mg/kg agalsidase beta + 150 mg migalastat HCl (N = 5)	877.31 (25.17)	2.00 (2.0;3.0)	3191.54 (27.48)	3.51 (1.304)	2.83 (30.94)
1.0 mg/kg agalsidase beta alone (N = 3)	1645.96 (26.63)	2.00 (1.5;3.0)	4765.43 (26.26)	5.33 (4.082)	
1.0 mg/kg agalsidase beta + 150 mg migalastat HCl (N = 3)	2291.63 (37.92)	2.22 (2.0;3.0)	9464.36 (30.60)	4.30 (1.712)	1.99 (16.85)
0.5 mg/kg agalsidase beta alone (N = 1)	684.36	3.00	2523.87	6.50	
0.5 mg/kg agalsidase beta + 450 mg migalastat HCl (N = 1)	1351.18	3.00	6197.71	3.49	2.46
1.0 mg/kg agalsidase beta alone (N = 6)	1655.35 (44.44)	2.25 (2.0;4.0)	4931.07 (65.14)	3.11 (1.875)	
1.0 mg/kg agalsidase beta + 450 mg migalastat HCl (N = 6)	2315.55 (31.39)	2.25 (2.0;4.0)	9676.48 (42.94)	4.96 (1.532)	1.96 (53.36)

<sup>a</sup> Geometric mean (% Coefficient of variation of geometric mean)

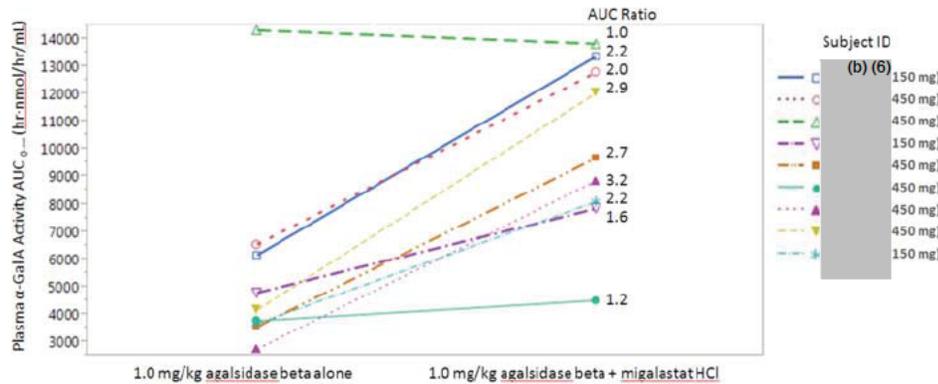
<sup>b</sup> Median (Range)

<sup>c</sup> Arithmetic Mean (Standard deviation)

Source: CSR AT1001-013, Table 9, page 60

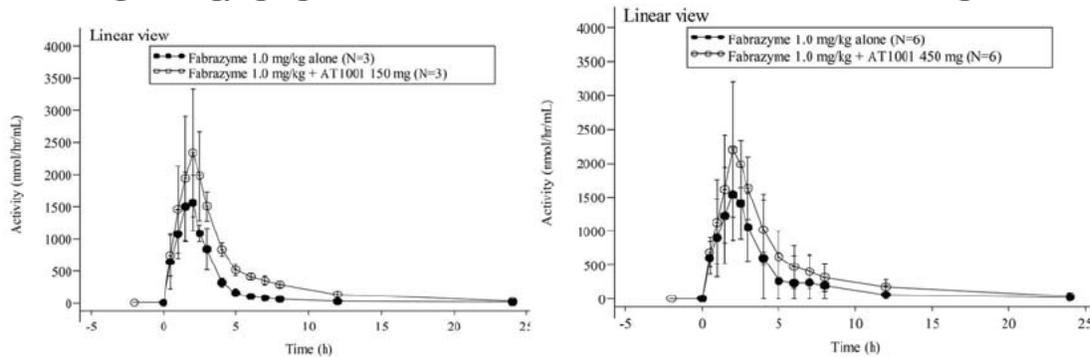
alpha-Gal A = alpha-galactosidase A, AUC = area under the curve, PK = pharmacokinetic

**Figure 23: Individual Plasma AUC for Active alpha-Gal A following 1.0 mg/kg Agalsidase Beta Administration With or Without Migalastat HCl**



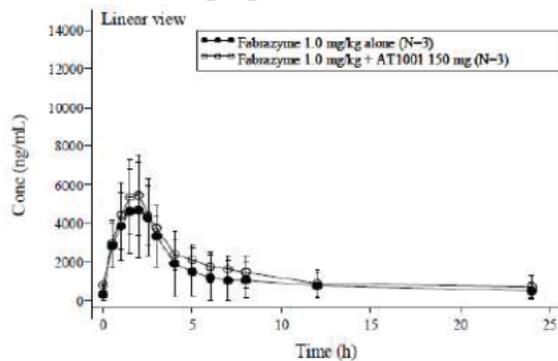
Source: CSR AT1001-013, Figure 5, page 58  
alpha-Gal A = alpha-galactosidase A, AUC = area under the curve

**Figure 24: Arithmetic Mean (SD) alpha-Gal A Enzyme Activity Time Profiles in Plasma following 1.0 mg/kg Agalsidase Beta Administration With or Without Migalastat HCl**



Source: CSR AT1001-013, Figure 6 on page 64 and Figure 7 on Page 68  
alpha-Gal A = alpha-galactosidase A, SD = standard deviation

**Figure 25: Arithmetic Mean (SD) Total alpha-Gal A Protein concentration-Time Profiles in Plasma following Agalsidase Administration With or Without 150 mg Migalastat HCl**



Source: CSR AT1001-013, Figure 8 on page 73  
alpha-Gal A = alpha-galactosidase A, SD = standard deviation

**Exploratory PD markers:**

No major changes in these exploratory PD markers were expected as this DDI study was a single dose study. In addition, because the study was a single-sequence cross-over, the potential effects of migalastat, if any is considered confounded with the number of agalsidase dose (migalastat was given with the second dose of agalsidase). Co-administration of migalastat with agalsidase did not significantly affect plasma lyso-Gb3, urinary GL-3, and urinary lyso-Gb3 while appeared to increase the change in alpha-Gal A enzyme activity from baseline in skin and WBC. High variability, the small sample size, and the study design issue prevent drawing a conclusion.

**6.5.7. Is the to-be-marketed formulation the same as the clinical trial formulation, and if not, are there bioequivalence data to support the to-be-marketed formulation?**

The to-be-marketed formulation has minor differences from the clinical trial formulation. The BE study was not deemed necessary for these minor differences. Migalastat is formulated as an immediate release, hard, gelatin capsule. There were minor changes (b) (4) from the phase 3 pivotal clinical trials formulation to to-be-marketed (TBM) formulation. Batch analysis data and dissolution data demonstrated that there is no impact of these minor changes on product quality or performance. Therefore, no bioequivalence study was considered necessary. Chemistry, manufacturing, and controls and biopharmaceutics reviewers agreed that a bioequivalence study is not necessary because the changes between the phase 3 formulation and to-be-marketed formulation were minor. Refer to chemistry, manufacturing, and controls and biopharmaceutics reviews.

## 7 Sources of Clinical Data and Review Strategy

### 7.1. Table of Clinical Trials

**Table 40: Migalastat Clinical Program**

Trial(s)	Phase/ Design	Dosing Regimen	Endpoints	Duration	No. of patients	Population
AT1001-011	Phase 3: Stage 1 randomized (1:1), double-blind, placebo-controlled Stage 2: open-label, treatment period	150 mg oral every other day	Pivotal efficacy and safety, tolerability	Stage 1: 6 months Stage 2: month 7-12 Open-label extension: months 13-24	67 (50 amenable, 45 with histology results)	Patients with Fabry disease
AT1001-012	Phase 3: Randomized (1:1), active control, parallel group	150 mg oral every other day	Pivotal efficacy and safety	18 months	60 (56 amenable)	Patients with Fabry disease
AT1001-41	Phase 3: Open-label extension for patients enrolled in FAB-CL-205*, AT1001-011 or AT1001-012	150 mg oral every other day	Long-term efficacy, PD and safety	23.5 months (median)	85	Patients with Fabry disease
AT1001-42	Phase 3: Open-label extension for patients enrolled in AT1001-012 and/or AT1001-41	150 mg oral every other day	Long-term efficacy, PD and safety	ongoing	Ongoing	Patients with Fabry disease
Phase 2 Trials:	6 trials	150-450 mg	Safety, tolerability, PK,	2 days – 48 months	50	Patients with Fabry disease
Phase 1 Trials:	10 trials	0.3 mg/kg-2000 mg	Safety, tolerability, PK, food effect, QTc	Single dose – 7 days	242	Healthy adults; renally impaired adults

PD = pharmacodynamic, PK = pharmacokinetic

\*FAB-CL-205 trial: phase 2 open-label, non-comparative trial assessing safety, tolerability, PK, and PD for 48 months in 23 FD patients

### 7.2. Review Strategy

For this NDA review, data on histological decrease in substrate deposition in KIC as well as biomarker data from trial AT1001-011 were reviewed in support of efficacy. Trials AT1001-011, AT1001-012, AT1001-041 and AT1001-042 were reviewed for safety. The tables and analyses presented in this review reflect the independent data analyses of the review team except

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where otherwise noted. Patient narratives of deaths, serious adverse events, and adverse dropouts related to the phase 3 trials were individually reviewed.

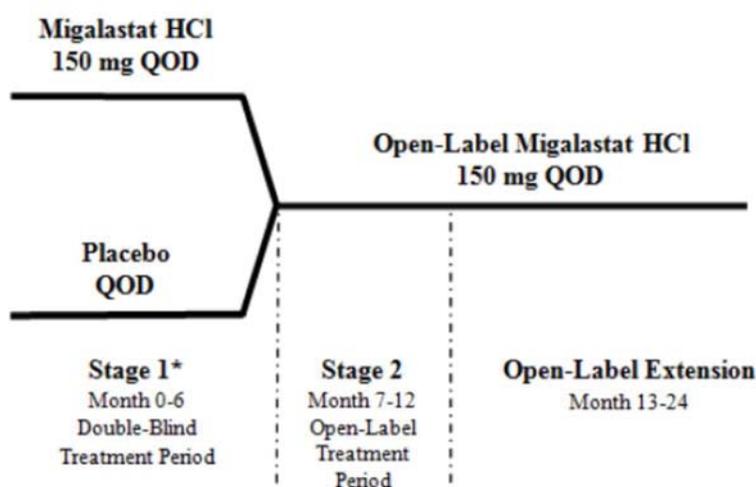
## 8 Statistical and Clinical Evaluation

### 8.1. Review of Relevant Individual Trials Used to Support Efficacy

#### 8.1.1. Trial AT1001-011

**Title: “A double-blind, randomized, placebo-controlled study to evaluate the efficacy, safety, and pharmacodynamics of AT1001 in patients with Fabry disease and AT1001-responsive *GLA* mutations.”**

Figure 26: AT1001-011 Trial Design



\* 1:1 Randomization; stratified by gender

HCl = hydrochloride; QOD = every other day.

Source: trial AT1001-011 CSR

Trial AT1001-011 was a randomized, placebo-controlled phase 3 trial which evaluated Galafold 150 mg every other day and consisted of a 6-month placebo-controlled portion (Stage 1) followed by an additional 6 months of open-label treatment (Stage 2). This was followed by an optional 12-month open label extension portion. The trial enrolled patients with Fabry disease who had “responsive” *GLA* mutations (as determined by the clinical trial HEK assay) and who had not received enzyme replacement therapy (ERT) for > 6 months or who had never received ERT. Stage 1 was a 6-month double blind, randomized, placebo-controlled portion with patients randomized 1:1 to either Galafold or placebo. The randomization was stratified by sex. Stage 2 was a 6-month extension portion where patients who received placebo during stage 1 were switched to Galafold and patients who received Galafold continued on Galafold.

## Key Objectives

### Stage 1:

- Primary: To compare the effect of Galafold versus placebo on kidney GL-3 deposition as assessed by histological scoring of the number of GL-3 inclusions per kidney interstitial capillary (KIC) after 6 months of treatment.
- Secondary:
  - To compare the effect of Galafold versus placebo on urine GL-3 levels, renal function (iohexol glomerular filtration rate (GFR), estimated glomerular filtration rate (eGFR), 24-hour urine protein)
  - To compare the safety and tolerability of Galafold versus placebo

### Stage 2:

- To assess the effect of Galafold on kidney GL-3 (e.g., interstitial capillary histology, other exploratory kidney histology assessments)
- To assess the effect of Galafold on urine GL-3 levels, renal function (iohexol GFR, eGFR, 24-hour urine protein), cardiac function (left ventricular mass, septal wall thickness, fractional shortening and ejection fraction as assessed by ECHO, with tissue Doppler imaging where available), patient-reported outcomes (SF-36 survey, Brief Pain Inventory short form, Gastrointestinal Symptoms Rating Scale), and on WBC alpha-Gal A activity.
- To assess the safety, tolerability, and PK of Galafold

## Study Centers and Number of Patients

Sixty-seven patients were enrolled by 39 investigators in 16 countries (Argentina, Australia, Belgium, Brazil, Canada, Denmark, Egypt, France, Germany, Italy, Netherlands, Poland, Spain, Turkey, United Kingdom, and the United States).

## Study Population

The study population was identified using the Clinical Trial (CT) HEK-293 in vitro assay which categorized *GLA* variants as either “responsive” to Galafold or “non-responsive” based on pre-specific biochemical criteria of alpha-Gal A enzyme activity (see sections 6.3 and 6.4). Following completion of study enrollment, the CT HEK-293 in vitro assay underwent bioanalytical validation in a qualified third-party laboratory in compliance with current regulatory guidance and relevant Good Laboratory Practice (GLP) regulations. The validated assay is referred to as the GLP HEK assay. *GLA* variants are categorized by the GLP HEK assay as “amenable” or “non-amenable” based on the same criteria as the CT HEK-293 assay. See Appendix 2 for details of the key differences between the two in vitro assays. Use of the GLP HEK assay changed the classification of 17 “responsive” mutations (based on the CT HEK assay) in patients in trial AT1001-011 to “non-amenable” mutations. As such, the final study population of patients with amenable *GLA* variants included 50 patients (out of 67 originally enrolled) with amenable *GLA* variants in trial AT1001-011. Of note, the reclassification of amenability for all *GLA* variants in trial AT1001-011 based on the GLP HEK assay occurred prior to data unblinding. Out of the 50 patients, 45 patients had renal histology data available both at baseline and at month 6 for analysis of the primary efficacy. The five remaining patients were missing baseline renal biopsy

data.

**Key inclusion criteria:**

- Male or female between 16 and 74 years inclusive, diagnosed with Fabry disease
- Confirmed *GLA* mutation that has been shown to be “responsive” to AT1001 in vitro based on the CT HEK assay
- Naïve to ERT or have not received ERT for at least 6 months before screening
- Urine GL-3  $\geq$  four times the upper limit of normal at screening
- Patients taking angiotensin converting enzyme (ACE) inhibitors or angiotensin receptor blockers (ARBs) must be on a stable dose for a minimum of 4 weeks before the baseline visit

Of note, the clinical trial did not have an inclusion criterion for the primary efficacy variable (GL-3 inclusions per kidney interstitial capillary). Absence of a specific threshold for the number of inclusions at baseline resulted in the enrollment of a patient population with mild histological manifestations of Fabry disease. This issue will be discussed in detail in the efficacy section.

**Key Exclusion criteria:**

- Patient has undergone or is scheduled to undergo kidney transplantation, or is currently on dialysis
- eGFR  $< 30$  mL/min/1.73m<sup>2</sup> (chronic kidney disease [CKD] Stage 4 or 5) based on Modification of Diet in Renal Disease [MDRD]) equation at Screening

**Dosing Regimen and Formulation**

Nonclinical studies found that a 30mg/kg dose of Galafold in mice resulted in a significant increase in alpha-Gal A activity and GL-3 substrate reduction. Refer to nonclinical section 5.5.1. A range of doses and regimens were explored in 27 patients enrolled in 5 phase 2 trials (refer to clinical pharmacology section 6.2.2 for further information). In these trials, the Applicant notes that “150mg migalastat QOD resulted in the best balance of substrate reduction (urine GL-3) and safety in patients with amenable mutations, compared to the other doses and regimens studied.”<sup>12</sup>

During stage 1 of trial AT1001-011, patients were randomized to either Galafold 123 mg once every other day or placebo. Patients were required to fast 2 hours before and 2 hours after taking each dose of study medication.

During stage 2 and the open-label treatment extension phase, patients took Galafold 123 mg once every other day. The use of ERT or substrate reduction therapies approved for Fabry

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<sup>12</sup> Applicant’s submission “Summary of Clinical Pharmacology Studies” 2.7.2 page 12/105

disease in the U.S. or elsewhere was not allowed. Specifically, the following medications were prohibited within 6 months of screening and at any time during study participation: Fabrazyme (agalsidase beta), Replagal (agalsidase alfa), Glyset (miglitol), Zavesca (miglustat).

## Endpoints

### Stage 1:

**Primary Efficacy Endpoint:** Proportion of patients with  $\geq 50\%$  reduction from baseline (visit 1) to month 6 (visit 4) in the average number of KIC GL-3 inclusions.

**Secondary Efficacy Endpoints:** Percent change from baseline (visit 1) in KIC GL-3 inclusions; urine GL-3, mGFRiohexol; eGFR; and 24-hour urine protein, urine albumin, and urine creatinine.

**Tertiary Efficacy Endpoints:** Percent KICs with zero GL-3 inclusions, ECHO, Short Form 36 v2, Brief Pain Inventory short form, Gastrointestinal Symptoms Rating Scale, WBC alpha-Gal A activity, and PK assessments.

**Exploratory efficacy endpoints:** Plasma lyso-Gb3, podocyte, mesangial cell, and endothelial cell GL-3; interstitial fibrosis and tubular atrophy; glomerular sclerosis

### Stage 2:

#### Key efficacy Endpoints:

- KIC GL-3 inclusions: durability of response by the mean change for patients with amenable mutations who received Galafold in stage 1 (Galafold-Galafold group)
- Mean change in KIC GL-3 inclusions in stage 2 for patients with amenable mutations who received placebo in stage 1 (placebo-Galafold group)
- Renal function (eGFR) annualized rate of change
- Changes in other exploratory kidney histology assessments (podocyte, mesangial cell, and endothelial cell GL-3)

**Exploratory Endpoint:** plasma lyso-Gb3

### Statistical Analysis Plan/Additional Analyses (based on re-classified patient population)

The primary endpoint analysis for stage 1 compared the proportion of successes ( $\geq 50\%$  reduction from baseline) in each treatment group using the exact Cochran-Mantel-Haenszel test stratified by sex. A p-value  $< 0.05$  (2-sided) was required to conclude a statistically significant treatment effect. The intent to treat (ITT) and intent-to-treat amenable (ITT-amenable) populations were used for the analysis of the primary efficacy endpoint. A supportive analysis was performed using similar methods for the modified intent to treat (mITT) and per protocol (PP) population. The same analyses were conducted for the proportion of patients with 25%, 35%, and 75% reduction from baseline to month 6 in the average number of KIC GL-3 inclusions. An additional

sensitivity analysis was conducted to impute missing month 6 kidney biopsy data. In this analysis, the numbers of successes in the placebo group (or Galafold group, respectively) were the number of successes observed plus the number of subjects with missing data in the same group times the upper (or the lower bound, respectively) of the 95% confidence interval of the percentage successes in the other group.

### **Timeline of HEK-293 Assay Validation**

The Applicant submitted the following timeline to describe the sequence of events during the phase 3 trials which led to the change in the final efficacy population.

The HEK-293 in vitro assay for amenability determination underwent GLP validation (while trial AT1001-011 was ongoing), which was completed on September 19, 2011. Reclassification of all trial AT1001-011 GLA variants was completed on May 16, 2012. Subsequently, stage 1 data unblinding occurred on November 13, 2012. Re-analyses of the primary efficacy endpoint based on the re-classified ITT-amenable population in trial AT1001-011 were conducted on April 11, 2014, after stage 1 data unblinding. In addition, in early 2013 and prior to the stage 2 data unblinding, additional efficacy analyses were included in an amended SAP (pre-specified) for assessment of efficacy at month 12 (stage 2) based on the re-classified ITT-amenable population. It is noteworthy that the in vitro assay (HEK-293) GLP validation for mutation amenability was completed prior to stage 1 data unblinding and, in addition, the biochemical criteria used for variant categorization (amenable or non-amenable) did not change from the CT HEK-293 assay used for patient enrollment.

### **Protocol Amendments**

Protocol amendments were reviewed and they appear to have been implemented prior to data unblinding and to improve patient safety. As such, these amendments appear to not have affected the efficacy assessments or analyses.

### **Compliance with Good Clinical Practices**

According to the submission (page 15 of the AT1001-011-body.pdf), "This study was conducted in compliance with the United States Food and Drug Administration regulations in 21 Code of Federal Regulations 56 and the International Conference on Harmonisation (ICH) Good Clinical Practice guidelines. For each site, the study protocol and informed consent form (ICF) were reviewed and approved by the appropriate Independent Ethics Committee (IEC)/Institutional Review Board (IRB). As needed, the IEC/IRB also reviewed and approved protocol amendments and any corresponding changes to the ICF." The signed ICF was placed in the study record for each subject, and a copy of the signed ICF was provided to the subject.

### **Financial Disclosure**

The Applicant provided a signed copy of FDA Form 3454 with a list of investigator names from each trial. This certified that they have not entered into any financial arrangement with their clinical investigators, whereby the value of compensation to the investigator could be affected by the outcome of the trial as defined in 21 CFR 54.2(a).

### **Primary Endpoint Assessment**

The average number of GL-3 inclusions per KIC was assessed by the BLISS histological methodology (see appendix 1). The methodology was based on using standardized digital images of renal biopsy specimen slides. One pathologist served as annotator and designated the 300 randomly selected capillaries within each slide to be scored. Two pathologists served as scorers; these pathologists completed blinded paired assessments (at baseline and at month 6) of the 300 annotated capillaries.

### **Analysis Populations:**

**Table 41: Efficacy Analysis Populations**

ITT population (intent to treat)	All randomized patients
ITT-amenable	All randomized patients with amenable <i>GLA</i> variants (based on GLP HEK assay)
ITT-amenable with available histology (AH)	All randomized patients with amenable <i>GLA</i> variants (based on GLP HEK assay) with available renal histology data both at baseline and at month 6
mITT population (modified intent to treat)	All randomized patients who received at least one dose of drug and underwent renal biopsy at baseline and month 6
Per protocol population	mITT population with no major protocol violations

GLP = good laboratory practice, HEK-293 = human embryonic kidney cell line 293, ITT = intent to treat, mITT = modified intent to treat

For the analysis of the primary endpoint in the ITT population, subjects with missing month 6 kidney histology data but who have a baseline measurement were considered as treatment failures. Subjects who had missing baseline measurements were not included in the analysis. No imputation was done for baseline values.

### **8.1.2. Trial AT1001-011 Results**

#### **Data Quality and Integrity**

The submitted datasets and definition files were accessible and the quality of the submitted datasets was acceptable.

#### **Patient Disposition**

A total of 180 patients consented to participate in the trial, and 67 patients were randomized in a 1:1 ratio to either receive Galafold (34 patients) or placebo (33 patients) in stage 1 of the trial. A total of 64 patients completed stage 1: 34 patients (100%) in the Galafold group and 30 patients (91%) in the placebo group. Of the three patients in the placebo group who discontinued during stage 1, two patients withdrew consent and one patient discontinued due to pregnancy. One patient in the Galafold group withdrew consent after completing stage 1.

A total of 63 patients entered stage 2: 33 patients in the Galafold-Galafold group and 30 patients in the placebo-Galafold group. A total of 60 patients completed stage 2: 31 patients (94%) in the Galafold-Galafold group and 29 patients (97%) in the placebo-Galafold group. Of the two patients in the Galafold-Galafold group who discontinued during stage 2, one withdrew consent and one discontinued due to an SAE (amyotrophic lateral sclerosis). One patient in the placebo-Galafold group discontinued due to an SAE of anaplastic large cell lymphoma.

**Table 42: Trial AT1001-011, Stage 1 and 2: Patient Disposition (ITT Population, N = 67)**

Reason for discontinuation	Galafold (n = 34), n (%)	Placebo (n = 33), n (%)
Adverse event	1 (3)	1 (3)
Lost to Follow-up	1 (3)	0
Pregnancy	1 (3)	1 (3)
Withdrawal by subject	2 (6)	3 (9)

Source: reviewer table  
ITT = intent-to-treat

**Table 43: Trial AT1001-011, Stage 1 and 2: Patient Disposition (ITT-Amenable Population, N = 50)**

Reason for Discontinuation	Galafold n = 28, n (%)	Placebo n = 22, n (%)
Adverse event	1 (4)	1 (5)
Pregnancy	1 (4)	1 (5)
Withdrawal by subject	2 (7)	1 (5)

Source: reviewer table  
ITT = intent-to-treat

### **Protocol Violations/Deviations**

Protocol deviations were comparable between both groups. A higher percentage of protocol deviations related to the “visit schedule criteria” was notable in the Galafold group (68%) versus placebo (59%). The majority of deviations were related to visits occurring outside the protocol-defined visit windows. Also, the majority of protocol deviations occurred in the placebo group.

**Table 44: Trial AT1001-011 Protocol Deviations (ITT-amenable population, N = 50)**

Deviation Type	Galafold	Placebo
	N = 28 n (%)	N = 22 n (%)
Informed consent	4 (14)	4 (18)
Administrative criteria	1 (4)	0
Source document criteria	0	1 (5)
Eligibility and entry criteria	0	1 (5)
Laboratory assessment criteria	10 (36)	11 (50)
Trial procedures criteria	14 (50)	18 (82)
Serious adverse event criteria	0	3 (14)
Randomization criteria	0	1 (5)
Visit schedule criteria	19 (68)	13 (59)
Investigational product compliance	12 (43)	10 (43)

Reviewer table  
ITT = intent to treat

### **Demographics/Baseline Characteristics**

Demographic characteristics were comparable between the two treatment groups: 64% of patients were female and most patients were white. There was only one patient < 18 years and one patient > 65 years in the Galafold group.

**Table 45: Trial AT1001-011: Demographic Characteristics (ITT-amenable population, N = 50)**

Characteristic		Galafold (N = 28)	Placebo (N = 22)
<b>Age</b>	Mean	41.5	45
	Min, Max	16, 68	25, 58
	> 65 years	1	0
<b>Sex, n (%)</b>	F	19 (68)	13 (59)
	M	9 (32)	9 (41)
<b>Race, n (%)</b>	White	26 (93)	22 (100)
<b>Ethnicity, n (%)</b>	Hispanic or Latino	0	3 (14)
	Not Hispanic or Latino	16 (57)	7 (32)
	Unknown	12 (43)	12 (55)

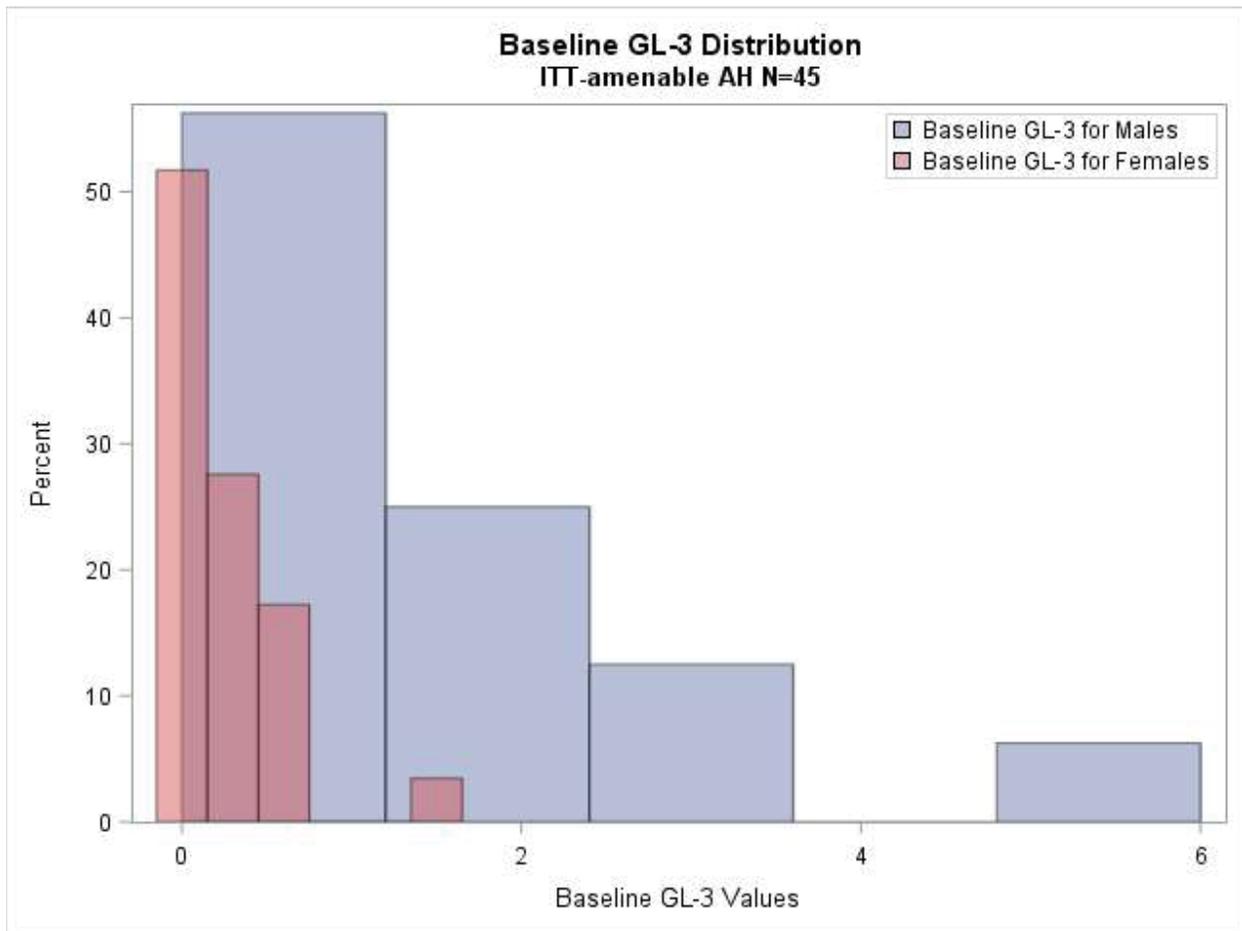
Reviewer's table  
ITT = intent to treat

### **Baseline GL-3 Inclusion Burden**

At baseline, there was a large difference between males and females in terms of GL-3 inclusion burden, as shown in the figure below. In general, males in trial AT1001-011 started with a higher baseline GL-3 inclusion burden as compared to the females, which is indicative of more extensive substrate deposition in KIC and, thus, more severe disease on a histologic level. This

discrepancy in baseline histological disease severity between males and females resulted in differential efficacy responses as described in the efficacy results section.

**Figure 27: Percent of Patients with Different Baseline GL-3 Inclusions per KIC by Sex (ITT-amenable AH Population, N = 45)**



AH = available histology, GL-3 = globotriaosylceramide, ITT = intent-to-treat, KIC = kidney interstitial capillary

### **Baseline Disease Characteristics and Concomitant Medications**

Important baseline characteristics in the Galafold and placebo groups are shown in the following table. Notably, placebo patients had a two-fold higher baseline urine GL-3 concentration than Galafold patients, which is of unclear clinical significance as GL-3 concentration in urine cannot reliably be correlated with disease severity in FD. There was also a higher amount of proteinuria in the placebo patients than in the Galafold patients, which may explain why more patients in the placebo group were treated with an ACE inhibitor or ARB during the trial. Notably, baseline renal function (as measured by eGFR) was similar in both groups.

**Table 46: Other Baseline Characteristics, Trial AT1001-011 (ITT-amenable, N = 50)**

Parameter		Galafold	Placebo
		(N = 28)	(N = 22)
<b>mGFR<sub>iohexol</sub></b> <b>(ml/min/1.73m<sup>2</sup>)</b>	n	27	21
	Mean	79.95	83.12
	Median	84.90	82.20
	Min, Max	4.6, 134.2	35.2, 122.0
<b>eGFR<sub>CKD-EPI</sub></b> <b>(ml/min/1.73m<sup>2</sup>)</b>	n	28	22
	Mean	94.36	90.57
	Median	96.55	93.53
	Min, Max	41.36, 151.35	55.71, 115.91
<b>Proteinuria (mg/24h)</b>	n	28	22
	Mean	373.68	584.55
	Median	206.50	302.00
	Min, Max	44, 1900	74, 2610
<b>Urine GL-3 (ng/mgcreat)</b>	n	28	22
	Mean	922.86	1071.87
	Median	235.40	530.85
	Min, Max	32.1, 4163.6	96.6, 5245.9
<b>*KIC GL-3 inclusions-all</b>	n	25	20
	Mean	0.65	0.49
	Median	0.22	0.25
	Min, Max	0.02, 5.70	0.03, 2.41
<b>*KIC GL-3 inclusions -male</b>	n	7	9
	mean	1.79	0.70
	median	1.22	0.16
	Min, Max	0.03, 5.70	0.03, 2.41
<b>*KIC GL-3 inclusions-female</b>	n	18	11
	Mean	0.21	0.32
	Median	0.14	0.29
	Min, Max	0.02, 0.60	0.09, 0.79
<b>Plasma lyso-Gb3 (nmol/L)</b>	n	18	13
	Mean	47.27	41.85
	Median	16.77	23.27
	Min, Max	1.19, 218.33	6.69, 113.33

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Parameter		Galafold	Placebo
		(N = 28)	(N = 22)
<b>WBC alpha-Gal A activity<sup>^</sup> (% of normal mean)- Male</b>	n	8	9
	Mean	6.17	2.33
	Median	1.09	1.91
	Min, Max	0.00, 41.44	0.00, 6.45
	< 1%	4 (50%)	3 (33%)
	≥ 1%	4 (50%)	6 (67%)
	< 3%	7 (88%)	7 (78%)
	≥ 3%	1 (13%)	2 (22%)
<b>Clinical phenotype</b>	n	28	22
	“Classic” FD-males <sup>a</sup>	7 (25%)	7 (32%)
	“Non-classic” FD-males	2 (7%)	2 (9%)
	“Classic” FD-females	0	0
	“Non-classic” FD Females	19 (68%)	13 (59%)
<b>GLA variant</b>	n	28	22
	Missense	28 (100%)	22 (100%)
<b>NSAID use during study</b>	Y	3 (11%)	3 (14%)
	N	25 (89%)	19 (86%)
<b>ACE inhibitor/ARB/RI use</b>	Y	6 (21%)	9 (41%)
	N	22 (79%)	13 (59%)
<b>History of ERT use</b>	Y	4 (14%)	7 (32%)
	N	24 (86%)	15 (68%)

<sup>a</sup> “classic male” FD clinical phenotype defined by the Applicant as: “male with multi-organ disease and WBC alpha Gal-A activity < 3% of wild type mean” Of note, there were no females with “classic FD” based on the Applicant’s definition.

\* Baseline Biopsy for KIC GL-3 inclusions performed in 45 patients

<sup>^</sup> converted WBC alpha Gal-A values are used for the analysis. Conversion formula: alpha Gal-A % Normal value = (WBC alpha Gal-A activity result/22) x 100

Source: table 1.1 Applicant information response submitted July 5, 2018

ACE = angiotensin converting enzyme, alpha-Gal A = alpha-galactosidase A, eGFR = estimated glomerular filtration rate, ERT = enzyme replacement therapy, GL-3 = globotriaosylceramide, lyso-Gb3 = globotriaosylsphingosine, NSAID = nonsteroidal anti-inflammatory drug, WBC = white blood cell, CKD-EPI = Chronic Kidney Disease Epidemiology Collaboration equation, MDRD = Modification of Diet in Renal Disease Study equation

### **Treatment Compliance, Concomitant Medications, and Rescue Medication Use**

Agalsidase beta, agalsidase alfa, miglitol, miglustat were prohibited six months prior to screening and at any time during study participation. Any other investigational/experimental therapy was prohibited 30 days prior to screening or at any time during study participation.

## **Efficacy Results**

### **Assessment of Substrate Burden**

Table 47 shows the results of the Applicant’s primary efficacy analysis in the ITT population, the proportion of subjects with  $\geq 50\%$  reduction from baseline (visit 1) to month 6 (visit 4) in the average number of GL-3 inclusions per KIC. As per the Applicant’s AT1001-011 SAP, for the primary efficacy analysis in the ITT population, subjects with month 6 missing renal histology data but with baseline measurement were considered as treatment failures. Subjects with missing baseline renal histology data were not included in the analysis. No imputation was performed for missing baseline values.

**Table 47: Trial AT1001-011 Stage 1: Primary Efficacy Analysis: 50% Reduction from Baseline to Month 6 in Average Number of GL-3 Inclusions per KIC (ITT Population, N = 64, Without Missing Data Imputation)**

<b>Response Threshold</b>	<b>Galafold Response Rate</b>	<b>Placebo Response Rate</b>	<b>Difference (Galafold-placebo) 95% CI</b>	<b>p-value</b>
$\geq 50\%$	13/32 (40.6%)	9/32 (28.1%)	(-13.4%, 37.3%)	0.30

Source: Applicant’s table 14.2.1.1-1 of at1001-011-body-section14.pdf

CI = confidence interval, GL-3 = globotriaosylceramide, ITT = intent to treat, KIC = Kidney Interstitial Capillary

As shown in Table 47, the difference between Galafold and placebo in the primary efficacy endpoint at stage 1 did not reach statistical significance at a two-sided significance level of 0.05 (p-value is 0.30). The Applicant conducted sensitivity analyses using other response thresholds, including 25%, 35% and 75% reduction from baseline, and none of the results reached statistical significance at a two-sided significance level of 0.05 (p-value range 0.08-0.14). In addition, the Applicant conducted a sensitivity analysis using their proposed imputation approach based on the confidence intervals (described previously in Section 8.1.1); the difference between Galafold and placebo did not reach statistical significance with a p-value of 0.5 and 95% CI of (-15.2%, 31.0%). Furthermore, the Applicant performed a similar set of analyses for the primary efficacy endpoint based on the mITT and PP populations; results from these analyses are similar to those from the primary analysis (two same p-values of 0.27 and two same 95% CIs as (-13.5%, 38.8%)).

As previously recommended by FDA, additional analyses using non-responder imputation for the primary endpoint are presented in Table 48, with similar results as those shown in Table 47.

**Table 48: Trial AT1001-011 Stage 1: Primary Efficacy Analysis: 50% reduction from baseline to Month 6 in Average Number of GL-3 Inclusions per KIC (ITT Population, N = 67, With Missing Data Imputation)**

Response Threshold	Galafold Response Rate	Placebo Response Rate	Difference (Galafold-placebo) 95% CI	p-value
≥ 50%	13/34 (38.2%)	9/33 (27.3%)	(-12.0%, 34.8%)	0.34

Source: Applicant's at1001-011-req-table1-kic-mod-resp-worst-case-2.pdf

CI = confidence interval, GL-3 = globotriaosylceramide, ITT = intent to treat, KIC = Kidney Interstitial Capillary

As described in Section 6.5, during the course of the trial, the CT HEK-293 in vitro assay (used for determination of *GLA* variant responsiveness to Galafold in vitro) underwent GLP validation, which included additional quality control measures to improve the performance characteristics of the assay. As a result of this validation, the *GLA* variants of 17 out of the enrolled 67 patients in trial AT1001-011 were reclassified as non-amenable based on the GLP HEK-293 in vitro assay. Based on the updated in vitro HEK assay, which was used to identify the patient population most likely to have a treatment effect, additional efficacy analyses were conducted in the 50 patients determined to have “amenable” *GLA* variants. These additional analyses are presented below.

Regarding the primary efficacy analysis, Table 49 presents the proportion of ITT-amenable subjects with at least 50% reduction from baseline (visit 1) to month 6 (visit 4) in the average number of GL-3 inclusions per KIC during stage 1.

**Table 49: Trial AT1001-011 Stage 1: Primary Efficacy Analysis: 50% reduction from baseline to Month 6 in Average Number of GL-3 Inclusions per KIC (ITT-amenable AH Population, N = 45)**

Response Threshold	Galafold Response Rate	Placebo Response Rate	Difference (Galafold-placebo) 95% CI	p-value
≥ 50% reduction	13/25 (52%)	9/20 (45%)	(-22.7%, 35.8%)	0.55

Source: table 1 of resp-info-req-q2.pdf and reviewer's analysis

AH = available histology, CI = confidence interval, GL-3 = globotriaosylceramide, ITT = intent to treat, KIC = Kidney Interstitial Capillary

In the SAP for post hoc stage 1 analyses, the analysis of the percent of subjects with a ≥ 50% reduction from baseline has been removed since at that time the stage 1 data had been unblinded and the results of the primary efficacy endpoint did not reach statistical significance. The Applicant chose to conduct analyses of the change from baseline in the average number of inclusions per capillary on a continuous scale using ANCOVA and MMRM models. According to the Applicant's stage 1 SAP, the originally planned model terms only includes sex and baseline values in the ANCOVA analysis. All the following analysis results are determined to be post hoc.

Since the normality assumption of ANCOVA did not hold for ITT-amenable population (p-value > 0.15), nonparametric method (SAS macro NparCov3) was applied and **Error! Reference source not found.** presents the analysis results.

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FDA further refined the final and most informative primary efficacy population to ITT-amenable patients with available histology (AH) data both at baseline and at month 6 (referred to as ITT-amenable AH, N = 45). Changes from baseline to month 6 in the average number of GL-3 inclusions per KIC in this population and subgroups by gender and baseline GL-3 inclusions per KIC (< 0.3 vs. ≥ 0.3) are shown in Table 50, below. The review team assessed the changes from baseline (as a continuous variable) to supplement the analyses using a responder criterion (at least 50% reduction from baseline).

**Table 50: Trial AT1001-011, Stage 1: Change from Baseline to Month 6 in ITT-Amenable AH Population (N = 45)**

<b>Group</b>	<b>Galafold n/N (%) with ≥ 50% reduction Median change from baseline (range)</b>	<b>Placebo n/N (%) with ≥ 50% reduction Median change from baseline (range)</b>
ITT-amenable AH patients (N = 45)	13/25 (52%) -0.04 (-1.94, 0.26)	9/20 (45%) -0.03 (-1.00, 1.69)
Females (N = 29)	8/18 (44%) -0.02 (-0.46, 0.26)	5/11 (46%) -0.03 (-0.35, 0.10)
Males (N = 16)	5/7 (71%) -1.10 (-1.94, -0.02)	4/9 (44%) -0.03 (-1.00, 1.69)
Patients with baseline GL-3 ≥ 0.3 (N = 17; 9 males, 8 females)	7/9 (78%) -0.91 (-1.94, 0.19)	2/8 (25%) -0.02 (-1.00, 1.69)
Patients with baseline GL-3 < 0.3 (N = 28; 7 males, 21 females)	6/16 (38%) -0.02 (-0.10, 0.26)	7/12 (58%) -0.05 (-0.16, 0.14)

AH = available histology, ITT = intent-to-treat, GL-3 = globotriaosylceramide

For the ITT-amenable AH population (5 out of 50 patients with missing baseline GL-3 values excluded), the review team used different responder thresholds, including 20%, 25%, 30%, 40%, 60% and 70%, to analyze the proportion of responders and change from baseline to month 6 in the number of GL-3 inclusions per KIC. In the ITT-amenable population without imputation, for 20%, 25%, 30%, 40% and 60% reduction, treated males appeared to show a larger response rate as compared to treated females. However, a similar response rate was observed in male patients and female patients when a 70% threshold for response was used.

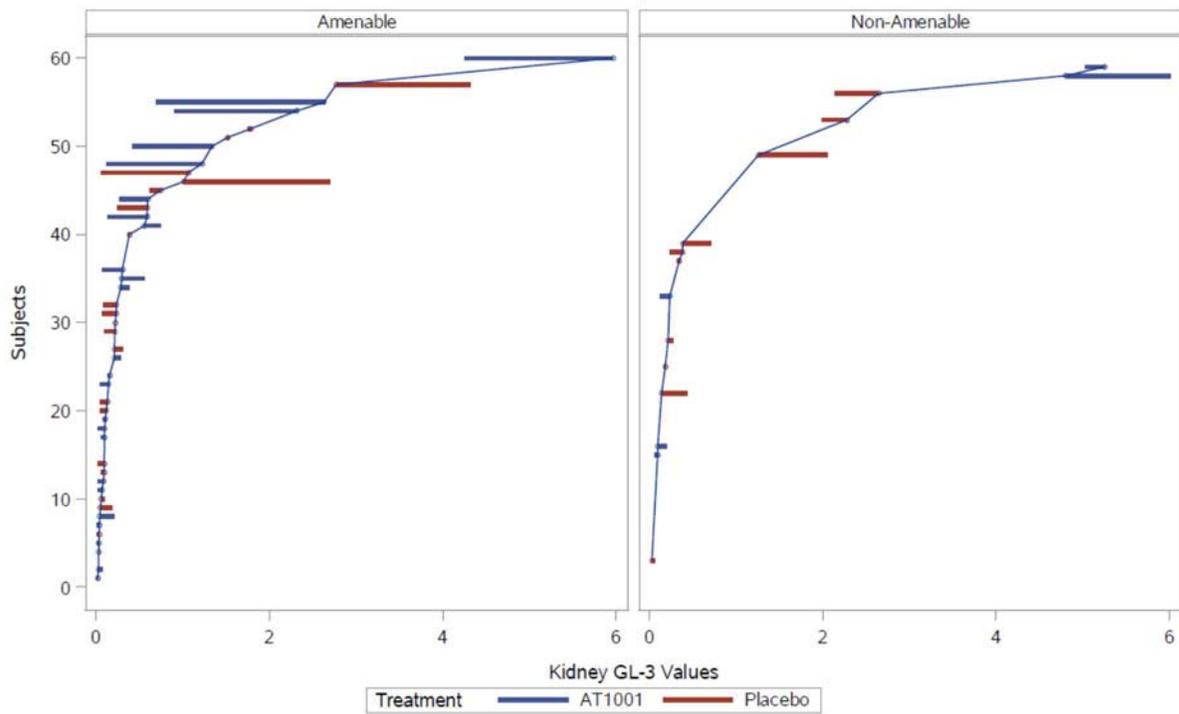
In the ITT-amenable population with non-responder imputation, similar response rate differences were observed between male patients and female patients when 20%, 25%, 30%, 40% and 50% were used. Male patients tend to have much larger response rate difference than female patients when 60% reduction was considered. However, when a 70% reduction threshold was used, females showed a larger response rate difference than male patients.

The mean change in GL-3 inclusions per KIC was -0.31 in Galafold group and 0.07 in placebo group in ITT-amenable AH population. The 2-sided p-value of the treatment difference is 0.04 in a post-hoc linear regression model.

As previously indicated, the clinical trial did not have an inclusion criterion for baseline KIC GL-3 inclusions, which resulted in the enrolment of a patient population with mild histological manifestations of Fabry disease. As shown in Table 46, the baseline KIC GL-3 inclusion mean/median was 0.65/0.22 in the Galafold group and 0.49/0.25 in the placebo group respectively. These values were close to the limit of detection of a signal with the histological assay used (per Applicant's description, values of 0.3 are close to the background noise in the histological method used). Therefore, the FDA conducted analyses in the limited number of patients with baseline GL-3 KIC values > 0.3 as such a value is consistent with a clear signal over background "noise" based on the histological assay methodology. Out of 45 evaluable patients for the primary efficacy endpoint (ITT-amenable AH), 17 patients had baseline GL-3 inclusions  $\geq$  0.3. Males tended to have a larger response rate difference between treated and placebo than female patients (except for the 70% reduction threshold as described in the previous section).

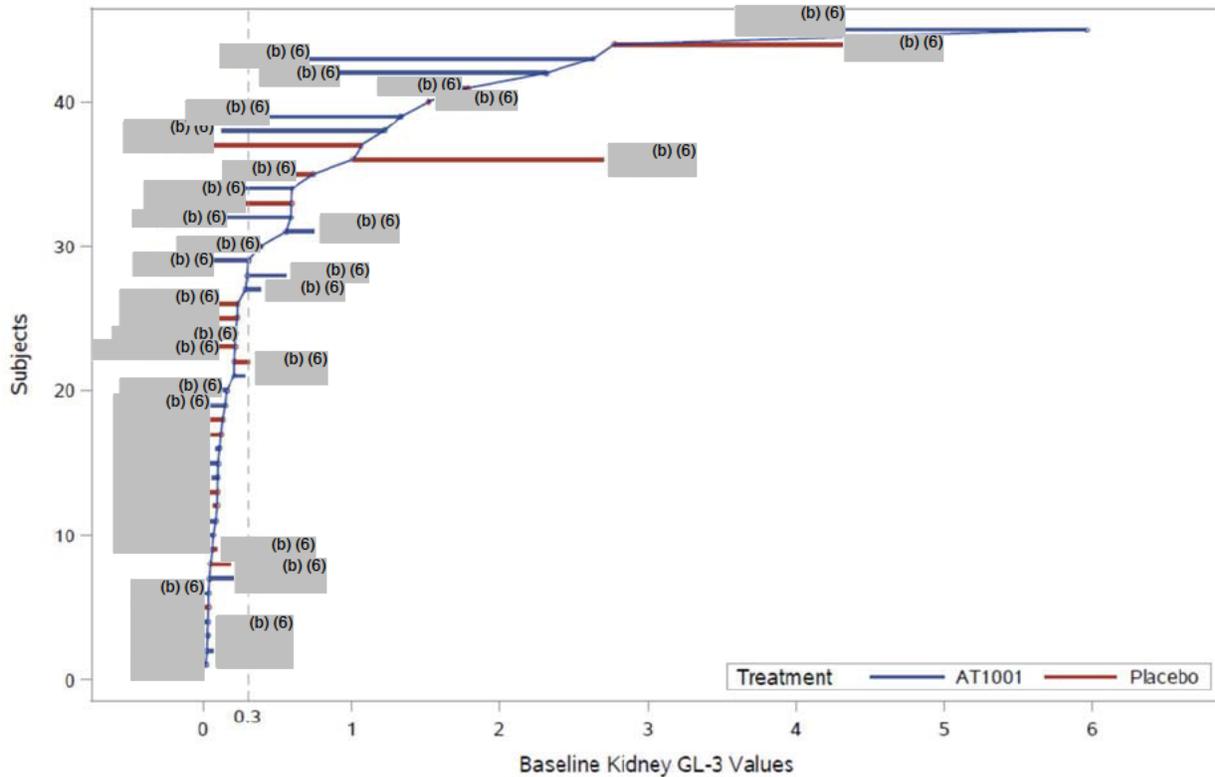
Patient profiles showing the change from baseline to month 6 in GL-3 inclusion burden are shown in Figure 28 and Figure 29 for trial AT1001-011, stage 1. As evident in these figures, most patients had a low baseline GL-3 KIC value and patients with relatively larger treatment effects were those with higher baseline GL-3 inclusion values. As expected, patients with low baseline GL-3 values, which constituted the majority, showed minimal to small changes in GL-3 inclusions after 6 months.

**Figure 28: Trial AT1001-011 Stage 1: Change from Baseline in Average Number of GL-3 inclusions per KIC in Individual Patients by Amenable Category (ITT Population, N = 67)**



Source: the Applicant's figure 1.1 of at1001-011-supporting-q1-kic-figure-1-1.pdf  
AT1001 = Galafold, GL-3 = globotriaosylceramide, ITT = intent-to-treat, KIC = kidney interstitial capillary

**Figure 29: Trial AT1001-011 Stage 1: Change from Baseline in Average Number of GL-3 Inclusions per KIC by Individual Patient and Baseline KIC GL-3 values (ITT-Amenable AH Population, N = 45)**



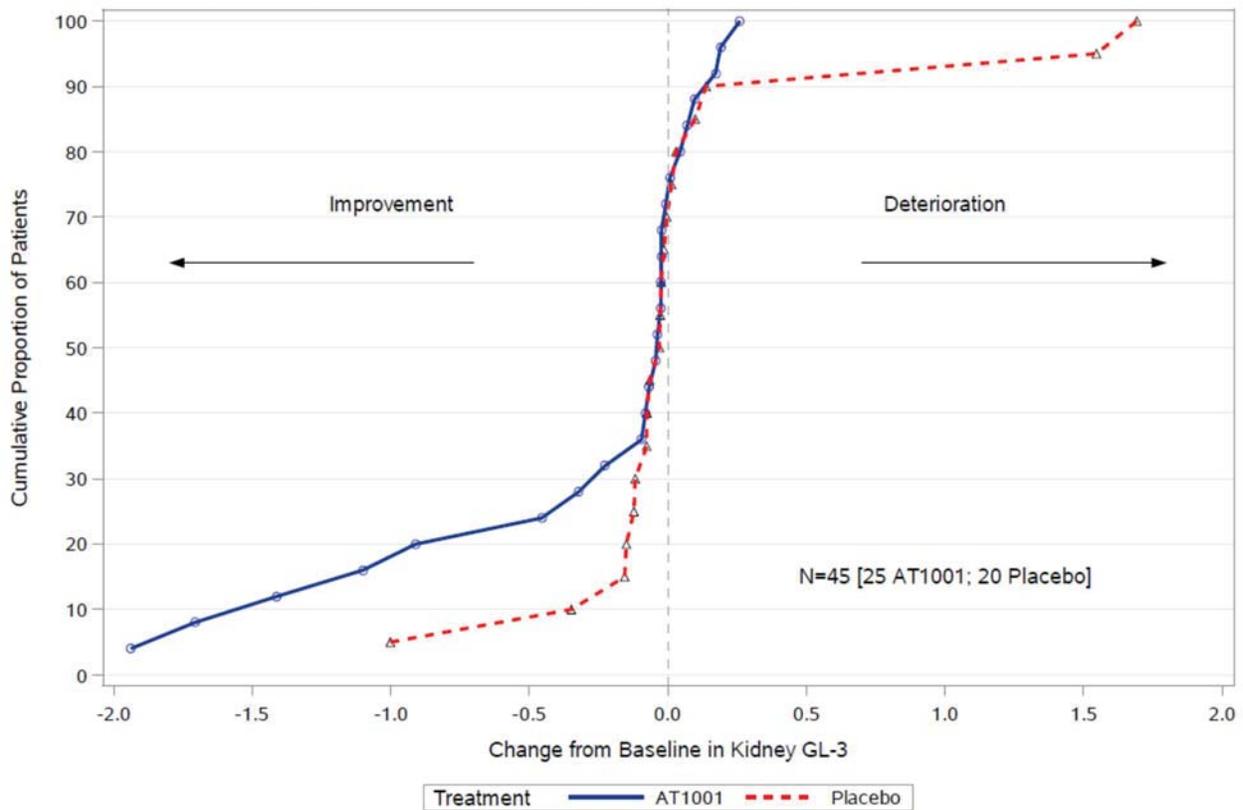
Source: the Applicant's figure 1.2 of at1001-011-supporting-q1-kic-figure-1-2.pdf  
AH = available histology, AT1001 = Galafold, GL-3 = globotriaosylceramide, ITT = intent-to-treat, KIC = kidney interstitial capillary

As shown in Figure 28 and Figure 29, patients with higher baseline GL-3 inclusion burden experienced a larger reduction in GL-3 inclusions at month 6.

To further explore the treatment effects, the cumulative distribution plots were generated to explore the treatment effect in subgroups, such as males versus females and baseline GL-3  $\geq 0.3$  per KIC versus baseline GL-3  $< 0.3$  per KIC. Figure 30 presents the plot for ITT-amenable AH population of change from baseline to month 6 in Average GL-3 Inclusions per KIC. No difference between Galafold and placebo was observed. Figure 31 and Figure 32 present the results for the ITT-amenable AH population without missing data imputation for GL-3 for males versus females and for patients with baseline GL-3  $\geq 0.3$  versus GL-3  $< 0.3$ , respectively. In male patients and patients with baseline GL-3  $\geq 0.3$ , separations in the estimated cumulative distributions between Galafold and placebo groups were observed in change from baseline to month 6 in average number of GL-3 inclusion per KIC. Furthermore, larger reductions were observed in the Galafold group in GL-3 at month 6 than placebo group in these subgroups. We should emphasize that the sample sizes in these subgroups are small with only 16 male patients and with 18 patients with baseline GL-3  $\geq 0.3$ . The estimated cumulative distributions have

large variabilities, preventing a definitive statistical conclusion. In contrast, the separation between Galafold and placebo groups was not observed in female patients or in patients with baseline GL-3 < 0.3 in the cumulative distributions.

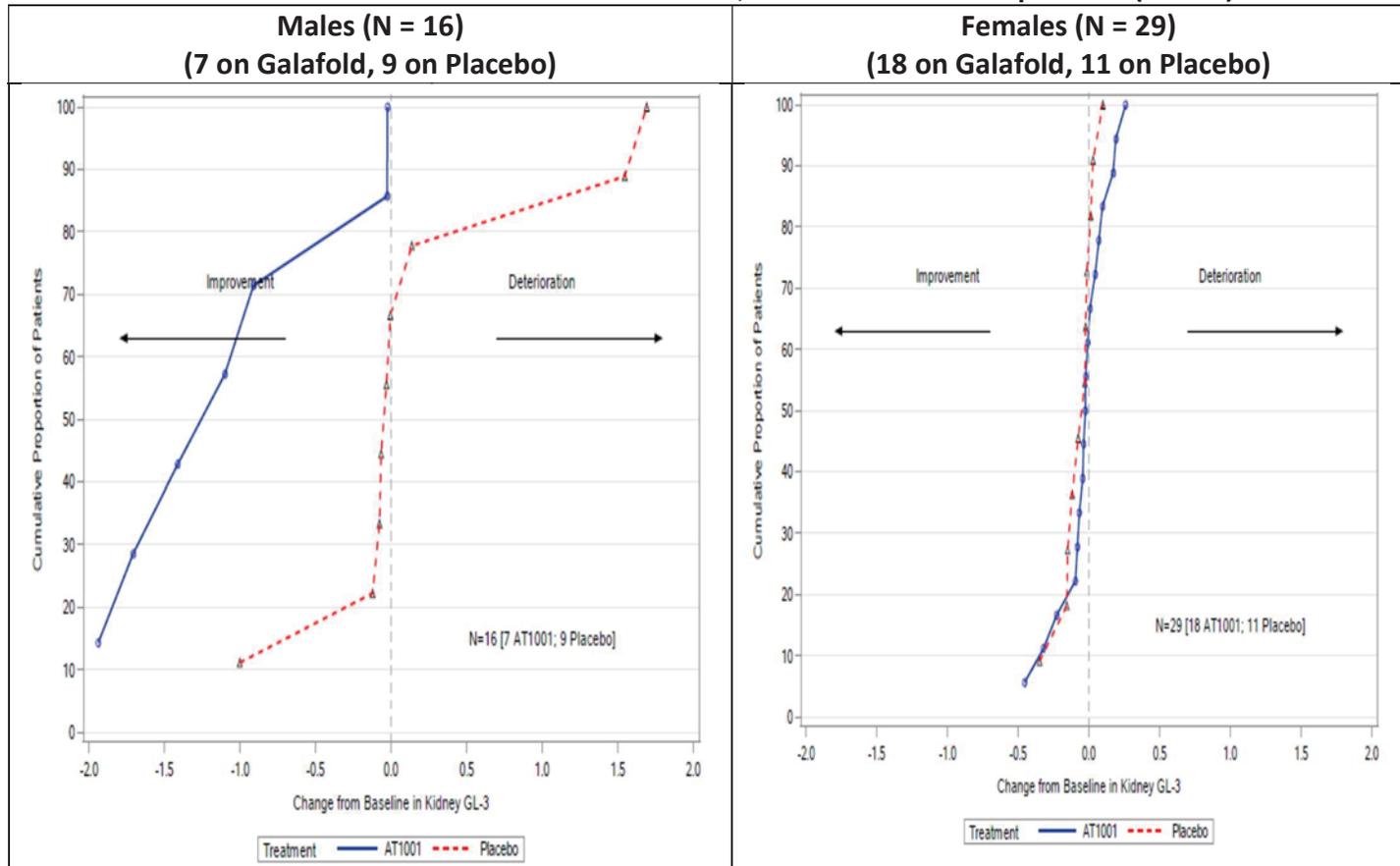
**Figure 30: Trial AT1001-011 Stage 1: Change from Baseline to Month 6 in Average Number of GL-3 Inclusions per KIC (ITT-amenable AH Population, N = 45)**



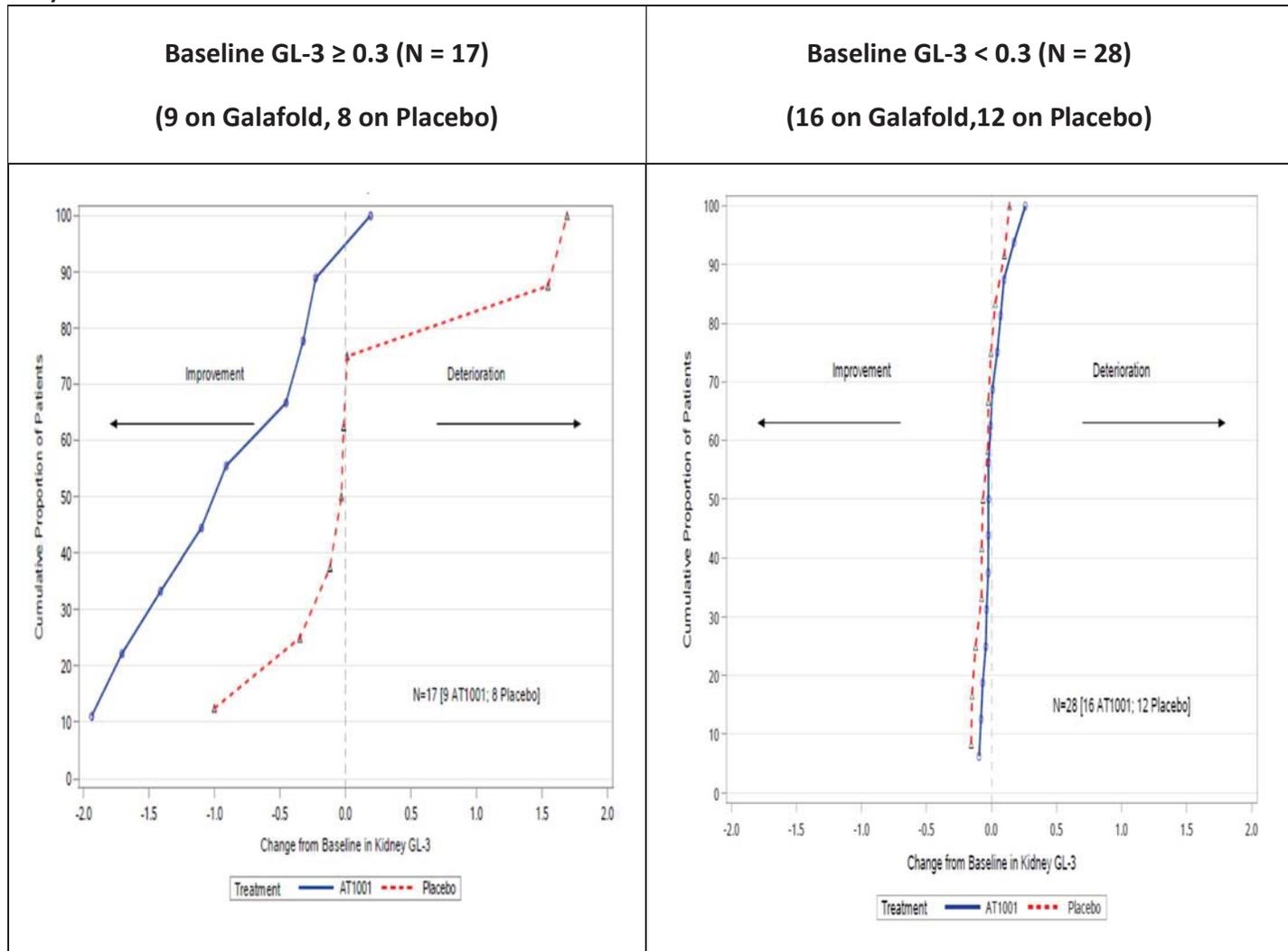
Source: Applicant's figure 2.2-1 IR-17Jul2018-KIC-Figure 2.2-1.pdf

AT1001 = Galafold, AH = available histology, GL-3 = globotriaosylceramide, ITT = intent-to-treat, KIC = kidney interstitial capillary

**Figure 31: Trial AT1001-011 Stage 1: Mean Change from Baseline to Month 6 in Average Number of GL-3 Inclusion Per KIC in Males and Females; ITT-amenable AH Population (N = 45)**



**Figure 32: Trial AT1001-011 Stage 1: Mean Change from Baseline to Month 6 in Average Number of GL-3 Inclusion Per KIC By Baseline GL-3 Inclusion Burden (ITT-amenable AH, N = 45)**

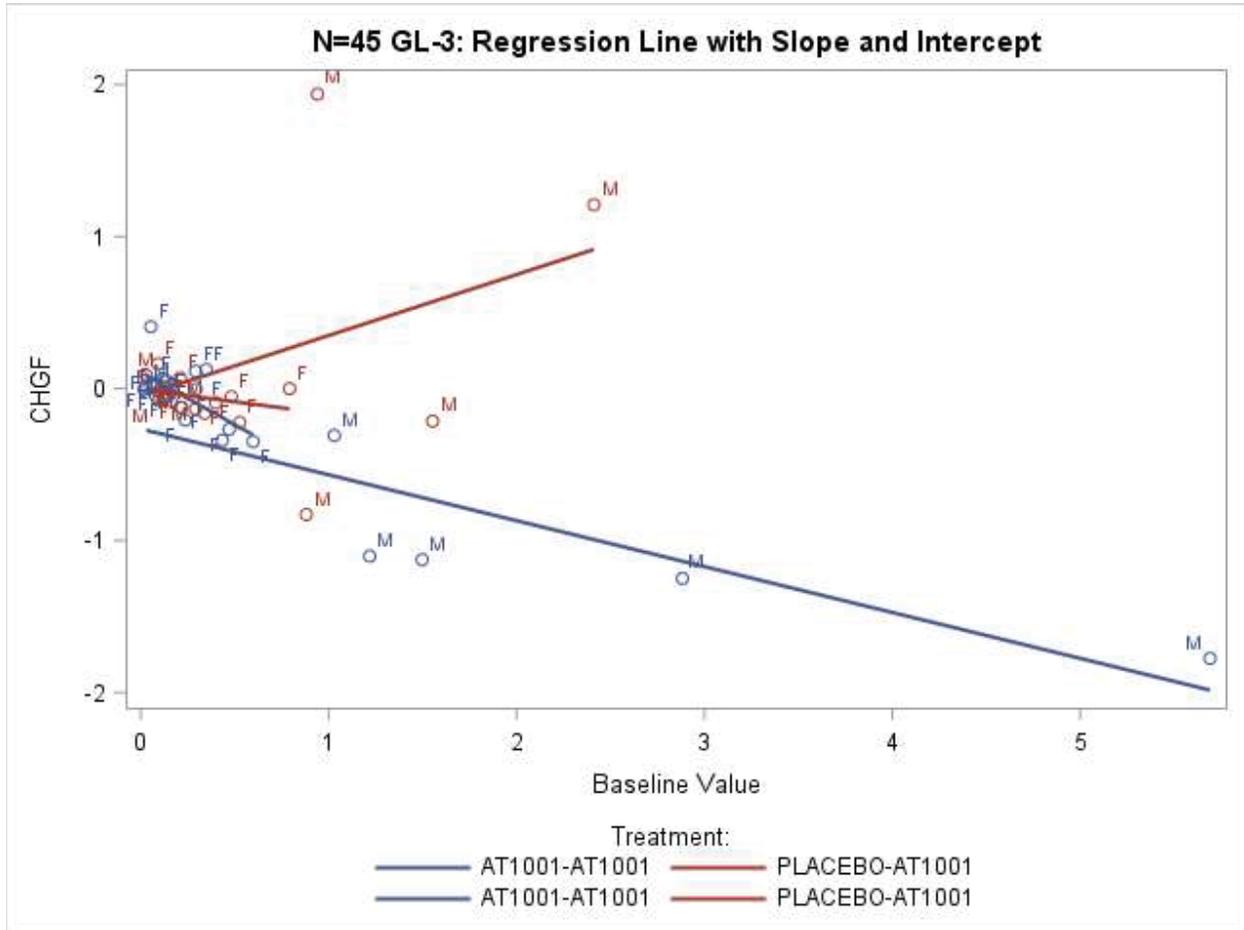


Source: the Applicant's Figures 2.2-6 of IR-17Jul2018-KIC-Figure2.2-6.pdf  
AT1001 = Galafold, AH = available histology, GL-3 = globotriaosylceramide, ITT = intent-to-treat, KIC = kidney interstitial capillary

### **Additional Analyses**

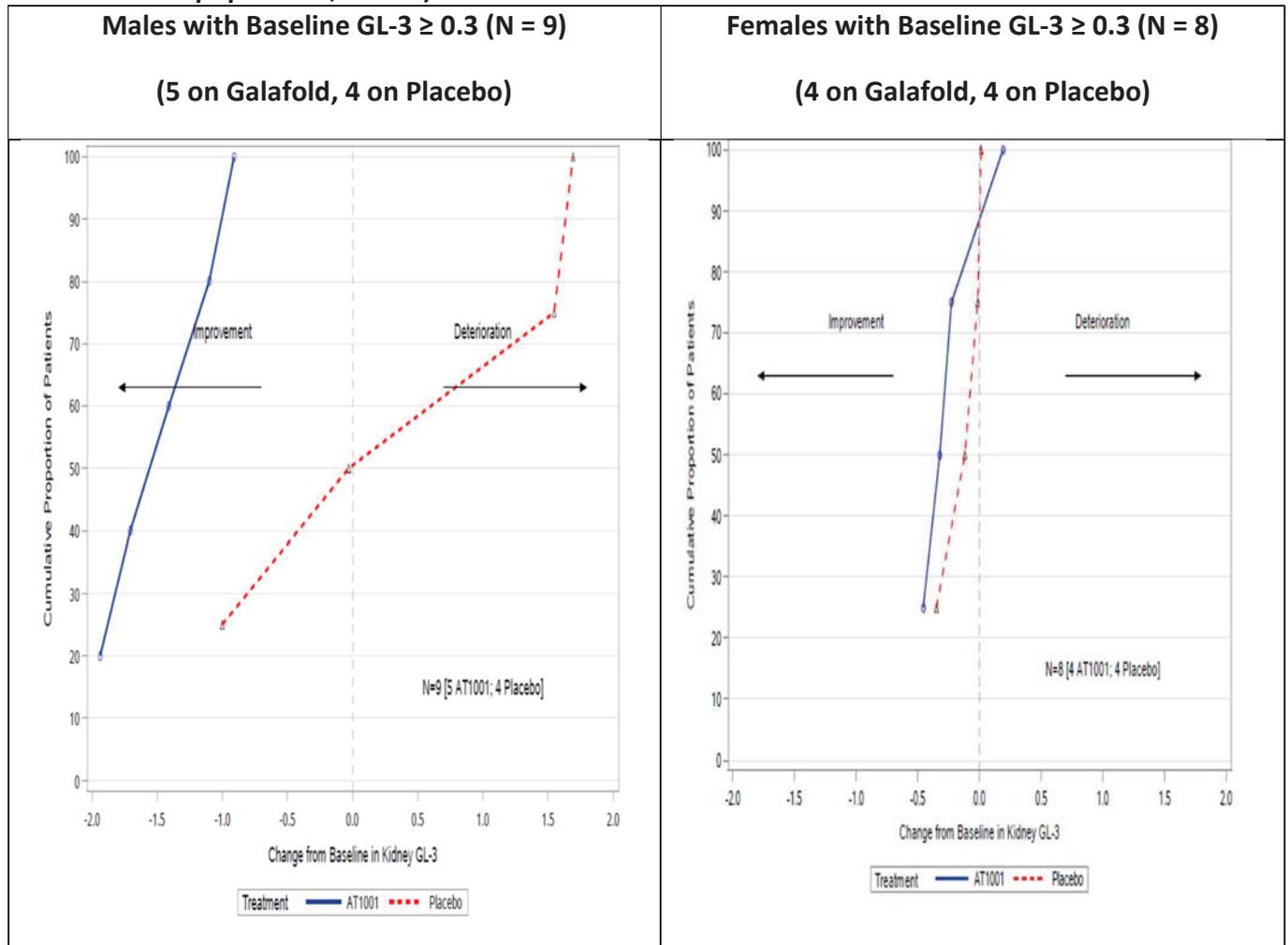
Figure 33 presents the exploration of whether there was significant interaction between sex and treatment groups. The p-value for the interaction term between sex and treatment group based on linear regression is 0.0014. These results are consistent with the results presented in Figure 34 of cumulative distribution of nine male and eight female patients with baseline GL-3  $\geq$  0.3, showing different treatment effects in male and female patients.

**Figure 33: Trial AT1001-011 Stage 1: Regression Lines for Mean Change from Baseline at Month 6 in Average Number of GL-3 Inclusion Per Kidney IC for Galafold vs. Placebo in Males and Females**



Source: Reviewer's analysis  
GL-3 = globotriaosylceramide, IC = interstitial capillary

**Figure 34: Trial AT1001-011 Stage 1: Mean Change from Baseline to Month 6 in Average Number of GL-3 Inclusion Per KIC in Males and Females With Baseline GL-3  $\geq 0.3$  per KIC (ITT-amenable AH population, N = 17)**



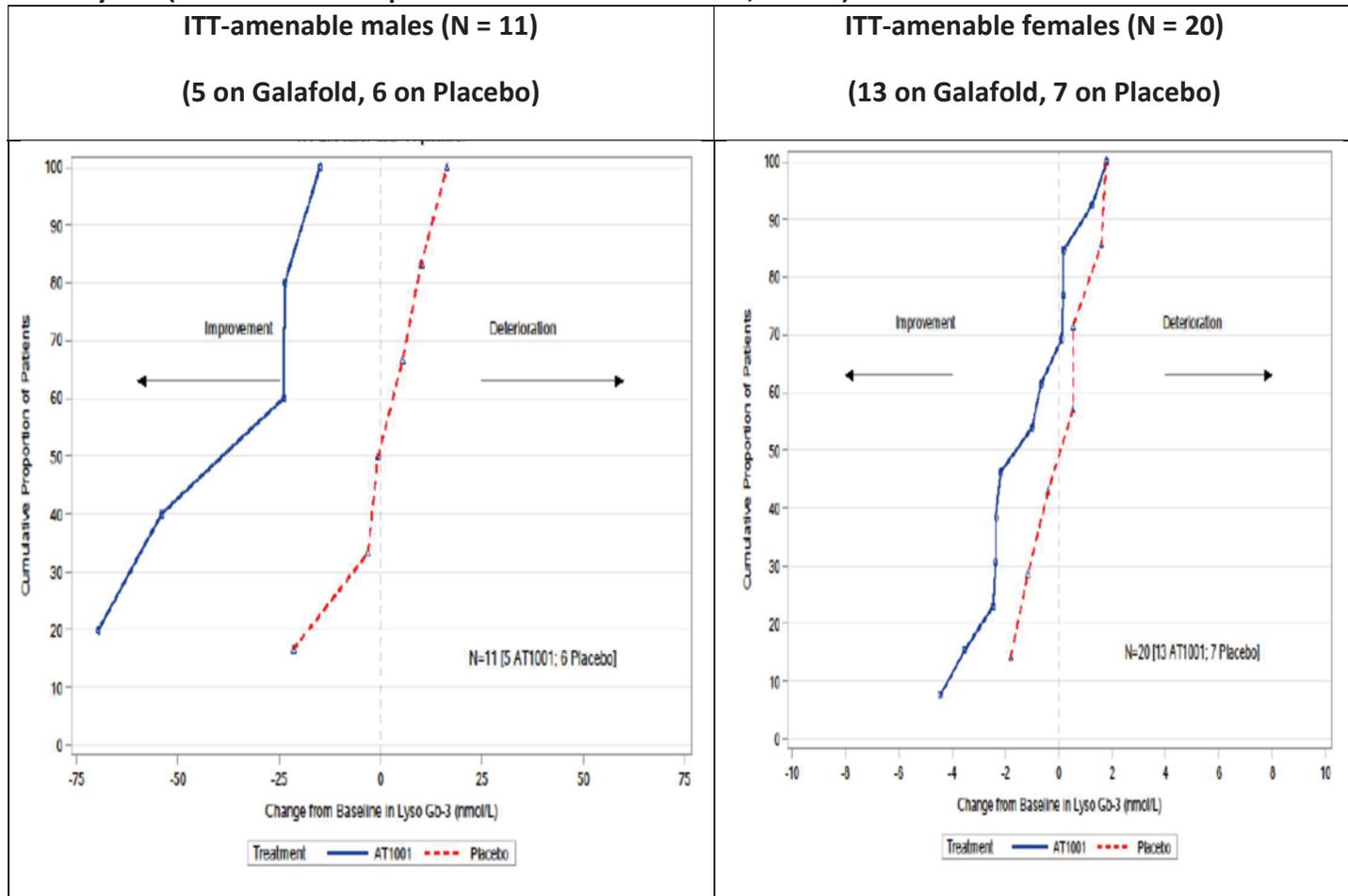
Source: Applicant's Figures 2.2-3 of IR-17Jul2018-KIC-Figure 2.2-3 and 2.2-5 of IR-17-Jul2018-KIC-Figure 2.2-5  
AH = available histology, GL-3 = globotriaosylceramide, ITT = intent-to-treat, KIC = kidney interstitial capillary

### **Plasma Lyso-Gb3**

Additional analyses were conducted for plasma lyso-Gb3, a FD-specific biomarker, thought to be indicative of overall disease severity. Notably, 31% of patients in trial AT1001-011 (14 out of 45) were missing lyso-Gb3 assessments both at baseline and at month 6 and, thus, 31 patients were included in the analysis of changes from baseline in plasma lyso-Gb3 (the majority of which were females). A more observable change in plasma lyso-Gb3 was noted in the 11 males with available data (who also started at a higher baseline lyso-Gb3 concentration than females in the trial) whereas the effect in females was minimal (as shown in Figure 35). This larger observed effect on plasma lyso-Gb3 in males is expected based on knowledge of the typically higher disease severity in affected males and the fact that males had a much higher baseline

concentration of the biomarker. Even though most patients with available data for lyso-Gb3 in this cohort were females, who showed minimal changes (partly due to starting at a low baseline), the larger observed effects with treatment in the Galafold-treated males and the demonstration of minimal changes in this biomarker in the placebo-treated males provide additional and supportive evidence for Galafold’s efficacy in this population.

**Figure 35: Trial AT1001-011 Stage 1: Mean Change from Baseline to Month 6 in Plasma Lyso-Gb3 By Sex (ITT-Amenable Population With Available Data, N = 31)**



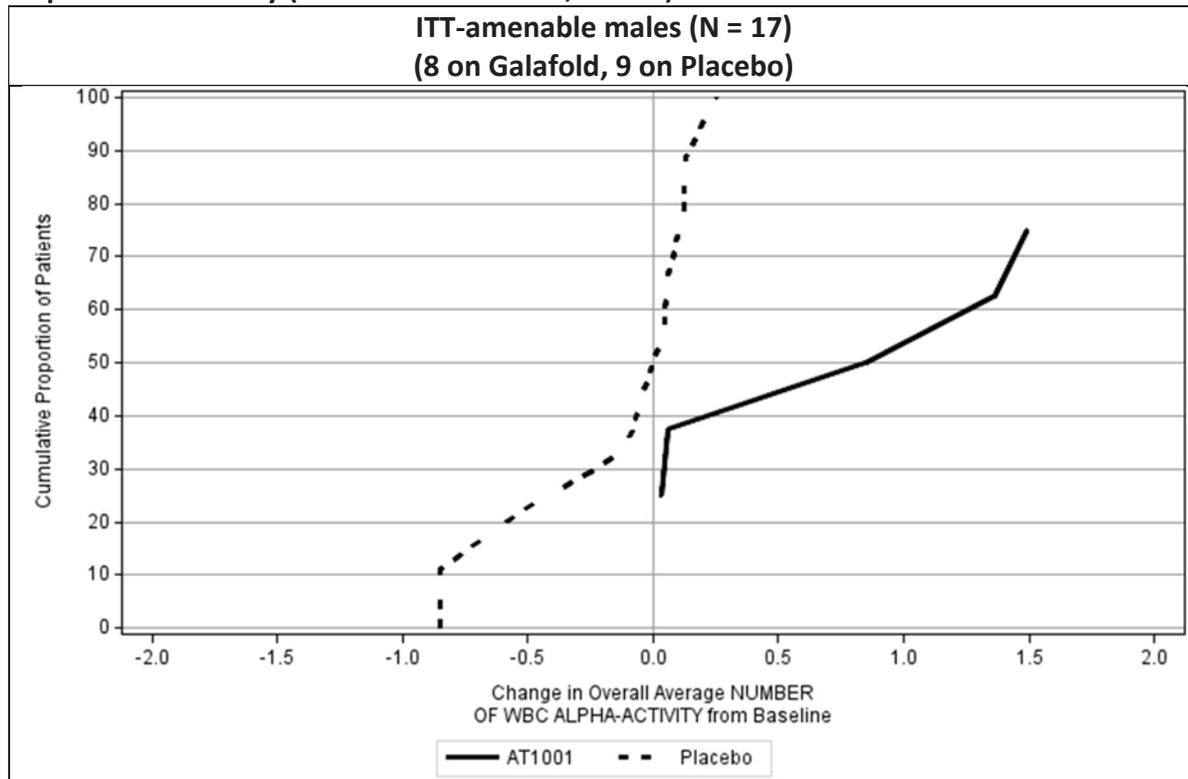
Source: Applicant’s figure 12 of clin-info-amend-20180601-q1.pdf  
ITT = intent-to-treat, lyso-Gb3 = globotriaosylsphingosine

**WBC residual alpha-Gal A activity (males)**

Figure 36 below illustrates the observed in vivo changes in alpha-Gal A activity in males with FD in trial AT1001-011. Data on eight Galafold-treated males show small increases in residual enzyme activity whereas there are also small changes in enzyme activity in the nine placebo-treated males. However, it is noteworthy that the Galafold-treated males appear to generally show a relatively greater increase in alpha-Gal A enzyme activity compared to the placebo-treated males as shown in Figure 36. Given the very limited sample size for this assessment and similar results in the two groups, clinical interpretation of these analyses is difficult. Of

note, changes in residual enzyme activity in females were not considered by the review team as alpha-Gal A enzyme values in females with Fabry disease are generally not reliable given the co-existence of WBCs with normal enzyme activity in peripheral blood (affected females with FD typically have a mixture of both normal and low alpha-Gal A activity in peripheral blood).

**Figure 36: Trial AT1001-011 Stage 1: Mean Change from Baseline to Month 6 in WBC residual alpha-Gal A activity (ITT-amenable Males, N = 17)**



Source: reviewer's analysis  
alpha-Gal A = alpha-galactosidase A, ITT = intent-to-treat, WBC = white blood cell

### **Exploratory Analyses in Males with Classic Fabry Disease**

Table 51 illustrates the changes from baseline to month 6 in the relevant disease-specific biomarkers (KIC GL-3 inclusions and plasma lyso-Gb3) in males with classic FD, defined by the review team as males having undetectable residual alpha-Gal A enzyme activity (< 1% of the normal mean) in trial AT1001-011. As shown, those males with classic FD had a higher baseline both for KIC GL-3 inclusion burden and for plasma lyso-Gb3 as compared to the ITT-amenable AH population and also compared to the whole male population in trial AT1001-011 (see Table 46). These males with "classic FD" experienced a significantly larger treatment effect in terms of both reductions in KIC GL-3 inclusion burden and plasma lyso-Gb3 compared to the whole ITT-amenable AH population (see Figure 32, Figure 34, and Figure 35). This provides additional evidence (albeit in this limited population) of the consistent pharmacodynamic effect of Galafold (when assessing multiple disease-specific biochemical outcomes) which appears to be

more pronounced in patients who have a greater baseline biochemical/histologic disease severity.

**Table 51: Trial AT1001-011 Stage 1: Median Change from Baseline to Month 6 in FD-specific Biomarkers (ITT-Amenable, Males With Classic FD\*)**

Treatment Group	n	Baseline Median (min, max)	Month 6 Median (min, max)	Change from baseline Median (min, max)
Average number of GL-3 inclusions per KIC (N = 13)				
Galafold	7	3.6 (0.2, 6.0)	2.6 (0.1, 6.0)	-0.7 (-1.7, 1.2)
Placebo	6	1.8 (0.1, 2.8)	2.0 (0.05, 4.3)	-0.04 (-0.5, 1.5)
Average plasma lyso-Gb3 (N = 4)				
Galafold	3	119.7 (81.7, 128)	104.0 (27.6, 104.7)	-24.0 (-54.1, -15.0)
Placebo	1	92.2 (92.2, 92.2)	89.0 (89.0, 89.0)	-3.2 (-3.2, -3.2)

Source: reviewer's analysis

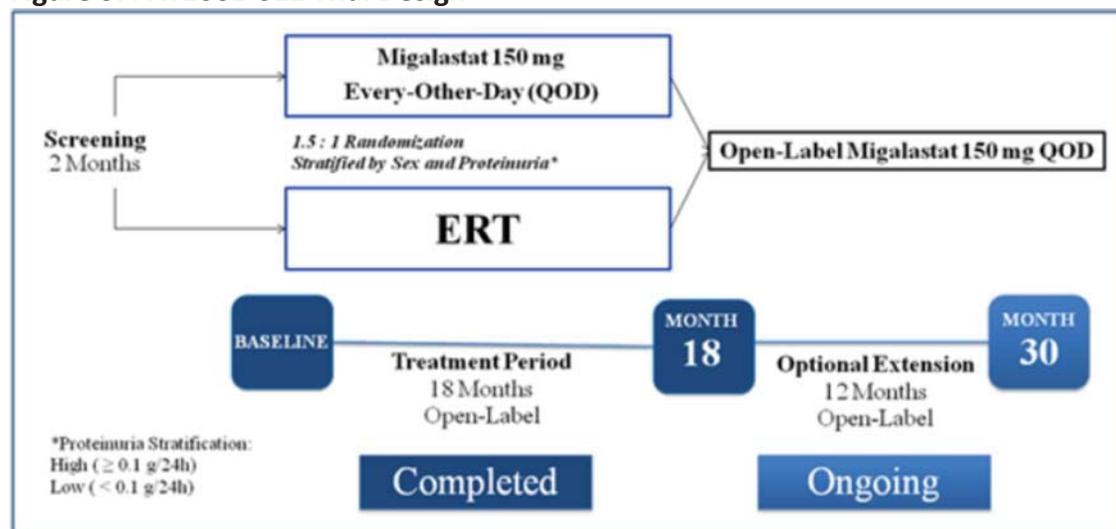
\* defined as males with residual WBC alpha-Gal A activity < 1% of normal mean.

FD = Fabry disease, GL-3 = globotriaosylceramide, ITT = intent to treat, lyso-Gb3 = globotriaosylsphingosine

### 8.1.3. Trial AT1001-012

**Title: "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."**

**Figure 37: AT1001-012 Trial Design**



Source: Applicant submission

ERT = enzyme replacement therapy, QOD = every other day

### Objectives

Compare the efficacy and safety of Galafold to enzyme replacement therapy (ERT) in patients with Fabry disease who were receiving ERT and who had "responsive" GLA variants.

### Statistical Analysis Plan:

There was no formal hypothesis testing in trial AT1001-012 and results were documented using descriptive statistics.

## 8.1.4. Trial AT1001-012 Results

### Patient Disposition

A total of 68 patients were enrolled in the study, and 60 patients with “responsive” *GLA* variants in the CT HEK assay were randomized (ITT population): 36 patients were randomized to the Galafold group, and 24 patients were randomized to the ERT group. Use of the GLP HEK assay changed the classification of four subjects with “responsive variants” (based on the CT HEK assay) to having “non-amenable” variants. As such, the final study population with amenable variants included 56 patients, 34 on Galafold and 22 on ERT.

### Demographic and Baseline Characteristics

Demographic characteristics were generally comparable between the treatment groups as shown in Table 52. Baseline renal function appears to be similar in the two groups. However, the ERT group included patients with a lower baseline residual enzyme activity who also had a higher baseline plasma lyso-Gb3 concentration. These factors both indicate a potentially more severely affected (at least from a biochemical perspective) population in the ERT group. It is unlikely that this baseline difference in disease severity affected the trial results, given that renal disease in FD progresses slowly over many decades, and no major changes in GFR would be expected over 18 months, even accounting for this mild imbalance.

**Table 52: Demographic Characteristics; Trial AT1001-012 (ITT-Amenable Population)**

Characteristic		Galafold (N = 34)	ERT (N = 22)
Age	Mean	51.18	44.68
	Min, Max	18, 70	18, 72
	≤ 65 years old	31	20
	> 65 years old	3	2
Sex, n (%)	F	19 (56)	13 (59)
	M	13 (38)	9 (41)
Race, n (%)	White	28	21
	Asian	5	1
	Multiple	1	0

Reviewer's table

ERT = enzyme replacement therapy

**Table 53: Baseline Disease Characteristics; Trial AT1001-012 (ITT-Amenable Population)**

Parameter	Galafold		ERT	
		(N = 34)		(N = 22)
<b>mGFR<sub>iohexol</sub> (ml/min/1.73m<sup>2</sup>)</b>	n	34	19	
	Mean	82.28	80.77	
	Median	81.30	74.30	
	Min, Max	52.8, 124.0	33.0, 132.2	
<b>eGFR<sub>CKD-EPI</sub>(ml/min1.73m<sup>2</sup>)</b>	n	34	19	
	Mean	88.70	94.38	
	Median	85.91	95.68	
	Min, Max	51.33, 132.47	44.83, 129.52	
<b>Proteinuria (mg/24h)</b>	n	34	19	
	Mean	259.59	395.42	
	Median	123.50	154.00	
	Min, Max	0, 2282	0, 3154	
<b>Urine GL-3 (ng/mgcreat)</b>	n	34	19	
	Mean	54.26	73.41	
	Median	25.65	25.50	
	Min, Max	6.1, 566.7	7.9, 408.6	
<b>Plasma lyso-Gb3 (nmol/L)</b>	n	32	17	
	Mean	9.06	17.65	
	Median	6.35	9.65	
	Min, Max	0.80, 59.07	0.85, 73.40	
<b>WBC alpha-Gal A (% of normal mean)- Male</b>	n	14	8	
	Mean	12.98	5.18	
	Median	8.36	1.36	
	Min, Max	0.73, 50.91	0.45, 30.50	

source: table 1.2 Applicant information response submitted July 5, 2018

alpha-Gal A = alpha-galactosidase A, CKD-EPI = Chronic Kidney Disease Epidemiology Collaboration equation, eGFR = estimated glomerular filtration rate, ERT = enzyme replacement therapy, GL-3 = globotriaosylceramide, lyso-Gb3 = globotriaosylsphingosine, WBC = white blood cell

## Efficacy Results

**Table 54: Trial AT1001-012: Annualized GFR from Baseline to Month 18 (ITT-Amenable Population, N = 52)**

Parameter	Galafold, N = 34 LS Mean ( $\pm$ SE) [95% CI]	ERT, N = 18 LS Mean ( $\pm$ SE) [95% CI]	Differences in LS Means
eGFR <sub>CKD-EPI</sub>	-0.4 (0.93) [-2.27, 1.48]	-1.0 (1.29) [-3.64, 1.58]	0.6
mGFR <sub>iohexol</sub>	-4.4 (1.64) [-7.65, -1.06]	-3.2 (2.27) [-7.81, 1.33]	-1.1

Source: Applicant's table 27 of summary-clin-efficacy.pdf

CI = confidence interval, CKD-EPI = Chronic Kidney Disease Epidemiology Collaboration equation, GFR = glomerular filtration rate, ITT = intent to treat, LS = least squares, SE = standard error

The mITT population, used for the analyses presented here for trial AT1001-012, was defined by the Applicant in the SAP as the population that “includes all randomized subjects with mutations amenable to Galafold in the GLP HEK assay that received at least one dose of study medication and have both baseline and a post-baseline efficacy measure of iohexol mGFR and a post-baseline measure of CKD-EPI eGFR.” Notably, out of the 60 enrolled patients with “responsive” *GLA* variants per the CT HEK assay, 56 patients had amenable *GLA* variants per the GLP HEK assay. For the analyses of GFR changes over 18 months of treatment with either Galafold or ERT, 52 patients had available data both at baseline and at month 18. At the end of 18 months, renal function appeared comparable between the two groups, a finding which is not unexpected given the slow renal disease progression seen in Fabry disease; this finding is also difficult to interpret in the absence of a placebo or no-treatment group. As such, the results of this trial, although considered in the general context of “totality of evidence” in this review, were not considered in the final determination of efficacy for Galafold.

In trial AT1001-012, 46 of 56 patients with amenable *GLA* variants (31 on Galafold, 15 on ERT) had lyso-Gb3 assessments available after 18 months of treatment. The median change from baseline to month 18 in plasma lyso-Gb3 (nmol/L) was 0.53 (range -2.27, 28.3) in Galafold-treated patients and -0.03 (range -11.9, 2.57) in ERT-treated patients. Overall, it appears the plasma lyso-Gb3 remained unchanged in both the Galafold and the ERT group over the 18 months of treatment, a finding that is difficult to clinically interpret in the context of the product's efficacy.

### 8.1.5. Assessment of Efficacy Across Trials

The efficacy assessment of Galafold was based primarily on AT1001-011 stage 1 efficacy data since only this 6-month period is placebo-controlled and double-blinded. In addition, there was no hypothesis testing in trial AT1001-012 and, as such, the efficacy results had limited contribution to the final determination of efficacy of Galafold.

### 8.1.6. Integrated Assessment of Effectiveness

The placebo-controlled phase (stage 1) of trial AT1001-011 provided the main source of evidence in support of the Applicant's claim of effectiveness for Galafold which was based on the reduction in histological disease burden in patients with FD and amenable *GLA* variants. Stage 1 of trial AT1001-011 was the only randomized, double-blind, placebo-controlled assessment in the whole Galafold clinical program. Data on the disease-specific biomarker, plasma lyso-Gb3, in a limited number of patients in the phase 3 trials provided additional supportive evidence of the drug's effectiveness. Lastly, in vivo increases in residual alpha-Gal A enzyme activity, observed in some treated males, was used as additional evidence of the drug's pharmacodynamic effect.

Interpretation of the efficacy data generated in the Galafold program presented significant challenges to the review team for multiple reasons. First, the in vitro amenability assay (CT HEK-293 assay), which was used to select patients with amenable *GLA* variants for trial enrollment, underwent GLP validation during the course of the phase 3 trial, which resulted in the reclassification of the final efficacy analysis population post randomization. Additional efficacy analyses of the stage 1 trial data (based on the redefined ITT-amenable population) occurred after unblinding of the stage 1 data. However, the biochemical criteria used for categorization of *GLA* variants into amenable and non-amenable remained unchanged between the CT HEK-293 assay (used for trial enrollment) and the GLP HEK-293 assay (used for the final efficacy analyses); in addition, the assay GLP validation was completed prior to stage 1 data unblinding. In view of this substantial change in the critical element for patient selection, concerns were raised by the review team throughout the clinical development program and during the NDA review regarding the validity and reliability of the final efficacy analyses in the ITT-amenable population. Taking all the limitations of this approach and the observed results across all biomarkers into account, the review team considers it to be highly unlikely that the observed results in the ITT-amenable population were significantly biased by the timing of the stage 1 data unblinding or by the methods used for the HEK-293 in vitro assay validation. All in all, the combination of the observed histological and biomarker effects after Galafold treatment, albeit heterogeneous and small in a heterogeneous and small enrolled patient population, especially when assessed in the most informative patient populations (i.e. males and patients with high baseline histological disease burden) provides evidence of a reasonably likely treatment benefit in this patient population.

Secondly, and no less importantly, the clinical trial did not use a quantitative inclusion criterion for the primary efficacy variable (histological GL-3 inclusion number) as patients were enrolled based on a baseline threshold of urinary GL-3 (the clinical significance of which remains unknown in Fabry disease) while the primary efficacy analysis measured renal interstitial tissue GL-3 inclusions in renal biopsy. Consequently, the trial randomized patients with a GL-3 KIC inclusion burden that was variable and mostly close to the limit of histological detection (defined as 0.3 GL3-inclusions per KIC based on the BLISS histological assay). In fact, the majority of patients in trial AT1001-011 had a baseline GL-3 inclusion burden < 0.3 per KIC indicating minimal baseline histological disease. There was a relatively small subgroup of patients with baseline KIC GL-3 inclusion burden over 0.3 GL-3 inclusions per KIC. In this

subgroup, Galafold reduced the average number of GL-3 inclusions per KIC by -0.91 after 6 months while the reduction in the placebo group was much smaller at -0.02. Treated males, who started with higher baseline KIC GL-3 inclusion burden than females, showed the greatest reduction in GL-3 inclusions.

Furthermore, trial AT1001-011 enrolled predominantly females with FD, who generally exhibit milder disease burden and follow a variable disease course as compared to males. Based on the known clinical heterogeneity in affected females with FD, it is conceivable that the females may also have a heterogeneous or slower treatment response accounting for such factors as the variably affected tissues at baseline, variable X chromosome inactivation in the tissues of interest such as the kidneys, and a slower overall disease progression. This significant trial limitation makes the finding of a non-statistically significant result for the primary efficacy outcome not entirely unexpected.

Results from Trial AT1001-012 were not part of the statistical assessment of efficacy as there was no formal hypothesis testing and the trial did not include a placebo group to allow for reliable comparisons against the disease natural progression. However, the descriptive analyses of renal function were taken into consideration in the overall conclusions of efficacy based on the finding that renal function did not deteriorate over 18 months of treatment with Galafold in patients who switched from Fabrazyme to Galafold.

## 8.2. Review of Safety

### 8.2.1. Safety Review Approach

The safety review approach focuses on the population of patients with Fabry disease, in particular the patients in the double blind, randomized, controlled trial AT1001-011. Trial AT1001-012, an open label trial with a comparison to ERT was also reviewed. The Applicant proposed safety population included all patients who received at least one dose of Galafold 123 mg in the completed trials and this population was also assessed. This review was based primarily on this reviewer's independent analysis of the data sets provided by the Applicant, and secondarily on the Applicant's trial reports. The tables and analyses presented in this report reflect the independent analysis of the reviewer except where otherwise noted. Narratives of deaths, serious adverse events, and adverse drop-outs were individually reviewed.

### 8.2.2. Review of the Safety Database

#### **Overall exposure**

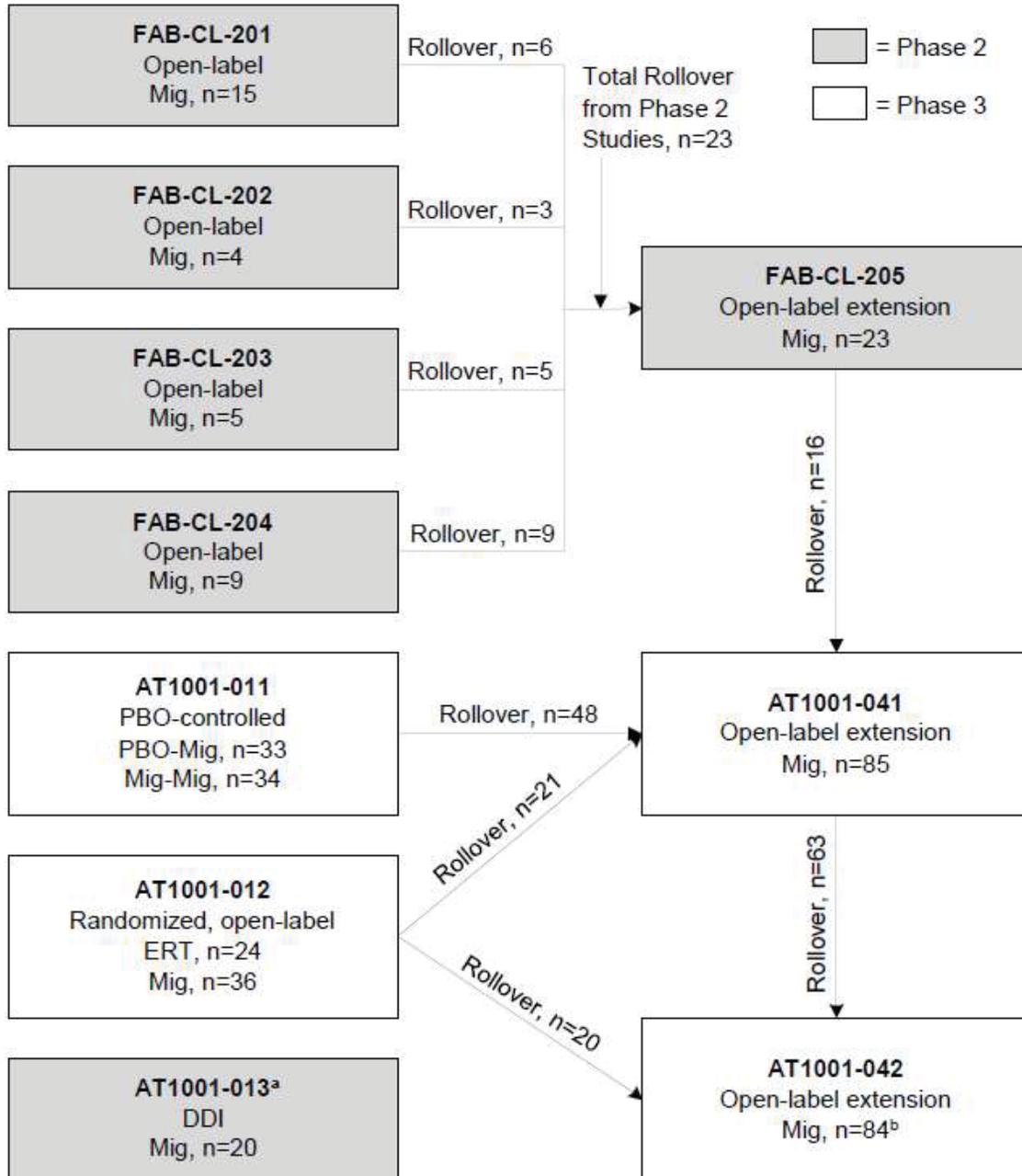
A total of 386 patients have been exposed to any dose of Galafold, including 194 healthy adults, 24 patients with renal impairment in phase 1 trials, and 168 patients with Fabry disease in phase 2 and phase 3 trials, of which a total of 160 patients received Galafold monotherapy. The total mean duration of exposure to Galafold 150mg QOD was 3.71 years. A total on 139 patients received Galafold 150mg QOD for 6 months and 132 patients received Galafold 150mg QOD for 18 months.

The Applicant proposed multiple pooling strategies for the assessment of safety. The first pool focused on the first 6 months of exposure to Galafold 150mg QOD in all trials in comparison to placebo. The next pool focused on the first 18 month of exposure to Galafold 150mg QOD in comparison to ERT. The third pool focused on total exposure of any patient that received at least one dose of 150mg Galafold. All three pools included phase 2 and phase 3 trials with open label extension trials.

The presentation of safety will focus on the first 6 months of exposure to Galafold compared to placebo in trial AT1001-011. Any serious adverse events (SAE), discontinuations due to AEs, and deaths reported in trials AT1001-011, AT1001-012, AT1001-041 and AT1001-042 will also be discussed.

Figure 38 provides the number of patients exposed to Galafold throughout the program.

**Figure 38: Safety Population**



Abbreviations: DDI=drug-drug interaction; ERT=enzyme replacement therapy; Mig=migalastat; PBO=placebo; SCS=Summary of Clinical Safety

<sup>a</sup> Of the 20 subjects who received migalastat in Study AT1001-013, 12 received migalastat monotherapy and are included in the SCS.

<sup>b</sup> One patient from expanded access Study AT1001-188 entered Study AT1001-042.

Source: Applicant's submission, review of clinical safety (30/138)

**Table 55: Drug Exposure in Phase 3 Trials**

Parameter	Individual Phase 3 Studies		Total Phase 3 Studies (N=115)
	Study 011 (011+041+042) (N=64)	Study 012 (012+041+042) (N=51)	
<b>Duration of Exposure (Years)<sup>a</sup></b>			
Mean (SD)	3.72 (1.970)	3.42 (1.408)	3.59 (1.742)
Median	4.23	4.0	4.14
Q1, Q3	1.98, 5.53	2.45, 4.59	2.07, 4.89
Min, Max	0.1, 6.8	0.1, 5.2	0.1, 6.8
<b>Duration of Exposure (Months)<sup>a</sup></b>			
Mean (SD)	44.65 (23.665)	41.15 (16.922)	43.10 (20.932)
Median	50.82	48.03	49.74
Q1, Q3	23.78, 66.40	29.41, 55.13	24.84, 58.78
Min, Max	1.2, 81.7	1.1, 62.6	1.1, 81.7
<b>Duration of Exposure (Categorical)</b>			
<3 months	1 (1.6%)	1 (2.0%)	2 (1.7%)
3 to <6 months	1 (1.6%)	1 (2.0%)	2 (1.7%)
6 to <12 months	4 (6.3%)	3 (5.9%)	7 (6.1%)
12 to <18 months	6 (9.4%)	2 (3.9%)	8 (7.0%)
18 to <24 months	5 (7.8%)	1 (2.0%)	6 (5.2%)
24 to <36 months	11 (17.2%)	9 (17.6%)	20 (17.4%)
36 to <48 months	2 (3.1%)	8 (15.7%)	10 (8.7%)
48 to <60 months	9 (14.1%)	24 (47.1%)	33 (28.7%)
60 to <72 months	18 (28.1%)	2 (3.9%)	20 (17.4%)
72 to <84 months	7 (10.9%)	0	7 (6.1%)
≥84 months	0	0	0

Source: adapted from Applicant summary of clinical safety (p40-41/138)  
Q1 = first quartile, Q3 = third quartile, SD = standard deviation

**Table 56: Safety Populations**

Populations	Trial	Galafold 150mg	Placebo	ERT	Total
AT1001-011, primary safety population	Phase 3	34	33	0	67
AT1001-012, secondary safety population	Phase 3	36	0	21	47
FAB-205, secondary safety population	Phase 2	23	0	0	23

Source: reviewer's table  
ERT = enzyme replacement therapy

The Applicant's pooled safety analysis included trials with different populations of patients including those who were ERT-naïve and ERT-experienced. This included patients who were off ERT prior to Galafold dosing, patients who did not have a washout period when switching to Galafold from ERT and patients who had received Galafold in the open label extension trials with no comparator. The ability to draw conclusions from comparisons to the ERT group is also limited because the trial was open-label and the investigators understood the safety profile of ERT and may have possibly underreported certain AEs that are known to be related to ERT. The pooled analysis also included duplicate patients who were identified as having participated in one or more trial sites or the extension trials. Therefore, focusing on the only blinded trial (AT1001-011) instead of pooling all trials will allow for a more accurate interpretation of primary safety for Galafold.

#### **Adequacy of the safety database**

In general, the submitted safety database was adequate in terms of duration of exposure and numbers of patients exposed to the to-be-marketed dose to allow for a thorough assessment of safety. The representation of different races in the trials may not represent the whole U.S. population as no African Americans or Asian Americans were enrolled in the trials. Regarding baseline characteristics, renal function was similar in both groups. Albuminuria and proteinuria are notable to be higher in the placebo group. ACE inhibitors were also more common in the placebo group. The higher prevalence of ACE inhibitors used in the placebo group may be associated with the increased proteinuria also seen in the placebo group. This may obscure potential safety signals of worsening renal function.

**Table 57: Baseline Characteristics, Safety Population, Trial AT1001-011**

Parameter		Galafold (N = 34)	Placebo (N = 33)
Age (years) mean ± SD		40 ± 13.3	44 ± 10.2
Sex, n (%)	Male	12 (35)	12 (36)
	Female	22 (65)	21 (64)
Ethnicity-U.S. sites only, n (%)	Hispanic or Latino	1 (10)	1 (10)
	Not Hispanic or Latino	9 (90)	9 (90)
Race, n (%)	White	32 (94)	33 (100)
	other	2 (6)	0
	Black	0	0
	Asian	0	0
mGFR <sub>iohexol</sub> (ml/min/1.732), mean ± SD		83 ± 30.3	86 ± 24.5
eGFR <sub>CKD-EPI</sub> (ml/min/1.732), mean ± SD		95 ± 28.5	94 ± 20.6
Urine protein: creatinine ratio (mg/mmol), mean ± SD		32 ± 44.3	41 ± 55.0
Urine albumin: creatinine ratio (mg/mmol), mean ± SD		19 ± 36.4	27 ± 47.3
Urine GL-3 (ng/mg creat), mean ± SD		875 ± 1165.4	1055 ± 1442.6
ACE inhibitor use, n (%)	Y	6 (18)	13 (39)
	N	28 (82)	20 (61)

Reviewer's table

ACE = angiotensin converting enzyme, CKD-EPI = Chronic Kidney Disease Epidemiology Collaboration equation, GFR = glomerular filtration rate, GL-3 = globotriaosylceramide, SD = standard deviation

### 8.2.3. Adequacy of Applicant's Clinical Safety Assessments

#### Issues Regarding Data Integrity and Submission Quality

The submitted safety datasets were sufficiently complete to allow for the review of safety. In addition, no data integrity concerns were reported after site inspection was completed as described in section 4.1.

#### Categorization of Adverse Events

The Applicant's process for recording, coding, and categorizing AEs met established standards. The Applicant provided accurate definitions of adverse events and serious adverse events in the protocols.

An Adverse event (AE) was defined as any untoward medical occurrence in a patient administered the trial drug, whether or not it had a causal relationship with the trial drug. A serious adverse event (SAE) was defined as any AE that resulted in death, was life threatening, required inpatient hospitalization, resulted in persistent or significant disability/incapacity, was a congenital anomaly/birth defect, or was an important treatment emergent medical condition considered serious by the investigator. From the time the patient provided written informed consent, AEs were recorded on appropriate page of the electronic case report form. Treatment

emergent adverse events (TEAE) were defined as occurring on or after the first dose of the drug and up to 30 days after the last dose of the drug or drug discontinuation.

Adverse event verbatim text was coded and classified by system organ class (body system) and preferred term (PT) using the MedDRA dictionary (version 16.1).

The Applicant used the following guidelines to grade the intensity of each reported AE:

- Mild: awareness of sign, symptom or event, but the AE was easily tolerated and did not interfere with daily activity
- Moderate: discomfort enough to cause interference with usual activity and could warrant intervention, but the patient was still able to function
- Severe: incapacitating with inability to do usual activities or significantly affected clinical status, and required medical intervention

To count the number of patients who experienced a TEAE, a patient experiencing the same TEAE multiple times was only counted once for the corresponding preferred term. If a patient experienced more than one TEAE of different intensity or relationship to the drug within the same system organ class/preferred term, only the worst case was reported.

### **Routine Clinical Tests**

Descriptive statistics for hematology, serum chemistry, and urinalysis were presented by treatment group and available visit. Change from baseline (visit 1) to post dose visit was also summarized. The physical examination included observation of general appearance, skin, neck (including thyroid), HEENT, abdomen, extremities, lymph nodes, lungs, and heart, as well as a brief neurological assessment. Any clinically significant abnormal observation was recorded in the case report form and was reported as an AE. Systolic and diastolic blood pressure measurement were obtained using the same arm, and respiratory rate and heart rate values were assessed. The measurements were taken with the patient in sitting or supine position after having rested for a 5-minute period. The same position was used at all visits. A standard 12-lead ECG was performed and read locally. Significant findings not present prior to start of treatment, which met the definition of an AE, were recorded in the case report form.

### **8.2.4. Safety Results**

#### **Deaths**

There were two deaths, both of which occurred in the open-label extension trial AT1001-041:

1. The first patient was a 63-year-old male with FD and history of obesity, obstructive sleep apnea, type 2 diabetes mellitus, hypercholesterolemia, cardiac stent placement, triple cardiac bypass surgery, cardiac pacemaker insertion, and Wolff-Parkinson-White syndrome. He was found dead at his home on day 21 of trial AT1001-041 (day 561 of open-label Galafold treatment) and it was reported that he had been dead for approximately 6 days at the time of discovery. The death certificate included coronary

artery disease and diabetes mellitus as the causes of death. The exact cause of death is unknown and an autopsy was not performed.

2. The second patient was a 64-year-old female with FD and history of endometriosis, hysterectomy, mucoid cysts in breast tissue, breast fibroadenoma, and right posterior fossa meningioma. She died on day 303 of trial AT100-041 (day 850 of open-label Galafold treatment). She had undergone a total mastectomy and Level III axillary lymph node clearance and subsequently experienced an SAE of liver infarction, which was ongoing at the time of the patient's death. The patient died 3 months after the diagnosis of metastatic breast cancer.

Both patients had multiple co-morbidities that likely contributed to their death. The available data cannot convincingly establish a link between Galafold and these events. However, it does not appear likely that Galafold was the cause of death in these cases.

### Serious Adverse Events

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Serious adverse events were evaluated during the blinded portion of trial AT1001-011 with comparator to placebo. There were more SAEs documented with placebo than with Galafold. See table below.

**Table 58: Serious Adverse Events, Safety Population First 6 Months AT1001-011**

Serious Adverse Event	Placebo (N = 33) n (%)	Galafold, (N = 34) n (%)
Anaplastic large cell lymphoma t- and null-cell types	1 (3.03)	0
Bacterial infection	1 (3.03)	0
Hydronephrosis	0	1 (2.94)
Meningitis viral	1 (3.03)	0
Postprocedural hematoma	0	1 (2.94)
Postprocedural hemorrhage	1 (3.03)	0
<b>Total</b>	<b>4 (12.12)</b>	<b>2 (5.88)</b>

Reviewer's table

The incidence of SAEs in trial AT1001-012 was similar between the Galafold and the ERT group as shown below.

**Table 59: AT1001-012: Serious Adverse Events Over 18 Months**

Serious Adverse Event	Galafold, (N = 36)	ERT, (N = 21)
	n (%)	n (%)
Chest pain	3 (8.33)	0
Obesity	2 (5.56)	0
Ventricular tachycardia	1 (2.78)	0
Bile duct stone	1 (2.78)	0
Pneumonia	1 (2.78)	0
Upper limb fracture	1 (2.78)	0
Phaeochromocytoma	1 (2.78)	0
Device malfunction	0	1 (4.76)
Atrial fibrillation	0	1 (4.76)
Cardiac failure chronic	0	1 (4.76)
Abdominal pain	0	1 (4.76)
Hernial eventration	0	1 (4.76)
Hypoaesthesia	0	1 (4.76)
Transient ischemic attack	0	1 (4.76)
Vertigo	0	1 (4.76)
Vision blurred	0	1 (4.76)
Dyspnea	0	1 (4.76)
<b>Total</b>	<b>10 (27.28)</b>	<b>10 (47.62)</b>

Reviewer's table

In trial AT1001-012, in the Galafold group, notable SAEs included three patients with chest pain who had pre-existing cardiac disease:

1. The first patient was a 50-year-old female with FD and history of chest pain, and cardiomyopathy with previous ICD placement. As part of her evaluation for chest pain, she had normal troponin levels and normal coronary arteries on angiogram.
2. The second patient was a 55-year-old female with FD and history of left ventricular hypertrophy and chronic chest pain. As part of her chest pain evaluation, she was found to have mild cardiac disease which was “not flow limiting” on cardiac angiogram.
3. The third patient was a 65-year-old male with FD and history of left ventricular hypertrophy who developed ventricular tachycardia and chest pain. Patient had an internal defibrillator placed.

Serious adverse events in all patients who received at least one dose of Galafold are shown below. This population includes patients dosed in the phase 2 trial FAB-CL-205, stage 2 of trial AT1001-011, trial AT1001-012 and the open label extension trials AT1001-041 and AT1001-042. Of note, there is no comparator in the above trials and, thus, safety results are reported only in Galafold-treated patients. The median duration of exposure in this population was 49.9 months with a maximum duration of 99.2 months.

**Table 60: Serious Adverse Events in Any Patient Who Received at Least One Dose of Galafold in Phase 2 and 3 Trials)**

<b>Serious Adverse Event</b>	<b>Galafold N = 139 n (%)</b>
Atrial fibrillation	5 (3.6)
Cerebrovascular accident	5 (3.6)
Pneumonia	4 (2.88)
Abdominal pain	3 (2.16)
Chest pain	3 (2.16)
Ventricular tachycardia	2 (1.44)
Lipomatosis	2 (1.44)
Obesity	2 (1.44)
Musculoskeletal chest pain	2 (1.44)
Headache	2 (1.44)
Dyspnea	2 (1.44)
Pneumothorax	2 (1.44)
Pulmonary embolism	2 (1.44)
Implantable defibrillator insertion	2 (1.44)

Reviewer's table

All patients who developed atrial fibrillation had a history of cardiomyopathy or arrhythmia. Patients who had a cerebrovascular accident also had a history of left ventricular hypertrophy or previous cerebrovascular accident/transient ischemic attack. The cardiovascular and cerebrovascular events noted above as SAEs can be part of FD. As such, and in the absence of a control group for comparisons of the incidence, causality cannot be determined. However, overall it appears that the observed SAEs are either part of the underlying disease or generally common in the general population (e.g. pneumonia, abdominal pain). Based on the team's review of the SAEs, no serious safety concerns are raised as associated with Galafold treatment.

### **Dropouts and/or Discontinuations Due to Adverse Events**

In trial AT1001-011, no patients discontinued treatment during stage 1, the randomized placebo controlled stage. In stage 2, two patients discontinued treatment. One patient that was initially randomized to Galafold discontinued due to diagnosis of amyotrophic lateral sclerosis. The other patient discontinued due to anaplastic large cell lymphoma which had begun while patient was receiving placebo in stage 1.

The table below shows all discontinuations due to an adverse event in phase 2, phase 3 and open extension trials.

**Table 61: Total Discontinuations Due to AEs (all treated patients)**

Adverse Event	Clinical Trial	Galafold (n = 139), n (%)
Amyotrophic lateral sclerosis	AT1001-011	1 (0.72%)
Anaplastic large cell lymphoma	AT1001-011	1 (0.72%)
Cerebrovascular accident	FAB-CL-205	1 (0.72%)
Death	AT1001-041	2 (1.44%)
Glomerular filtration rate decreased	AT001-042	1 (0.72%)
Metastatic squamous cell carcinoma	AT1001-041	1 (0.72%)
Proteinuria	AT1001-042	1 (0.72%)
Ventricular fibrillation	FAB-CL-205	1 (0.72%)
<b>Total</b>		<b>9 (6.47%)</b>

Reviewer table

AE = adverse event

### **Treatment Emergent Adverse Events**

Headache was the most common AE reported during treatment with Galafold and was seen with a higher incidence in Galafold-treated patients than placebo-treated patients over the first 6 months of treatment. There were no deaths or trial discontinuations due to an AE during stage 1 of trial AT1001-011.

**Table 62: Most Frequent TEAEs (> 5% in Either Group): Trial AT1001-011 Stage 1 (6 Months)**

Adverse event	Galafold n = 34 n (%)	Placebo n = 33 n (%)
Headache	12 (35.29)	7 (21.21)
Nasopharyngitis	6 (17.65)	2 (6.06)
Abdominal pain	6 (17.65)	4 (12.12)
Urinary tract infection*	5 (14.71)	0
Nausea	4 (11.76)	2 (6.06)
Pyrexia	4 (11.76)	1 (3.03)
Diarrhea	3 (8.82)	1 (3.03)
Myalgia	3 (8.82)	1 (3.03)
Back pain	3 (8.82)	0
Oropharyngeal pain	3 (8.82)	2 (6.06)
Epistaxis	3 (8.82)	1 (3.03)
Cough	3 (8.82)	0
Hematuria	3 (8.82)	0
Insomnia	3 (8.82)	2 (6.06)
Dizziness	2 (5.88)	1 (3.03)
Hypoaesthesia	2 (5.88)	0
Asthenia	2 (5.88)	1 (3.03)
Chest pain	2 (5.88)	1 (3.03)
Weight increased	2 (5.88)	1 (3.03)
Torticollis	2 (5.88)	0
Atrial fibrillation	2 (5.88)	0

Reviewer's table

Includes: urinary tract infection, kidney infection and cystitis

TEAE = treatment emergent adverse event

There was a higher incidence of urinary tract infections seen in the Galafold group as compared to the placebo group during the first 6 months of treatment. However, there were no SAEs related to urinary tract infection (UTI). No further information is provided regarding how the UTIs were diagnosed (symptoms, urinalysis, urine culture, or a combination). There is also no information on how UTIs were treated. Of the patients diagnosed with UTI, most were female and two were males over 60 years old.

### **Laboratory Findings**

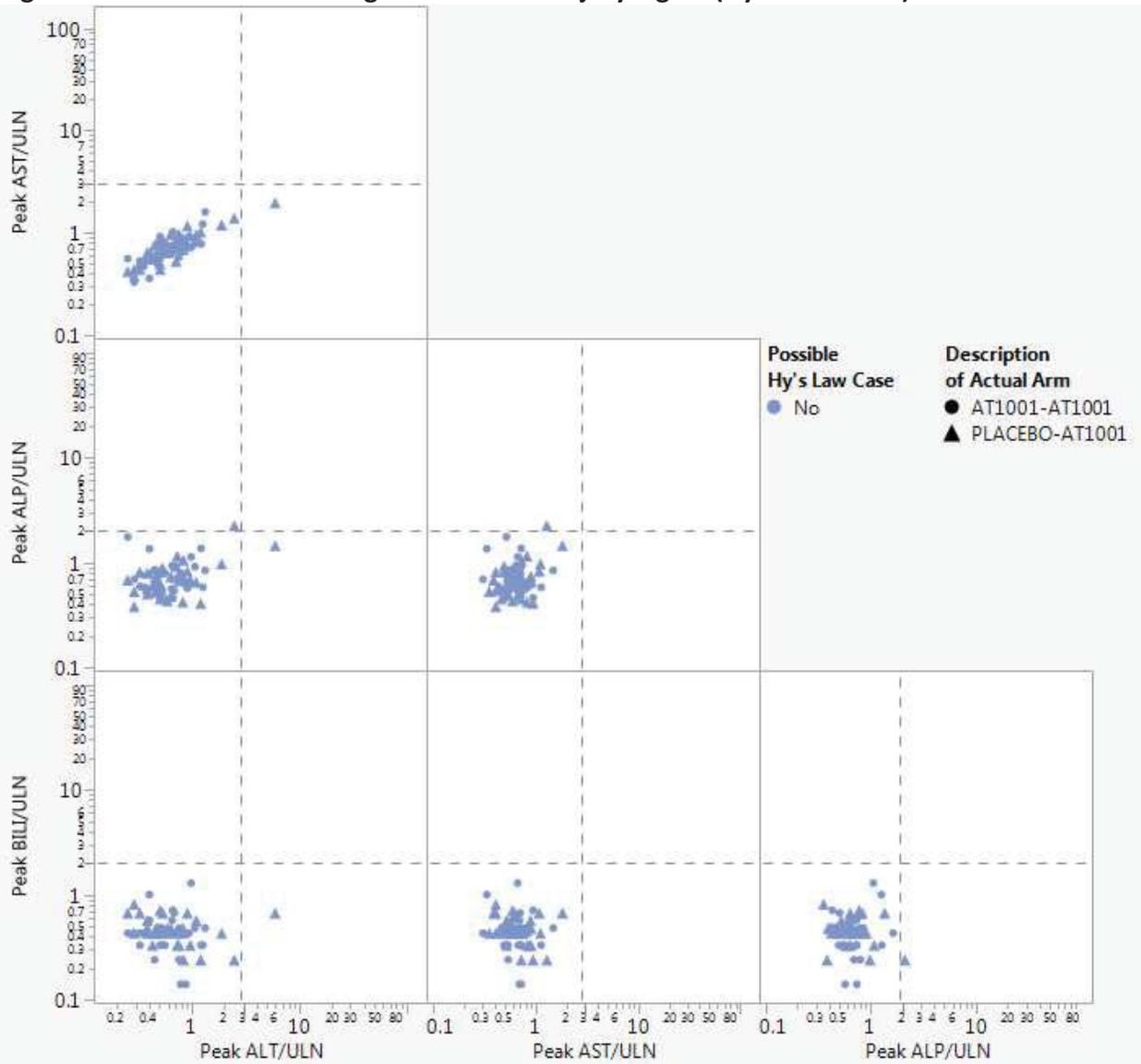
Laboratory tests were reviewed from the placebo controlled trial AT1001-011 and from the active control trial AT1001-012.

### **Chemistry Labs**

Mean and median values and change from baseline for each chemistry lab was similar in the Galafold group compared to placebo in trial AT1001-011 and was similar in the Galafold group

compared to ERT in trial AT1001-012. No TEAEs associated with clinical chemistry test results were reported for patients that received Galafold in trial AT1001-011. No Hy's law cases of DILI (drug induced liver injury) were seen, as shown below.

**Figure 39: Assessment for Drug-Induced Liver Injury Signal (Hy's Law cases): trial AT1001-011**



Reviewer's figure

ALP = alkaline phosphatase, AST = aspartate aminotransferase, BILI = bilirubin, ULN = upper limit of normal

Potentially clinically significant (PCS) laboratory values, as defined by the Applicant in the AT1001-011 SAP, were summarized by patients with PCS-low or PCS-high values at any time during the trial. The PCS values are defined by the following table.

**Table 63: PCS table for chemistry and Hematology**

Parameter	SI Units	PCS Low	PCS High	Sex
Hemoglobin	g/L	<95	-	F
	g/L	<115	-	M
Hematocrit	%	<32	-	F
	%	<37	-	M
RBC count	10 <sup>12</sup> /L	-	>10	F
	10 <sup>12</sup> /L	-	>8	M
WBC count	10 <sup>9</sup> /L	<2.8	>16	B
Neutrophils (total)	GI/L	≤ 1.5	≥ 13.0	B
Lymphocytes	%	-	≥ 75	B
Monocytes	%	-	≥ 15	B
Eosinophils	%	-	≥ 10	B
Basophils	%	-	≥ 10	B
Platelet count	10 <sup>9</sup> /L	<75	>700	B
BUN (NOT urea)	mg/dL	-	≥30	B
Serum creatinine	mg/dL	-	≥2.0	B
Total bilirubin	mg/dL	-	≥2.0	B
Alkaline phosphatase	IU/L	-	>390	B
AST	IU/L	-	>150	B
ALT	IU/L	-	>165	B

F=Female, M=Male, B=Both

Applicant's table: AT1001-011 SAP p 29/132.

ALT = alanine aminotransferase, AST = aspartate aminotransferase, BUN = blood urea nitrogen, PCS = potentially clinically significant, RBC = red blood cell, WBC = white blood cell

**Table 64: PCS Chemistry for Trial AT1001-011**

Parameter	Galafold		Placebo	
		N = 34, n (%)	N = 33, n (%)	
ALT	Low	0	0	
	High	0	0	
ALP	Low	0	0	
	High	0	0	
AST	Low	0	0	
	High	0	0	
Bilirubin	Low	0	0	
	High	0	0	
Blood urea nitrogen	Low	0	0	
	High	0	2 (6)	
Creatinine	Low	0	0	
	High	1 (3)	0	

Adapted from Applicant table: AT1001-011 CSR table14.3.3.3.3

ALP = alkaline phosphatase, ALT = alanine aminotransferase, AST = aspartate aminotransferase, PCS = potentially clinically significant

**Table 65: PCS Chemistry for Trial AT1001-012**

Parameter	Galafold		ERT	
		N = 36, n (%)	N = 21, n (%)	
ALT	Low	0	0	
	High	0	0	
ALP	Low	0	0	
	High	0	0	
AST	Low	0	0	
	High	0	0	
Bilirubin	Low	0	0	
	High	0	0	
Blood urea nitrogen	Low	0	0	
	High	1 (3)	1 (5)	
Creatinine	Low	0	0	
	High	0	0	

Adapted from Applicant Trial report body Table 14.3.3.3.3

ALP = alkaline phosphatase, ALT = alanine aminotransferase, AST = aspartate aminotransferase, PCS = potentially clinically significant

One patient in trial AT1001-011 with a clinically significant abnormality had started with a baseline creatinine of 2.0 with elevation to 2.3 by the end of 6 months and stayed stable at 2.3 by the end of 12 months.

### **Hematology Labs**

Mean and median values and change from baseline for each hematology lab were similar in the Galafold group compared to placebo in trial AT1001-011 and were similar in the Galafold group compared to ERT in trial AT1001-012. No trends were seen over time. No TEAEs associated with hematology test results were reported for patients that received Galafold in trial AT1001-011. TEAEs associated with hematology tests document one patient with a decrease white blood cell count. This patient was noted to have a low WBC at baseline which increased throughout the trial to a normal limit. Patients with clinically significant abnormalities were noted to be comparable in placebo and Galafold. Four patients in trial AT1001-012 were noted to have a low hematocrit. Three patients started with a baseline low hematocrit which improved throughout the trial and stayed within normal limits. One patient had a low but within normal limit hematocrit that stayed stable throughout trial.

**Table 66: PCS Hematology for Trial AT1001-011**

Parameter	Galafold		Placebo
		N = 34, n (%)	N = 33, n (%)
Hemoglobin	Low	1 (3)	2 (6)
	High	0	0
Hematocrit	Low	2 (6)	6 (18)
	High	0	0
Leukocytes	Low	0	0
	High	1 (3)	0
Neutrophils	Low	0	1 (3)
	High	0	0
Platelets	Low	0	0
	High	0	0

Adapted from Applicant table AT1001-011 CSR table 14.3.3.2.3

PCS = potentially clinically significant

**Table 67: PCS Hematology for Trial AT1001-012**

Parameter,	Galafold		ERT
		N = 36, n (%)	N = 21, n (%)
Hematocrit	Low	4 (11)	1 (5)
	High	0	0
Hemoglobin	Low	1 (3)	1 (5)
	High	0	0
Leukocytes	Low	0	0
	High	0	0
Neutrophils	Low	0	0
	High	0	0
Platelets	Low	0	0
	High	0	0

Adapted from Applicant's AT1001-012 trial report Table 14.3.3.2.3

PCS = potentially clinically significant

### **Vital Signs**

Vital Signs were reviewed from the placebo controlled trial AT1001-011 and from the active control trial AT1001-012.

Mean and median values and change from baseline showed comparable results in the Galafold group compared to placebo in trial AT1001-011 and were similar in the Galafold group compared to ERT in trial AT1001-012. TEAEs associated with vital signs were reported for three patients that received Galafold in trial AT1001-011. One was related to blood pressure, the other two were related to weight gain. The patient with elevated blood pressure was noted to have one elevated blood pressure reading above the patient's baseline but was still within normal limits.

Potentially clinically significant (PCS) values were summarized by patients with PCS-low or PCS-high values at any time during the trial. Blood pressure and pulse were noted to be comparable between both groups. Increase in weight was noted in AT100-012, with a > 7% change from baseline seen in 4 of 36 (11%) in the Galafold group and 1 of 21 (5%) in the ERT group.

However, no change was noted when compared with placebo in trial AT1001-011.

### **Electrocardiograms (ECGs)**

Patients had ECGs done at screening, baseline, and at months 1, 3, and 6: RR, QT, QTc, QRS, HR were recorded. Changes from baseline were computed. ECGs were read locally. There were no changes in mean values from baseline between Galafold and placebo.

Potentially clinically significant (PCS) ECG values were summarized by patients only in trial AT1001-011 with PCS-low or PCS-high values at any time during the trial. QRS duration, heart rate and QTc measurements were found to be comparable between Galafold and placebo.

### **QT**

The TQT trial AT1001-010 looked at 52 healthy adults with 26 males and 26 females randomly assigned to receive four dose groups in one of four treatment sequences. Patients received either 150mg or 1250mg of Galafold. The QT trial demonstrated that Galafold did not have an effect on cardiac repolarization at clinical and supra-therapeutic doses. There was no effect on QT/QTc prolongation. See Janell E. Chen's QT-IRT consult for more information.

### **Immunogenicity**

Not applicable.

## **8.2.5. Analysis of Submission-Specific Safety Issues**

### **Urinary Tract Infections**

An increase in urinary tract infections was found in the Galafold group compared to placebo in trial AT1001-011. In trial AT1001-012, there was also a higher percentage urinary tract infections in the Galafold group compared to ERT. Urinary tract infections that developed in patients who received at least one dose of Galafold 150mg in the phase 2 FAB-CL-205, phase 3 and extension trials occurred with a frequency of 11 out of 139 (7.9%). The rate of infections overall in trial AT1001-011 with Galafold was 25 (74%) versus placebo 15 (45%). The rate of infections in Galafold excluding urinary tract infection was 18 (53%) versus placebo 15 (45%). In trial AT1001-012, five patients developed a UTI versus two in the ERT group. See table below for all adverse events in trial AT1001-012.

**Table 68: TEAE > 5 Events; Trial AT1001-012**

Adverse events	Galafold (N = 36), n (%)	ERT (N = 21), n (%)
Nasopharyngitis	14 (38.89)	9 (42.86)
Headache	11 (30.56)	6 (28.57)
Abdominal pain	7 (19.44)	3 (14.29)
Dizziness	7 (19.44)	2 (9.52)
Influenza	5 (13.89)	4 (19.05)
Urinary tract infection	5 (13.89)	2 (9.52)
Diarrhea	5 (13.89)	2 (9.52)
Nausea	5 (13.89)	2 (9.52)
Myalgia	5 (13.89)	2 (9.52)

Reviewer table (kidney infection, cystitis and urinary tract infections were grouped together under urinary tract infection)  
ERT = enzyme replacement therapy, TEAE = treatment emergent adverse event

In trial AT1001-012, there were 43 (83%) infections in Galafold patients versus 34 infections in (62%)ERT patients. Infections in Galafold excluding urinary tract infections were 38 versus 32 in the ERT group. Since there was no general increased infection rate with Galafold (other than URIs and nasopharyngitis), there is no convincing evidence of possible immunosuppression. Of note, there were no imbalances between groups for severe infections.

Although there were more females enrolled in the trials, they were equally distributed between Galafold, placebo and ERT. Two males in the Galafold group developed a UTI but all others were female in both the Galafold and ERT group. Both males that developed a UTI were over the age of 60. One male also had an adverse event of renal hematoma and hydronephrosis. No UTIs were documented in the placebo group. All patients that developed a UTI had amenable mutations.

Only one AE of pyelonephritis was documented but no SAE cases of pyelonephritis.

Since all UTIs were documented as AEs, it's unknown how the UTI was diagnosed and whether the diagnosis was made on the basis of urine culture or symptoms. The imbalance of UTIs does not have a clear explanation at this point and is treatable.

## 8.2.6. Safety Analyses by Demographic Subgroups

### **Sex**

In trial AT1001-011, there were 22 (64.7%) females and 12 (35.3%) males enrolled in the Galafold group. Overall, a total of 120 treatment adverse events were recorded in females and 45 adverse events in recorded with males. One male and one female had a serious adverse event. The most frequently reported adverse events in females versus males were headache (36.36% versus 33.33%), abdominal pain (27.27% versus 8.33%), fatigue (18.18% versus 0), nasopharyngitis (22.73% versus 8.33%), paresthesia (18.18% versus 0), nausea (13.64% versus 8.33%), and urinary tract infection (18.18% versus 16.67%).

In trial AT1001-012, there were 20 (55.6%) females and 16 (44.4%) males enrolled in the Galafold group. There was a total of 199 adverse events recorded for females and 114 recorded in males. The most common AEs in females versus males were nasopharyngitis (65% versus 93.75%), headache (45% versus 37.50%), abdominal pain (45% versus 6.25%), diarrhea (25% versus 6.25%), nausea (15% versus 12.50%), and urinary tract infection (20% versus 6.25%).

### **Age**

In trial AT1001-011, two patients were < 18 years old, and 1 patient > 65 years of age. Nine TEAEs were noted in the patients < 18 years old (diarrhea, abdominal pain, headache, mitral valve incompetence, pilonidal cyst, red blood cells in urine, sinus arrhythmia); and in the patient > 65 years of age had four TEAEs (abdominal pain, fatigue, pyelonephritis, auricular perichondritis). No serious adverse events were documented in either age groups.

In trial AT1001-012 three patients were > 65 years and no patients were < 18 years of age. One patient had a SAE of pneumonia. Most common treatment adverse events were comparable to those expected in the general population (headache, nasopharyngitis, abdominal pain).

Gastrointestinal symptoms were more common adverse events seen in females in both trial AT1001-011 and trial AT1001-012 compared to males. This may be related to the natural history of FD in females as it has been reported that females with FD have a slightly higher incidence of GI manifestations, or may be simply an imbalance observed in the small group of non-randomized patients.<sup>13</sup> Also, UTIs were reported mostly in females. The incidence of all adverse events in any patient that received Galafold is comparable between both genders (98.7% in females and 96.7% in males). The assessment of safety by age group is limited as only two patients were < 18 years and only four patients were > 65 years.

### **8.2.7. Specific Safety Studies/Clinical Trials**

Not applicable

### **8.2.8. Additional Safety Explorations**

#### **Human Carcinogenicity or Tumor Development**

There were four cases of cancer diagnosed in patients treated with Galafold during the phase 3 trials and one case in a placebo-treated patient:

1. One case of malignancy occurred in trial AT1001-011. A 50-year-old female developed anaplastic large cell lymphoma while she was on placebo in stage 1. The drug was

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<sup>13</sup> Zar-Kessler, C, A Karaa, K Bustin Sims, V Clarke, and B Kuo, 2016, Understanding the gastrointestinal manifestations of Fabry disease: promoting prompt diagnosis, *Ther Adv Gastroenterol*, 9(4):626-634.

discontinued.

2. A 64-year-old female with a history of endometriosis, hysterectomy, mucoid cysts in breast tissue, breast fibroadenoma, was enrolled in trial AT1001-41 and had participated in trial AT1001-011. She was diagnosed with grade III metastatic breast cancer on day 1022 of treatment. The patient passed away during the trials.
3. A 49-year-old male with a history of premature atrial contractions (PACs), hypertriglyceridemia, tobacco and alcohol use participated in trial AT1001-041 and had participated in two previous phase 2 open-label Galafold trials, FAB-CL-201 and FAB-CL-205. On day 2736 of Galafold treatment, he was diagnosed with metastatic squamous cell carcinoma of the neck. The drug was discontinued.
4. A 49-year-old female who enrolled in trial AT1001-041 and had participated in trial AT1001-011 was diagnosed with papillary thyroid carcinoma on day 764 of Galafold treatment. The drug was continued.
5. A 69-year-old female enrolled in trial AT1001-42 and had participated in trials FAB-CL-204 and FAB-CL-205. She was diagnosed with breast cancer during routine mammography on day 3833 of Galafold treatment. The drug was continued.

As described above, four patients (ages 49 to 69 years old) treated with Galafold who participated in the long-term extension trials AT1001-041 and AT1001-042 reported a diagnosis of cancer during the study, two of which were breast cancer (with the other two being squamous cell carcinoma of the neck and papillary thyroid carcinoma). One placebo-treated patient was diagnosed with lymphoma during the first 6 months of treatment in trial AT1001-011. Additional details on the patients' family history and other risk factors for the development of cancer were not available. Given the limited details provided, the absence of a control group in the extension trials, and the high prevalence of breast cancer and other cancers of epithelial origin in this age group (49 to 69 years old), it is difficult to infer causality for these cases. Carcinogenicity studies done in animals did not suggest any increased risk of malignancy. This potential risk should be further monitored in the postmarketing setting through routine pharmacovigilance.

### **Human Reproduction and Pregnancy**

During the clinical trials, pregnancy was reported in three female patients who received Galafold (one patient in trial AT1001-011, one patient in AT1001-012 and one patient in trial AT1001-041). All three pregnant women stopped the trial drug at the time pregnancy was confirmed. All three babies were healthy, two were delivered vaginally and one via a planned caesarian section. One patient enrolled in trial AT1001-011 became pregnant while receiving placebo and vaginally delivered a healthy baby.

Data is limited to make a conclusion regarding use of Galafold in pregnancy. The long-term safety of Galafold use during pregnancy and lactation as well as effects on the developing fetus and newborn will be assessed in the postmarketing setting in a patient registry.

#### **Pediatrics and Assessment of Effects on Growth**

Trial AT100-011 enrolled two patients who were 16 years of age (one with amenable *GLA* variant and another with a non-amenable *GLA* variant). Trial AT1001-012 did not include any patients under 18 years old. were enrolled in. APPEARS THIS WAY ON ORIGINAL

Assessment of safety in the pediatric population is limited due to the small number of pediatric patients 16-18 years old enrolled in the trials. Moreover, linear growth is 90% complete by 14 years in girls and 16 years in boys – (b) (4)

#### **Overdose, Drug Abuse Potential, Withdrawal, and Rebound**

Not applicable

### **8.2.9. Safety in the Postmarket Setting**

#### **Safety Concerns Identified Through Postmarket Experience**

Galafold was approved by the EMA in 2016. As on October 1, 2017, 26 adverse drug reactions were reported from post marketing sources. One notable observation is a case of posttransplant lymphoproliferative disorder which has not been previously seen.

#### **Expectations on Safety in the Postmarket Setting**

Many subpopulations were not well represented in the safety database, including pediatric patients (including adolescents), patients older than 65 years of age, and patients of different ethnicities. With respect to older patients and different ethnicities, important differences in the safety profile are not anticipated in the post market setting. (b) (4)

(b) (4) In addition, identified non-serious AEs, such as headache and UTIs, can be further assessed and monitored through routine pharmacovigilance in the postmarketing setting

### **8.2.10. Integrated Assessment of Safety**

A total of 386 patients were exposed to any dose of Galafold, including 194 healthy adults, 24 patients with renal impairment in phase 1 trials, and 168 patients with Fabry disease in phase 2 and phase 3 trials, of which a total of 160 patients received Galafold monotherapy. The mean duration of exposure to Galafold 123 mg QOD was 3.71 years. A total of 139 patients received Galafold 123 mg QOD for 6 months and 132 patients received Galafold 123 mg QOD for 18

months. The overall safety database appears adequate for assessment of safety of Galafold in the patient population studied given the rarity of the disease.

No serious safety signals were identified as convincingly associated with Galafold treatment in the phase 3 trials as described in more detail above. The most common treatment-emergent adverse events were headache, nasopharyngitis, urinary tract infections, nausea, and pyrexia, and they were more frequent in the Galafold arm than in the placebo arm in stage 1 of trial AT1001-011. In trial AT1001-012, AEs occurred with comparable frequencies between the ERT and Galafold groups except for urinary tract infections which were more common in the Galafold group than in the ERT group. There were no clinically significant laboratory or vital sign changes noted in the phase 3 trials. Lastly, no cardiac safety signals were identified in association with Galafold treatment through ECG assessments in the phase 3 trials and through the completed thorough QT study.

Limited data are available on the use of Galafold during pregnancy and any effects on the developing fetus and newborn. Despite the lack of safety signals in pregnant animals and their offspring exposed to migalastat in the nonclinical program and given that women with Fabry disease who are of reproductive age will be treated with Galafold, safety data from the use of Galafold during pregnancy and lactation and data on its potential effects on the developing fetus and newborn are still needed. This will be accomplished via a postapproval observational study (as part of a FDAAA PMR) that the Applicant has agreed to conduct (see section 13).

In summary, the available safety database in patients exposed to Galafold 123 mg every other day in the completed trials provide a sufficient basis for the conclusion of safety for Galafold for the granted indication. In general, it appears that no serious safety risks have been reported as convincingly related to short-term or long-term Galafold treatment and the most frequent AEs reported with Galafold will be communicated through patient and prescriber labeling. Continued safety monitoring in treated patients in the postmarketing setting is recommended through routine pharmacovigilance. Safety data of the use of Galafold during pregnancy and lactation will be collected through the agreed-upon postapproval study.

## SUMMARY AND CONCLUSIONS

### 8.3. Statistical Issues

The Applicant submitted one adequate and well-controlled trial (AT1001-011) to support the efficacy of Galafold in patients with Fabry disease and amenable *GLA* variants. During the drug development, FDA had informed the Applicant their concern about using 50% reduction in KIC GL-3 as a responder criterion. The treatment difference in the pre-specified primary endpoint in the ITT population was not statistically significant (p-value 0.3). As discussed in Section 8.1.6, failing to show a statistically significant reduction in KIC GL-3 burden in the phase 3 trial was not entirely unexpected due to some major trial limitations.

As the Applicant later found out that not all study patients had amenable *GLA* variants, they re-analyzed the trial data using the amenable patients only in Trial AT1001-011. The new analysis was based on two variables: the later-defined amenable criteria for the protocol-specified primary endpoint and the change from baseline to month 6 in average number of GL-3 inclusions per KIC. For the ITT-amenable AH population, the results for the primary endpoint remained non-statistically significant.

Of note, the changes from baseline to month 6 in the average number of GL-3 inclusion per KIC for the entire ITT-amenable patients appeared to be driven by the male patient population and by patients with baseline GL-3 inclusions  $\geq 0.3$  per KIC, which were two highly correlated patient subgroups. Consistent with the current knowledge of the pathophysiology and natural history of Fabry disease, we did not observe any difference between Galafold and placebo in the female patient population or in patients with baseline GL-3 inclusions  $< 0.3$  per KIC. The detailed results are included in Table 50. Favorable trends were also observed in other biomarkers, including lyso-Gb3 and WBC alpha-Gal activity in males only.

#### 8.4. Conclusions and Recommendations

The determination of efficacy for Galafold in adults with Fabry disease was primarily based on phase 3 renal histological evidence of a treatment effect in patients with amenable *GLA* variants supported by limited, disease-specific, biomarker evidence of an effect (plasma lyso-GB3). These effects appeared to be overall more pronounced in treated males who started at a higher baseline of both histological and biochemical disease severity. The observed treatment effects in the analyzed populations were overall small. Factors which rendered statistical analyses and clinical interpretation challenging in this application included the following:

1. Selection of a patient population in trial AT1001-011 with overall minimal to small histological disease burden at baseline which did not allow for substantial changes to be demonstrated over the short duration of the placebo-controlled phase (6 months);
2. Inclusion of both males and females with Fabry disease, which likely introduced an additional element of variability in the study results based on the known disease heterogeneity and milder baseline severity in affected females with FD;
3. The majority of patients in trial AT1001-011 appeared to have “late-onset” FD (baseline enzyme activity  $> 1$  to 3% of the normal mean), which itself may have contributed to a limited histological treatment effect as late-onset FD patients tend to exhibit high heterogeneity in disease severity and rate of progression (which tends to be slower than patients with “classic” Fabry disease);
4. About one third of patients in trial AT1001-011, which provided the histological data supporting the primary efficacy outcome, were missing data for the disease-specific biomarker (plasma lyso-Gb3). Similarly, data (with appropriate comparator group) on in vivo changes in alpha-Gal A enzyme activity were limited to males in trial AT1001-011 (17 males in the ITT-amenable population) as relevant changes in the enzyme are

unreliable in females (for the reasons described above). These challenges limited the ability to truly integrate the biomarker responses with the observed histological responses in the small patient population on which the primary efficacy was based (N = 45 patients).

Overall, the totality of data in the Galafold application demonstrated a reduction in disease-specific biomarkers in treated patients with the primary efficacy being based on a previously accepted surrogate endpoint which is reasonably likely to predict clinical benefit in FD, namely GL-3 inclusion in kidney interstitial capillaries. The available safety database presented in this NDA shows an acceptable safety profile for Galafold in the population studied. As such, the review team recommends accelerated approval of Galafold with a plan for a confirmatory trial to verify and describe the clinical benefit in adults with Fabry disease and amenable *GLA* variants.

## 9 Advisory Committee Meeting and Other External Consultations

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The application for Galafold was not referred to an FDA advisory committee because outside expertise was not necessary; there were no controversial issues that would benefit from advisory committee discussion.

## 10 Pediatrics

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The granted indication includes only adults with FD (b) (4)  
(b) (4)  
(b) (4). In addition, Galafold has orphan drug designation in Fabry disease and is, thus, exempt from PREA requirements.

## 11 Labeling Recommendations

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### 11.1. Prescription Drug Labeling

The final agreed-upon prescribing information (PI) for Galafold reflects multiple changes made from the originally submitted, Applicant- proposed PI. Notable changes and critical elements that were discussed with the Applicant during the NDA review include the following:

1. Final granted indication

2. Patient selection for treatment based on an amenable *GLA* variant which, in the context of the individual patient (and in consultation with a clinical genetics professional, if needed) appears to cause Fabry disease (pathogenic or likely-pathogenic interpretation of the *GLA* variant).

(b) (4)

4. List of amenable *GLA* variants based on the in vitro HEK-293 assay
5. Non-amenable *GLA* variants not included in the PI (appropriate contact information provided for physicians/patients to obtain information on *GLA* variants not included in the PI).
6. Indication that limited available data exist on the use of Galafold in pediatric patients and those > 65 years old.

(b) (4)

8. Description of pharmacodynamic (plasma lyso-Gb3) and histological (GL-3 inclusions in KIC) changes in patients with amenable *GLA* variants in trials AT1001-011 and AT1001-012 (sections 12 and 14 of the PI).

## 12 Risk Evaluation and Mitigation Strategies

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A risk evaluation and mitigation strategy is not necessary for approval of Galafold. The potential risks associated with Galafold treatment, which are non-serious based on our current understanding and review, can be conveyed via standard product labeling and can be monitored in the postmarketing setting through routine pharmacovigilance.

## 13 Postmarketing Requirements and Commitment

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This NDA will receive accelerated approval based on a surrogate endpoint that is reasonably likely to predict clinical benefit; two postmarketing requirements will be conducted to describe and verify the clinical benefit of Galafold in patients with Fabry disease and amenable *GLA* variants. An additional post-marketing requirement will be conducted to evaluate the safety of Galafold in pregnant women and their offspring.

### Agreed-upon PMRs:

1. A phase 4, randomized, double-blind, placebo-controlled trial evaluating the clinical efficacy, safety, and pharmacodynamics of Galafold in adults with a confirmed diagnosis of Fabry disease and amenable *GLA* variants. This trial will verify and describe the

clinical benefit of Galafold in adult patients with Fabry disease and amenable *GLA* variants.

2. A prospective, observational study evaluating the clinical effects of Galafold in adults with a confirmed diagnosis of Fabry disease and amenable *GLA* variants. The study will collect and analyze data on laboratory parameters (e.g., alpha-Gal A activity, lyso-Gb3, serum and urine creatinine, eGFR, urine protein, and urine albumin) and on major renal, cardiac, and cerebrovascular outcomes in treated and untreated patients with Fabry disease.
3. A worldwide, prospective, single-arm, observational study in women exposed to Galafold (migalastat) during pregnancy and lactation to assess: risks of pregnancy complications, adverse effects on the developing fetus and neonate, and adverse effects on lactation and the breastfed infant. Pregnancy exposures and outcomes will be reported voluntarily by providers and patients (e.g., telephone contact number and/or website will be provided in the product's prescribing information). Complete data will be captured regarding pregnancy outcomes and any adverse effects in offspring. Results will be analyzed and reported descriptively. The study will collect information for a minimum of 10 years. Interim reports on the cumulative findings and analyses will be submitted annually. Data collected retrospectively from other sources will be analyzed separately and reported with the interim and final study reports.

**Agreed-upon PMCs:**

1. A PK/PD trial to determine the appropriate dosing regimen of Galafold in patients with Fabry disease and severe renal impairment or who are on kidney dialysis.
2. A study to generate additional DNA sequencing data for all recombinant *GLA* cDNA constructs (amenable and non-amenable) used to generate in vitro alpha-galactosidase A (alpha-Gal A) enzyme data from HEK-293 cells in support of NDA 208623. All cDNA constructs must be re-sequenced using additional bi-directional primers internal to the *GLA* cDNA sequence. The newly generated forward and reverse primer sequences should be of sufficient sequence quality and length to provide the necessary base or sequence information that is currently missing from the full length forward and reverse cDNA single strands for all amenable and non-amenable *GLA* variants. The newly generated sequencing data should be appropriately matched and aligned with the previously submitted data in order to complete the bidirectional analysis of the full length of the double-stranded *GLA* cDNA and to produce a consensus *GLA* cDNA sequence for each construct. To confirm the presence of a specific *GLA* mutation as well as the base context of the cDNA, the consensus sequence generated from matched strands from each construct should be compared to the NCBI Consensus Coding Sequence (CCDS) reference sequence.

## 14 Division Director (DGIEP)

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I concur with the recommendation for approval of Galafold for the indication of “treatment of adults with a confirmed diagnosis of Fabry disease and an amenable galactosidase alpha (*GLA*) variant based on in vitro assay data.”

Migalastat, the active ingredient in Galafold, is a new molecular entity. Structurally, it is a small molecule which functions as a pharmacological chaperone to alpha-galactosidase A (alpha-Gal A), the enzyme whose deficiency is responsible for Fabry disease. Mechanistically, migalastat was designed to bind to the active site of alpha-Gal A in the endoplasmic reticulum and facilitate its trafficking to the lysosomes. The evidence of efficacy of Galafold comes primarily from a subgroup analysis conducted in a single randomized, placebo-controlled, phase 3 clinical trial (Trial AT1001-011).

There were two important issues related to the design of this trial, and both of them have had an impact on the assessment of efficacy. First, the trial enrolled patients on the basis of substrate elevation in the urine (urine GL-3 had to be  $\geq 4$  X upper limit of normal at screening), while its prespecified efficacy analysis assessed histologically the change in the same substrate in the kidney. The unanticipated consequence of not having an inclusion criterion specific for the primary efficacy variable was that the patient population enrolled in the trial had a baseline kidney GL-3 interstitial cell inclusion burden that was low and close to the limit of histological detection; as such, in most patients, a histological effect of treatment could not be assessed. Another trial design issue was that a key in vitro assay that was used to confirm patient eligibility for trial enrollment and treatment assignment was modified postrandomization, and this resulted in a redefinition of the primary analysis population.

Figure 29 of this review best illustrates both the challenge of conducting efficacy analyses for the KIC endpoint in the overall patient population (most patients had mild renal disease) and the need to focus on the only patient group in which a treatment effect could be seen. It is in this relatively small subgroup (defined as patients with amenable mutations and KIC inclusions  $> 0.3$ ) that a treatment difference was identified. While such an analysis is post hoc, analyzing this subgroup is justified by the fact that one needs a patient population with distinct elevation in the variable of interest (KIC) at baseline to see a treatment effect. Of interest, this subgroup of 17 patients includes mostly males, and males are the patients with Fabry disease who have the most severe disease manifestations. The treatment difference relative to placebo is biologically plausible based on both the mechanism of action of the drug and the patient subpopulation in which the treatment effect was identified.

Reduction of kidney GL-3 deposition via histology is a surrogate endpoint that is reasonably likely to predict clinical benefit in Fabry disease. Based on the known disease pathophysiology, it has been previously used for accelerated approval of agalsidase beta, the only approved treatment in patients with Fabry disease. The Applicant has demonstrated that Galafold reduces substrate (GL-3) deposition in kidney interstitial capillaries, as assessed by light microscopy of renal biopsy samples. As such, I concur that Galafold should be approved under the accelerated approval regulations (Subpart H), given that the risk-benefit analysis is

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favorable (the safety profile of the 150 mg of migalastat hydrochloride was found to be acceptable within the limitations of the size of the currently available dataset). This recommendation should not be seen as an endorsement of post hoc subgroup efficacy analyses as a basis for drug approval across the board, but rather as a flexible application of current regulations in the context of a rare disease.

The Applicant will conduct a postapproval study to verify and describe the clinical benefit of Galafold in adult patients with Fabry disease and amenable *GLA* variants.

## 15 Office Director (Office of Drug Evaluation III)

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I concur with the recommendation of the Division of Gastroenterology and Inborn Errors Products (DGIEP) for accelerated approval for NDA 208623 Galafold (migalastat) under 21 CFR 314.510 (subpart H). Galafold is an alpha-galactosidase pharmacological chaperone indicated for the treatment of adults with a confirmed diagnosis of Fabry disease and an amenable *GLA* variant. It is administered orally once every other day. Galafold represents the first pharmacological chaperone approved for this indication. Currently, only agalsidase beta, an enzyme replacement therapy, is approved in the U.S. for Fabry disease. Galafold offers a new treatment alternative for Fabry disease patients with amenable *GLA* variants.

The efficacy of Galafold was demonstrated based on its effect on a surrogate endpoint (renal globotriaosylceramide, GL-3, deposition), that is reasonably likely, based on pathophysiologic considerations, to predict clinical benefit in patients with Fabry disease and which has previously served as the basis for accelerated approval of agalsidase beta. After 6 months of treatment, Galafold reduced GL-3 deposition in kidney interstitial capillaries relative to placebo. Substrate reduction was greater in males and in patients with greater substrate deposition at baseline (defined as  $GL-3 \geq 0.3$ ). Reduction in plasma lyso-Gb3 and increased WBC alpha-Gal A activity from baseline, indicative of the pharmacodynamic effects of Galafold, were also demonstrated in males. Galafold treatment was well tolerated.

The Applicant will be required to conduct postmarketing studies to verify and describe the clinical benefit of Galafold in adult patients with Fabry disease and amenable *GLA* variants. These studies will also provide safety and pharmacodynamic information. Additional postmarketing studies will address concerns raised during the review regarding gene sequencing techniques, and will provide pregnancy exposure data, evaluate effects on lactation, and explore dosing in patients with severe renal impairment which can occur in patients with Fabry disease progression.

The product labeling for Galafold will list the amenable *GLA* variants identified to date using an in vitro assay as described in the Clinical Pharmacology section of the PI, under Mechanism of Action 12.1. Updates to labeling will occur as new amenable *GLA* variants are identified. A risk evaluation and mitigation strategy or REMS will not be required.

## 16 Appendices

### 16.1. Appendix 1: BLISS and Genzyme Histological Methods for Analysis of Kidney Interstitial Capillary Cell GL3-Inclusions

Table 69: BLISS and Genzyme Histological Methods for Analysis of Kidney Interstitial Capillary Cell GL-3 Inclusions

	Comparative Histological Methodology	
	Migalastat (BLISS Methodology)	Fabrazyme (Genzyme Methodology)
Scoring system	Quantitative	Semi-quantitative
Format of specimens used by scorers	Digital images of slides	Glass slides
Number of interstitial capillaries scored	300 or until all available images have been assessed.	≥50 (Steps 2 and 3; see below)
Annotation of capillaries to be scored	Capillaries in up to 8 images per subject are designated. Ratio of maximum and minimum diameter of each perpendicular vessel must be < 4; capillary diameter must be > tubular cell nucleus	None
Scoring	Continuous: Average number of GL-3 inclusions per interstitial capillary	Categorical: Step 1: Qualitative; scores: 0-3 (lowest to highest number of inclusions). Step 2: Quantitative –for specimens scored as 0 or 1 in Step 1. Step 3: Additional quantitative criteria if Score of 0 obtained in Step 2.
Definition of “Score 0”	Zero GL-3 inclusions in any interstitial capillary	≥50% of interstitial capillaries have no GL-3 inclusions AND <5% of interstitial capillaries have a score of ≥1 (more than 2 or 3 inclusions).

GL-3 = globotriaosylceramide

## 16.2. Appendix 2: In Vitro Assays of alpha-Gal A Mutant Proteins and Classification Criteria

**Table 70: In Vitro Assays of alpha-Gal A Mutant Proteins and Classification Criteria**

Assay	Terminology	Implementation	Criteria
NIH <i>ex vivo</i> Patient-derived T-cell Assay	Enhanceable	Phase 2 (Inclusion criterion)	$\geq 1.20$ -fold increase above baseline in total $\alpha$ -Gal A activity <sup>a</sup>
HEK (non-GLP) <i>in vitro</i> Assay (Referred to as Clinical Trial HEK Assay)	Responsive	Phase 3 (Inclusion criterion)	$\geq 1.20$ -fold increase above baseline in mutant $\alpha$ -Gal A activity Absolute increase of $\geq 3.0\%$ of wild-type $\alpha$ -Gal A activity
HEK (GLP) <i>in vitro</i> Migalastat Amenability Assay (Referred to as GLP HEK Assay) <sup>b</sup>	Amenable	Phase 3 (Efficacy analyses)	$\geq 1.20$ -fold increase above baseline in mutant $\alpha$ -Gal A activity Absolute increase of $\geq 3.0\%$ of wild-type $\alpha$ -Gal A activity

alpha-Gal A = alpha-galactosidase A, GLP = good laboratory practice, HEK-293 = human embryonic kidney cell line 293

### 16.3. Appendix 3: Trial AT1001-011 Stage 1 Schedule of Procedures and Assessments

Table 71: Trial AT1001-011 Stage 1 Schedule of Procedures and Assessments

Study Month #	7 (±7 days)	9 (±7 days)	12 (±7 days)	13 Follow Up (±7 days) <sup>1</sup>
Study Period	Stage 2 - Active Treatment			
Clinic Visit	5	6	7	8 <sup>1</sup>
Assessments				
PHYSICAL EXAM			X	X
PREGNANCY TEST <sup>2</sup>	X	X	X	X
CONCOMITANT MEDICATIONS	X	X	X	X
VITAL SIGNS (BP, HR, RR)	X	X	X	X
WEIGHT	X	X	X	X
HEIGHT			X	
12 Lead ECG	X	X	X	X
LABORATORY TESTS	X	X	X	X
URINE GL-3 <sup>3</sup>	X	X	X	
eGFR calculation <sup>4</sup>	X	X	X	
ADVERSE EVENTS	X	X	X	X
WBC α-GAL A ACTIVITY	X	X	X	
Iohexol GFR			X	
KIDNEY BIOPSY			X	
24-HOUR URINE PROTEIN, ALBUMIN AND CREATININE			X	
SF-36 SURVEY			X	
BRIEF PAIN INVENTORY (BPI) short form			X	
GASTROINTESTINAL SYMPTOM RATING SCALE (GSRS)			X	
ECHOCARDIOGRAPHY (with tissue Doppler imaging where available) <sup>5</sup>			X	
PK SAMPLING <sup>6</sup>	X			
STUDY TREATMENT				
SUPPLY/RESUPPLY/RETURN <sup>7</sup>	X	X	X	X

<sup>1</sup> Subjects who participate in the Open-Label Treatment Extension phase will not have a Follow Up Visit at Month 13/Visit 8; their Follow-Up Visit will be scheduled after the last study visit is performed if they prematurely discontinue or elect not to enter a study extension offered under a separate protocol (Table 3). The assessments to be performed if a subject prematurely discontinues participation during Stage 1 or Stage 2 are discussed in Sections 8.6, 10.1.4 and 10.1.6; their Follow-Up Visit (Section 10.1.8) should be scheduled within of one month (±7days) from the date of last study visit.

<sup>2</sup> For female subjects of child-bearing potential, urine pregnancy test to be performed.

<sup>3</sup> Urine GL-3 will be assessed using a 24 hour sample at Month 12/Visit 7;  
Urine GL-3 will be assessed using first morning urine collections at all other visits.

<sup>4</sup> eGFR will be calculated centrally, not at the site level.

<sup>5</sup> Left ventricular mass, septal wall thickness, fractional shortening and ejection fraction.

<sup>6</sup> Blood samples will be collected before dosing and at four time points after dosing (2, 3, 4 and approximately 8 hr post-dose). A ±10 min window is permitted on blood draws. This visit must occur on a dosing day.

<sup>7</sup> Drug to be administered during visit if visit occurs on a dosing day. A six-month supply of study treatment will be dispensed at Month 12/Visit 7 only to subjects who participate in the Open-Label Treatment Extension phase. The return of unused supplies at a Follow-Up Visit should occur if a subject did not return the supplies at a prior visit.

ECG = electrocardiogram, eGFR = estimated glomerular filtration rate, GL-3 = globotriaosylceramide, PK = pharmacokinetic, SF-36 = short form 36, WBC = white blood cell

#### 16.4. Appendix 4: Financial Disclosure

##### Financial Disclosure

**Covered Clinical Trial (Name and/or Number):** FAB-CL-101, FAB-CL-102, FAB-CL-103, FAB-CL-104, AT1001-010, AT1001-188, FAB-CL-201, FAB-CL-202, FAB-CL-203, FAB-CL-204, FAB-CL-205, AT1001-011, AT1001-012, AT1001-013, AT1001-014, AT1001-015, AT1001-016, AT1001-018, AT1001-041, AT1001-042,

Was a list of clinical investigators provided:	Yes <input checked="" type="checkbox"/>	No <input type="checkbox"/> (Request list from Applicant)
Total number of investigators identified: <u>1096</u>		
Number of investigators who are Sponsor employees (including both full-time and part-time employees): <u>0</u>		
Number of investigators with disclosable financial interests/arrangements (Form FDA 3455): <u>1</u>		
<p>If there are investigators with disclosable financial interests/arrangements, identify the number of investigators with interests/arrangements in each category (as defined in 21 CFR 54.2(a), (b), (c) and (f)):</p> <p>Compensation to the investigator for conducting the trial where the value could be influenced by the outcome of the trial: <u>0</u></p> <p>Significant payments of other sorts: <u>1</u></p> <p>Proprietary interest in the product tested held by investigator: <u>0</u></p> <p>Significant equity interest held by investigator in Sponsor of covered trial: <u>0</u></p>		
Is an attachment provided with details of the disclosable financial interests/arrangements:	Yes <input checked="" type="checkbox"/>	No <input type="checkbox"/> (Request details from Applicant)
Is a description of the steps taken to minimize potential bias provided:	Yes <input checked="" type="checkbox"/>	No <input type="checkbox"/> (Request information from Applicant)
<p>Number of investigators with certification of due diligence (Form FDA 3454, box 3): Up to date financial disclosure information from <u>23</u> investigators who participated in the covered studies was not obtainable by Amicus Therapeutic, Inc. Internal review of investigator grants provided by Amicus Therapeutics, Inc. did confirm that the clinical investigators have no financial interests or arrangements as described in 21 CFR Part 54.4 (a)(3), and thus meet the criteria described in 21 CFR Part 54.4(a)(1). In order to complete due diligence, Amicus contacted all clinical investigators by their provided email addresses two separate times, and a third time by mail requiring signature upon delivery. The study coordinator and principal investigator were also contacted to receive updated contact information with no success. Amicus attempted to contact the clinical investigators through web searches, LinkedIn, and social media and also attempted to locate their new places of employment with no success.</p>		
Is an attachment provided with the reason:	Yes <input checked="" type="checkbox"/>	No <input type="checkbox"/> (Request explanation from Applicant)

## Reviewers of Multi-Disciplinary Review and Evaluation

---

DISCIPLINE	REVIEWER	OFFICE/DIVISION	SECTIONS AUTHORED/ ACKNOWLEDGED/ APPROVED	AUTHORED/ ACKNOWLEDGED/ APPROVED
Pharmacology Reviewer (separate memo-to-file; Reference ID: 4292378)	Vinay Patil	OND/ODEIII/DGIEP	<b>Sections: 5</b>	<b>Select up to two:</b> <input checked="" type="checkbox"/> Authored <input type="checkbox"/> Acknowledged <input type="checkbox"/> Cleared
	<b>Signature: Vinay A. Patil -S</b> <small>Digitally signed by Vinay A. Patil -S            DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Vinay A. Patil -S,            0.9.2342.19200300.100.1.1=2001232169            Date: 2018.08.02 10:13:35 -04'00'</small>			
Pharmacology Reviewer (separate memo-to-file; Reference ID: 4292378)	Ke Zhang	OND/ODEIII/DGIEP	<b>Sections: 5</b>	<b>Select up to two:</b> <input checked="" type="checkbox"/> Authored <input type="checkbox"/> Acknowledged <input type="checkbox"/> Cleared
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Lead Pharmacologist (separate memo-to-file; Reference ID: 4292378)	David Joseph	OND/ODEIII/DGIEP	<b>Sections: 4.2, 5</b>	<b>Select up to two:</b> <input checked="" type="checkbox"/> Authored <input type="checkbox"/> Acknowledged <input checked="" type="checkbox"/> Cleared
	<b>Signature: David B. Joseph -S</b> <small>Digitally signed by David B. Joseph -S            DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People,            0.9.2342.19200300.100.1.1=1300134835, cn=David B. Joseph            -S            Date: 2018.08.02 11:07:47 -04'00'</small>			
ODE Associate Director for Pharmacology and Toxicology (Acting) (separate memo-to-file; Reference ID: 4292378)	Ronald Wange	OND	<b>Sections: 4.2, 5</b>	<b>Select up to two:</b> <input checked="" type="checkbox"/> Authored <input type="checkbox"/> Acknowledged <input checked="" type="checkbox"/> Cleared
	<b>Signature: Ronald L. Wange -S</b> <small>Digitally signed by Ronald L. Wange -S            DN: c=US, o=U.S. Government, ou=HHS, ou=FDA,            ou=People, 0.9.2342.19200300.100.1.1=1300236480,            cn=Ronald L. Wange -S            Date: 2018.08.02 11:18:24 -04'00'</small>			

**Multi-Disciplinary Review  
NDA 208623 GALAFOLD (migalastat)**

DISCIPLINE	REVIEWER	OFFICE/DIVISION	SECTIONS AUTHORED/ACKNOWLEDGED/APPROVED	AUTHORED/ACKNOWLEDGED/APPROVED
In vitro cell based assay Reviewer (*separate memo-to-file)	Paula Hyland	OTS/OCP/DARS	<b>Sections: 6 Appendix 5</b>	<b>Select up to two:</b> <input checked="" type="checkbox"/> Authored <input type="checkbox"/> Acknowledged <input type="checkbox"/> Cleared
	<b>Signature:</b> Paula L. Hyland -S <small>Digitally signed by Paula L. Hyland -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=0013899128 cn=Paula L. Hyland -S Date: 2018.08.03 15:3900 -0400</small>			
In vitro cell based assay Reviewer (*separate memo-to-file)	James Weaver	OTS/OCP/DARS	<b>Sections: 6 Appendix 5</b>	<b>Select up to two:</b> <input checked="" type="checkbox"/> Authored <input type="checkbox"/> Acknowledged <input type="checkbox"/> Cleared
	<b>Signature:</b> James L. Weaver -S <small>Digitally signed by James L. Weaver -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=1300052681, cn=James L. Weaver -S Date: 2018.08.03 15:14:58 -0400</small>			
Pharmacometrics Reviewer (*separate memo-to-file)	Justin Earp	OTS/OCP/DPM	<b>Sections: 6 Appendix 7</b>	<b>Select up to two:</b> <input checked="" type="checkbox"/> Authored <input type="checkbox"/> Acknowledged <input type="checkbox"/> Cleared
	<b>Signature:</b> Justin C. Earp -S <small>Digitally signed by Justin C. Earp -S Date: 2018.08.03 16:21:27 -0400</small>			
Pharmacometrics Team Leader (*separate memo-to-file)	Lian Ma	OTS/OCP/DPM	<b>Sections: 6 Appendix 7</b>	<b>Select up to two:</b> <input checked="" type="checkbox"/> Authored <input type="checkbox"/> Acknowledged <input checked="" type="checkbox"/> Cleared
	<b>Signature:</b> Lian Ma -S <small>Digitally signed by Lian Ma -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Lian Ma -S, 0.9.2342.19200300.100.1.1=2000825336 Date: 2018.08.03 16:06:08 -0400</small>			

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NDA 208623 GALAFOLD (migalastat)**

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Clinical Pharmacology Reviewer (*separate memo-to-file)	Dilara Jappar	OTS/OCP/DCPIII	<b>Sections: 6 Appendix 6 &amp; 8</b>	<b>Select up to two:</b> <input checked="" type="checkbox"/> Authored <input type="checkbox"/> Acknowledged <input type="checkbox"/> Cleared
	<b>Signature: Dilara Jappar -S</b> <small>Digitally signed by Dilara Jappar -S  DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People,  cn=Dilara Jappar -S, 0.9.2342.19200300.100.1.1=2000371317  Date: 2018.08.08 09:32:08 -04'00'</small>			
Clinical Pharmacology Team Leader (*separate memo-to-file)	Insook Kim	OTS/OCP/DCPIII	<b>Sections: 6 Appendix 5-8</b>	<b>Select up to two:</b> <input checked="" type="checkbox"/> Authored <input type="checkbox"/> Acknowledged <input checked="" type="checkbox"/> Cleared
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Associate Director for Genomics and Targeted Therapy (OCP) (*separate memo-to-file)	Michael Pacanowski	OTS/OCP/GTTG	<b>Sections: 6 Appendix 5-8</b>	<b>Select up to two:</b> <input checked="" type="checkbox"/> Authored <input type="checkbox"/> Acknowledged <input checked="" type="checkbox"/> Cleared
	<b>Signature: Michael Pacanowski -S</b> <small>Digitally signed by Michael Pacanowski -S  DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People,  0.9.2342.19200300.100.1.1=2000350707, cn=Michael Pacanowski -S  Date: 2018.08.08 09:04:15 -04'00'</small>			

**Multi-Disciplinary Review  
NDA 208623 GALAFOLD (migalastat)**

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Statistical Reviewer	Min Min	OTS/OB/DBIII	Sections: 8	<b>Select up to two:</b> <input checked="" type="checkbox"/> Authored <input type="checkbox"/> Acknowledged <input type="checkbox"/> Cleared
	Signature: <b>Min Min -S</b>		 <small>Digitally signed by Min Min -S            DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Min Min -S, 0.9.2342.19200300.100.1.1=1300432372            Date: 2018.08.09 08:40:23 -04'00'</small>	
Statistical Team Leader	Yeh-Fong Chen	OTS/OB/DBIII	Sections: 8	<b>Select up to two:</b> <input checked="" type="checkbox"/> Authored <input type="checkbox"/> Acknowledged <input checked="" type="checkbox"/> Cleared
	Signature: <b>Yehfong Chen -S</b>		 <small>Digitally signed by Yehfong Chen -S            DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Yehfong Chen -S, 0.9.2342.19200300.100.1.1=1300157970            Date: 2018.08.09 09:22:32 -04'00'</small>	
Deputy Director (Acting) (DBIII)	Lei Nie	OTS/OB/DBIII	Sections: 1, 7 and 8	<b>Select up to two:</b> <input type="checkbox"/> Authored <input type="checkbox"/> Acknowledged <input checked="" type="checkbox"/> Cleared
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**Multi-Disciplinary Review  
NDA 208623 GALAFOLD (migalastat)**

DISCIPLINE	REVIEWER	OFFICE/DIVISION	SECTIONS AUTHORED/ACKNOWLEDGED/APPROVED	AUTHORED/ACKNOWLEDGED/APPROVED
Regulatory Affairs/ Project Management	Hong Vu	OND/ODEIII/DGIEP	<b>Sections: All</b>	<b>Select up to two:</b> <input type="checkbox"/> Authored <input checked="" type="checkbox"/> Acknowledged <input type="checkbox"/> Cleared
	<b>Signature: Hong Vu -S</b>			<small>Digitally signed by Hong Vu -S            DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Hong Vu -S, 0.9.2342.19200300.100.1.1=2000546905            Date: 2018.08.08 07:19:05 -04'00'</small>
Application Technical Lead	Hitesh Shroff	OND/DNDP/Branch V	<b>Section: 4.2</b>	<b>Select up to two:</b> <input checked="" type="checkbox"/> Authored <input type="checkbox"/> Acknowledged <input checked="" type="checkbox"/> Cleared
	<b>Signature: Hitesh N. Shroff -S</b>			<small>Digitally signed by Hitesh N. Shroff -S            DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=2000348333, cn=Hitesh N. Shroff -S            Date: 2018.08.08 10:10:34 -04'00'</small>
Clinical Reviewer	Anita Zaidi	OND/ODEIII/DGIEP	<b>Sections: 1.1, 2, 3, 7, 8, 9, 10, 11, 12, 13</b>	<b>Select up to two:</b> <input checked="" type="checkbox"/> Authored <input type="checkbox"/> Acknowledged <input type="checkbox"/> Cleared
	<b>Signature: Anita A. Zaidi -S</b>			<small>Digitally signed by Anita A. Zaidi -S            DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Anita A. Zaidi -S, 0.9.2342.19200300.100.1.1=2002237277            Date: 2018.08.08 11:11:23 -04'00'</small>
Clinical Team Leader and Cross Discipline Team Leader	Patroula Smpokou	OND/ODEIII/DGIEP	<b>Sections:</b> <b>Authored: sections 1.2, 1.3</b> <b>Cleared: all sections</b>	<b>Select up to two:</b> <input checked="" type="checkbox"/> Authored <input type="checkbox"/> Acknowledged <input checked="" type="checkbox"/> Cleared
	<b>Signature: Patroula I. Smpokou -S</b>			<small>Digitally signed by Patroula I. Smpokou -S            DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=2002001766, cn=Patroula I. Smpokou -S            Date: 2018.08.08 12:00:57 -04'00'</small>

**Multi-Disciplinary Review  
NDA 208623 GALAFOLD (migalastat)**

DISCIPLINE	REVIEWER	OFFICE/DIVISION	SECTIONS AUTHORED/ACKNOWLEDGED/APPROVED	AUTHORED/ACKNOWLEDGED/APPROVED
Division Director (Acting) (DGIEP)	Dragos Roman	OND/ODEIII/DGIEP	Sections: Authored: Section 14 Cleared: All	Select up to two: <input checked="" type="checkbox"/> Authored <input type="checkbox"/> Acknowledged <input checked="" type="checkbox"/> Cleared
	<b>Signature:</b> Dragos G. Roman - S <small>Digitally signed by Dragos G. Roman -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=1300160397, cn=Dragos G. Roman -S Date: 2018.08.08 18:23:48 -04'00'</small>			
ODE III Office Director	Julie Beitz	OND/ODEIII	Sections: Authored: Section 15 Cleared: All	Select up to two: <input checked="" type="checkbox"/> Authored <input type="checkbox"/> Acknowledged <input checked="" type="checkbox"/> Cleared
	<b>Signature: See DARRTS electronic signature page</b>			

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HONG VU  
08/10/2018

JULIE G BEITZ  
08/10/2018



DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration  
Center for Drug Evaluation and Research  
Silver Spring, MD 20993

<b>NDA or BLA Number</b>	NDA 208623
<b>Link to EDR</b>	<a href="\\CDSESUB1\evsprod\NDA208623\208623.enx">\\CDSESUB1\evsprod\NDA208623\208623.enx</a>
<b>Submission Date</b>	12/13/2017
<b>Submission Type</b>	Original submission, Priority Review
<b>Brand Name</b>	Galafold
<b>Generic Name</b>	Migalastat
<b>Dosage Form and Strength</b>	Capsule, 123 mg
<b>Route of Administration</b>	Oral
<b>Proposed Indication</b>	For the treatment of adults with Fabry disease and an amenable alpha-galactosidase A gene variant
<b>Applicant</b>	Amicus Therapeutics
<b>OND Division</b>	Division of Gastroenterology and Inborn Errors Products
<b>Associated IND</b>	IND 68,456
<b>OCP Review Team</b>	DCP3: Dilara Jappar, Insook Kim (TL) DARS: Jim Weaver, Paula Hyland DPM: Justin Earp, Lian Ma (TL)
<b>OCP Final Signatory</b>	Michael A Pacanowski (Associate Director, GTTG)

The Office of Clinical Pharmacology (OCP) review is complete and has been added to the multidisciplinary review and evaluation document. OCP's review is based on the information currently in the administrative record. If we must review information that is subsequently added to the administrative record, we will update my part of the multidisciplinary review and evaluation document accordingly.

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## 5. Appendix 5: Enrichment, Stratification, and/or Biomarker-Based Assessment

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DARS/Office of Clinical Pharmacology review of the GLP HEK-293 assay for determination the amenability of Fabry disease mutations to migalastat (AT1001)

James L. Weaver & Paula L. Hyland

### Executive summary

The Applicant designed and used an *in vitro* assay to screen over 700 individual galactosidase alpha (*GLA*) gene mutations to determine which Fabry disease mutations have the potential to respond to treatment with the investigational drug migalastat (AT1001). In turn, the Applicant used the *in vitro* data to identify patients with amenable mutations or mutant alpha-galactosidase A (alpha-Gal A) that should respond to the drug. *GLA* mutations that did not respond to migalastat *in vitro* assay were classified as nonamenable.

The assay used a conventional molecular biology and transient transfection methods to produce each mutant alpha-galactosidase alpha-Gal A enzyme in HEK-293 cells. Following treatment with migalastat, residual enzyme activity was measured in extracted lysates and activity was normalized to total protein content.

Our evaluation determined that the design of the assay is appropriate to the disease. (b) (4)  
(b) (4) the assay was properly validated and can be performed in a reproducible manner. Two review issues were identified.

Analyses of sequence data for wild-type (WT) and mutant *GLA* cDNA constructs (b) (4)  
(b) (4) indicated that bidirectional analysis of full length *GLA* was not completed by the Applicant. Further, 498 of 753 individual *GLA* cDNA mutations of the recombinant mutant constructs were confirmed by the Applicant using data from only one primer DNA strand because both the universal T7 (forward) and BGH (reverse) (referred to as BGHR by Applicant) primer reads were not long enough to cover the entire length of the double stranded cDNA target. Using matched data from both strands (as indicated in the Applicant's criteria) or bidirectional sequencing is the standard sequencing approach and best practice. Bidirectional sequencing is required to confirm each mutation by matching data from both strands as well as the base context of the entire cDNA sequence (which is double-stranded). Both, research and clinical mutations, as well as the DNA integrity of the target gene of interest are confirmed using bidirectional sequencing.

However, independent sequence data were generated for each of the 753 mutant *GLA* constructs by the Applicant via other sequencing laboratories (b) (4)  
(b) (4) While these independent data neither alone or in combination with the previous data from (b) (4) provide the required bidirectional sequence analysis, as discussed in detailed elsewhere in the appendix (see page 7), collectively these data

can provide sufficient interim confidence of mutation confirmation and DNA integrity to allow inclusion of 348 amenable mutations in labeling pending prospective completion of bidirectional analysis. As such, we recommend that all 753 recombinant *GLA* cDNA constructs (amenable and nonamenable) used to generate *in vitro* enzyme data from HEK-293 cells in support of NDA 208623 should be sequenced by the Applicant using additional internal bidirectional *GLA* primers.

The second issue relates to the clinical biomarker assays that were used to determine the *in vitro* to *in vivo* correlation. The Applicant used cutoff values that did not take into account the analytical performance of the assay. Using adjusted cutoff values, the analysis showed that the correlation of the HEK-293 assay with clinical biomarker response is only moderate at best. Overall, a mutation classified as amenable by the HEK-293 assay had around a 50% chance of showing a response for any one of the three clinical biomarkers evaluated. Because the assay does not have high positive or negative predictive value, a therapeutic trial period with measurement of WBC alpha-Gal A and plasma lyso-GB3 should be considered.

### **Background**

Fabry disease (FD) [MIM: 301500] is a rare X-linked inherited lysosomal storage disorder caused by mutations in *GLA* which encodes the enzyme alpha-Gal A. The enzyme is synthesized as a 429 amino acid precursor from which the signal peptide is removed to yield a 398 amino acid glycoprotein forming a homodimer. Glycosylation of alpha-Gal A allows transport to lysosomes by mannose-6-phosphate receptors (M6PR). Mutations in the *GLA* gene lead to a deficiency in enzyme activity that results in accumulation of intra-lysosomal globotriaosylceramides (GL-3) deposits in various cell types and/or organs. Clinically the most affected organs include the kidney, heart, brain/central nervous system, gastrointestinal tract, and skin. To date, 922 *GLA* mutations have been described and 865 are reported as Fabry- or disease-causing mutations by The Human Gene Mutation Database (HGMD<sup>®</sup> Professional 201.1; <http://www.hgmd.cf.ac.uk/ac/index.php>) with an additional 54 reported as possible FD-causing mutation. The severity of disease and phenotype vary with the specific mutation and by sex. Females carrying a mutated allele can demonstrate a wide range of disease severity, from virtually asymptomatic to the more classical severe phenotype because females will have a mosaic of healthy and diseased cells. This mosaicism occurs in somatic cells because one of the two X chromosomes in females is randomly and permanently inactivated in cells.

The Applicant, Amicus Therapeutics has submitted NDA 208623 seeking approval for the use of the small molecule drug migalastat as a treatment for FD. Migalastat (can act as a competitive inhibitor of alpha-Gal A at high doses, but at lower doses acts as pharmacological chaperone that reversibly binds to the active site of alpha-Gal A, stabilizing the enzyme, allowing it to move from the endoplasmic reticulum to the lysosomes. Once in the lysosomes which has higher accumulated level of substrate and a lower pH (resulting in lower binding affinity), migalastat can dissociate from the alpha-Gal A, allowing the enzyme to bind and break down globotriaosylceramide (GL-3) and related substrates. Mutation of the *GLA* gene, which encodes alpha-Gal A, can result in the production of mutant forms of the enzyme that retain enzymatic activity but are abnormally folded and less stable, resulting in less of the enzyme reaching the

lysosomes where it functions. Specific alpha-Gal A mutants, the genotypes of which are referred to as amenable can be stabilized by migalastat *in vitro* increasing cellular levels and the enzymatic activity of these mutant forms.

The Applicant has submitted one placebo-controlled clinical trial with 62 total FD patients with 38 unique mutations. These small numbers reflect the rarity of the disease and low prevalence.

(b) (4)

**Description of the GLP HEK-293 assay**

This GLP HEK-293 assay is a multi-step system that uses conventional molecular, cellular and enzymatic methods.

(b) (4)

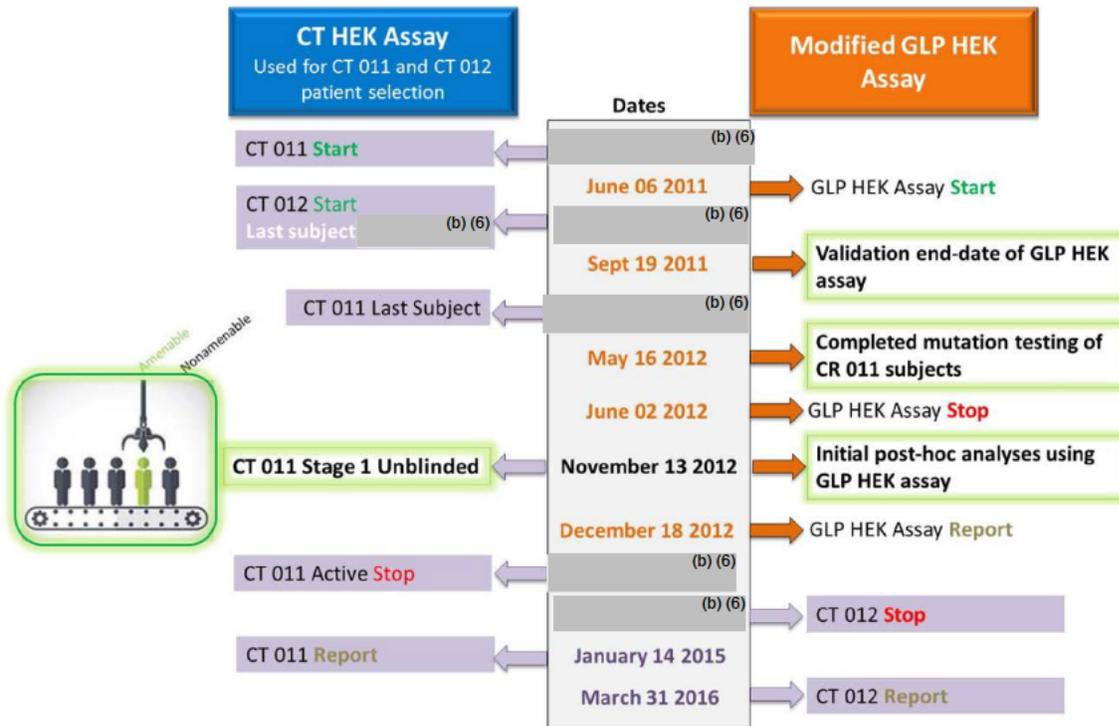
(b) (4)

Final residual alpha-Gal A enzyme activity was reported as the average ( $\pm$  SEM) in nmol/mg/hour for twenty data points for each treatment. If the value was below 142 nmol/mg/hour (pg. 58, RR10001-26) then the average value was reported as below the limit of detection (BLD). Residual enzyme activity was reported as fold change compared to base line and absolute enzyme activity in the presence of migalastat compared to percentage of the WT (untreated) control. The Applicant set thresholds of an increase of 3% or more in absolute enzyme activity and a 1.2-fold (20%) increase compared to untreated baseline to be declared amenable. For BLD mutants only the absolute activity increase was used to determine amenability.

### **History of the assay**

The iteration of the HEK-293 assay used to select patients for CT-011 is referred to as the Clinical Trial (CT) HEK-293 assay. This assay entered development on 5/1/2007. Active work was reported to have ended on 3/31/2012 and the report was issued 7/30/2012 (RR1001-19). During the discussion with the Agency, the Agency recommended the formal validation of the HEK-293 assay and recruitment based on the validated assay. Clinical trial AT1001-11 (CT-011) began on (b) (6) and the last patient was recruited (b) (6). The final version of the

HEK-293 assay is referred to as the GLP HEK-293 assay. Work began on 6/6/2011 and ended 2/6/2012. The results of the GLP work resulted in reclassification of some mutations in both directions. These data were available on 5/6/2012. The data from Stage 1 of CT-011 was unblinded on 11/13/2012, thus all patients recruited into CT-011 were recruited based on the data from the CT version. See Figure 2 below for the complete timeline.



**Figure 2: Timeline of HEK-293 assay development relative to clinical trials 11 and 12**

Recruiting of CT12 was begun using the CT assay classifications but was switched to the GLP assay when the data became available.

### Initial evaluation

The initial evaluation of the suitability of the assay is based on the specific factors listed in a previous publication (Durmowicz et al, 2018).

*Is the understanding of the disease sufficiently well documented in the scientific literature so that the consequences of individual mutations can be understood in most cases?*

Fabry disease is caused by any one of over 800 mutations in the *galactosidase alpha (GLA)* gene (<http://www.hgmd.cf.ac.uk>) which is located on the long arm of the X chromosome at position 22.1. Mutations are widely distributed throughout the structure of the protein (Garman, 2007). In male patients, the severity of the disease correlates with a decrease or deficit of residual alpha-Gal A activity measured in peripheral blood leukocytes. In females, the correlation is less certain due to random X-chromosome inactivation.

*Is the mechanism of action of the drug sufficiently well understood and consistent with the known mechanism of the disease?*

Mutation of the *GLA* gene which encodes alpha-Gal A can result in the production of mutant forms that retain enzymatic activity, but are abnormally folded and less stable resulting in less of the enzyme reaching the lysosomes where it functions. The drug binds to the active site of the enzyme which stabilizes the mutant (and WT) form allowing it to be trafficked to the lysosomes. Once in the lysosomes where there is a higher accumulated level of substrate and a lower pH (resulting in a high *K<sub>i</sub>*), the drug dissociates, allowing the enzyme to bind and break down its substrates. An X-ray crystallography structure of the drug bound to the active site of alpha-Gal A has been published (Yu et al, 2014).

*Is there sufficient clinical data from both drug-responsive and drug-nonresponsive mutations to allow adequate evaluation of the predictive power of the *in vitro* assay?*

Data are available from the AT1001-011 phase 3 clinical trial that enrolled 62 migalastat-treated patients and 31 patients in the placebo group. Of the treated patients, 47 (76%) were classified as amenable by the *in vitro* GLP HEK-293 assay with the remaining 15 classified as nonamenable. A total of 38 unique mutations were found among the 62 patients. A total of 30 were classified as amenable by the GLP HEK-293 assay and the remaining 8 were nonamenable. Additional patients were studied in the AT1001-012 clinical trial but the data are not useful for evaluating the predictive value of the *in vitro* assay as only two nonamenable mutations were found among the 51 treated patients.

*Does the *in vitro* assay directly measure the function of the human protein affected by the disease?*

The assay measures the ability of recombinant WT and mutant alpha-Gal A to cleave the fluorogenic substrate 4-methylumbelliferyl-alpha-D galactopyranoside (4-MUG) *in vitro*. The enzyme assay for evaluating function *in vitro* incorporates a widely used standard test for measuring alpha-Gal A activity that was originally published in 1981 (Mayes et al, 1981). A caveat of the assay is the fact that measurement of enzyme activity is carried out in a tube or cell-free environment using total extracted protein, and does not inform on the requirement for the amenable or stabilized protein to be correctly localized to the lysosomes as a prerequisite for function and amenability *in vivo*.

*Are the full performance metrics of the assay reported and has the assay been formally validated?*

The assay is well characterized and formal validation of all assay stages (except for DNA sequencing of *GLA* cDNA plasmids/vectors in Step 1) has been performed and is reported in the NDA as: "AT1001: a bioanalytically validated, HEK-293 cell-based assay to identify mutant forms of alpha-Gal A that respond to AT1001, RR1001-26 Pharmacology report".

*Are the raw instrument data available so that the entire pathway from raw data to the results reported in the NDA tables can be recreated?*

Raw instrument data were requested and received from the Applicant. The recreation is described in detail below.

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### **Evaluation of predictive power versus clinical biomarkers**

The Applicant used three clinical biomarkers to evaluate the predictive power of the HEK-293 assay. These were residual alpha-Gal A activity in white blood cells (WBC), kidney capillary GL-3 inclusions and plasma lyso-GB3. For each clinical biomarker we will discuss cutoffs, 2x2 table results and numerical correlations.

#### **WBC Alpha-Gal A enzyme activity**

Residual alpha-Gal A activity in WBC is measured using the same fluorescent substrate used in the HEK-293 assay system. Data are reported as nM product, i.e., 4-MU produced per hour per mg of total cell protein. The Applicant reports that 22 nM/hr/mg protein is the value for WBC from healthy volunteers (Report RR1001-34, page 25). Baseline values for male patients ranged from 0 to 41% of WT but with all but one patient less than 5% of WT. For female patients, baseline values ranged from 6.7% to over 100% of the WT value.

#### *Applicant cutoffs for responsive vs. nonresponsive – alpha-Gal A*

The cutoff used for the WBC alpha-Gal A activity assay was reported as percent of WT activity. An increase of 2% over baseline measurement was declared to be a clinical response for this

biomarker in later studies such as RR1001-66. In earlier studies, such as RR1001-22, a cutoff of 3% was used. The Applicant stated that non-classical patients have WBC alpha-Gal A levels of 1-5% whereas classic patients are < 1%. Based on these values they claim that an increase of 2% represents a clinically meaningful change in WBC alpha-Gal A activity. This threshold does not consider the analytical variability of the WBC alpha-Gal A assay.

*FDA’s alternate cutoffs for responsive vs. nonresponsive – alpha-Gal A*

The method validation report (Study AA42942-05, Appendix 2) includes data on the WT WBC lysate quality control standard. Across 5 batches with 18 samples, the inter-run mean was 44.9 µM of 4-MU produced with a standard deviation of 1.75 and a CV of 3.6%. Based on these data, the threshold was set at a value of 4% although higher values could be defended. Sensitivity analysis (Table 11) shows that the 2x2 table outcome parameters are relatively insensitive to changes in the cutoff for this biomarker.

**Table 11: Effect of changing the delta % change cutoff value for WBC alpha-Gal A activity on 2x2 table outcome measures.** Open box – Applicant cutoff, pale green box – adjusted cutoff by FDA

		Parameter Sensitivity Analysis								
		CT 11								
		GLP HEK vs WBC GLA								
		Male Patients								
		0.50%	1.00%	2.00%	3.00%	4.00%	5.00%	6.00%	8.00%	10.00%
Sensitivity		0.89	0.94	0.91	0.90	0.89	0.88	0.88	0.86	0.83
Specificity		0.75	0.67	0.36	0.33	0.31	0.29	0.29	0.27	0.25
PPV		0.94	0.88	0.59	0.53	0.47	0.41	0.41	0.35	0.29
NPV		0.60	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
% discordant:		13.6%	13.6%	36.4%	40.9%	45.5%	50.0%	50.0%	54.5%	59.1%

*2 x 2 table evaluations – alpha-Gal A*

A simple evaluation of predictive ability is the standard 2x2 table. Table 12 shows the 2x2 table and associated outcome values for the comparison between the GLP HEK-293 assay call and the WBC alpha-Gal A call using the adjusted cutoff values. This table is for all patients in CT11.

**Table 12: GLP HEK-293 assay calls versus CT-011 WBC alpha-Gal A activity for all treated patients, adjusted cutoff set at greater than or equal to 4% change**

CT 11		4% call	
GLP HEK vs WBC GLA			
ALL Patients		Clinical Effect	
		Yes	No
In vitro effect	Yes	30	16
	No	9	6
	Sensitivity	0.77	
	Specificity	0.27	
	PPV	0.65	
	NPV	0.40	
	Total pts	61	
	Total discordant:	25	
	% discordant:	41.0%	

Table 13 shows the same type of analysis but this time divided by the sex of the patient.

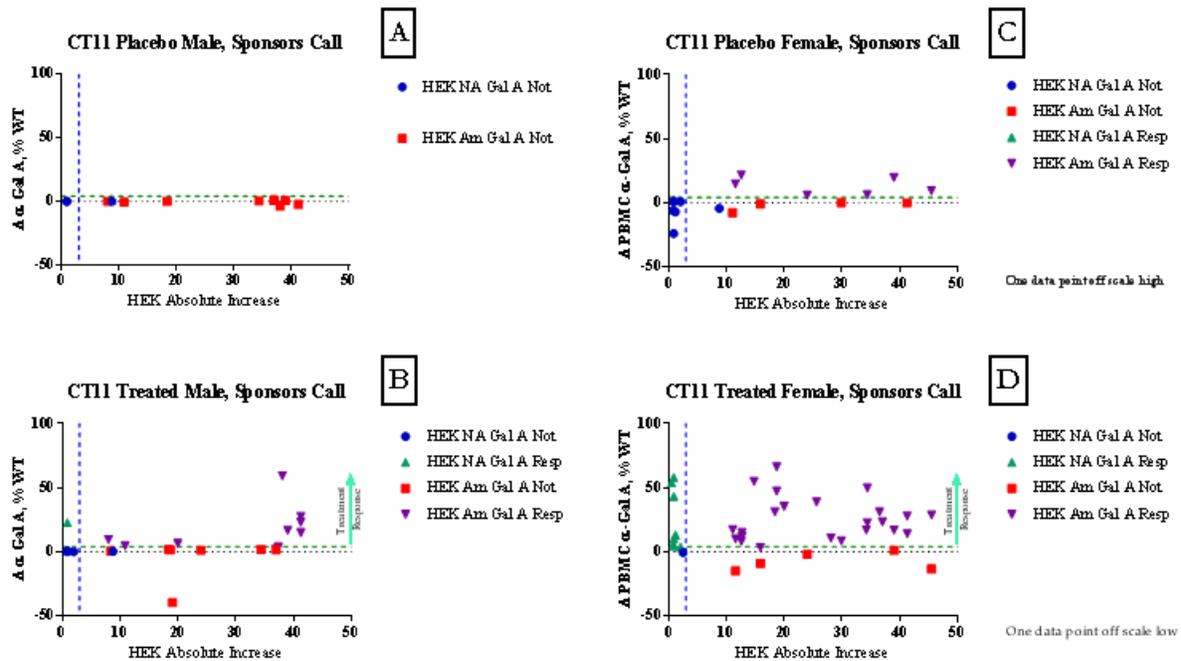
**Table 13: GLP HEK-293 assay calls versus CT11 WBC alpha-Gal A activity calls for treated patients grouped by sex, adjusted cutoff set at  $\geq$  4% change from baseline. A – treated Male patients, B – treated Female patients**

A				B			
CT 11		4% call		CT 11		4% call	
GLP HEK vs WBC $\alpha$ -Gal A				GLP HEK vs WBC $\alpha$ -Gal A			
Male Patients		Clinical Effect		Female Patients		Clinical Effect	
		Yes	No			Yes	No
In vitro effect	Yes	8	9	In vitro effect	Yes	22	7
	No	1	4		No	8	2
	Sensitivity	0.89			Sensitivity	0.73	
	Specificity	0.31			Specificity	0.22	
	PPV	0.47			PPV	0.76	
	NPV	0.80			NPV	0.20	
	Total pts	22			Total pts	39	
	Total discordant:	10			Total discordant:	15	
	% discordant:	45.5%			% discordant:	38.5%	

In the analysis by sex, we observed the expected dichotomy in male versus female responses. As the in vivo enzyme activity in the males is due to mutant enzyme, the negative prediction of the HEK-293 assay is reasonable. In contrast, the NPV in female patients is poor due to the confounding effect of the activity of normal or WT enzyme as a result of expression from the active (nonmethylated) X allele. PPV is notably better for female than for male patients and this is likely due to the stabilizing effect of migalastat on the WT alpha-Gal A protein. Because X inactivation results in mosaics comprising a mixture of both WT and mutant alpha-Gal A cells in female FD patients, neither the baseline activity nor the effect of migalastat on the mutant alpha-Gal A can be accurately determined.

### Numerical correlations – alpha-Gal A

The relationship between the GLP HEK-293 absolute increase and the change in WBC alpha-Gal A activity is shown in Figure 5. The scatter in the data in placebo-treated patients between baseline and six months are shown in plot A for male patients and plot C for female patients (Right panel). The corresponding plots for migalastat-treated male patients are in plot B and female patients in plot D (Left panel). In treated patients, there is no relationship between the HEK absolute increase and the WBC alpha-Gal A enzyme activity. In fact, clear changes in alpha-Gal A biomarker activity are seen with mutations reported as nonamenable by the HEK-293 assay.



**Figure 5:** Relationship between WBC alpha-Gal A enzyme activity and GLP HEK-293 assay absolute activity. A: Male placebo, B: Male migalastat-treated, C: Female placebo, D: Female migalastat-treated. Color/shape coding for the symbols is per the Applicant’s calls for each parameter. HEK NA-nonamenable; HEK Am-amenable; Gal-A Not - not responsive; Gal-A Resp – responsive. The adjusted cutoffs are shown by the horizontal green dashed lines. The vertical blue dashed line shows the Applicant’s amenable versus nonamenable cutoff value.

### Kidney capillary GL-3 inclusions

Kidney capillary GL-3 inclusions are inclusion bodies of globotriaosylceramides that are found in the cytoplasm of kidney capillary endothelial cells among other locations. These are measured by counting inclusions in sections cut from plastic-embedded needle biopsy specimens. Slides are scored by two pathologists who examine the tissue sections at 1000x. From 50 to 100 capillaries are scored per slide. Data are reported as inclusions per capillary, the data at baseline range from 0.03 to 5.7.

#### *Applicant cutoffs for responsive vs. nonresponsive – kidney GL-3 inclusions*

For kidney GL3 inclusions, the Applicant called any negative change in the number of inclusions/capillary as a clinical response for this biomarker.

#### *Alternate cutoffs for responsive vs. nonresponsive – kidney GL-3 inclusions*

As these counts were made by only two pathologists, usual measures of variability are not appropriate. However, the Applicant did report counts by pathologist for a total of 43 individual patient biopsy samples from phase 2 trials 202, 203 and 204. Overall, the agreement between the two pathologists is reasonable ( $r^2=0.89$ ). When examined at the individual sample level the average disagreement amounted to 75% of the mean count. This value is high due to the significant number of low absolute count values. For example, if the individual counts are 0.1 for pathologist A versus 0.2 for pathologist B then the percent difference is 66%. To somewhat compensate for this exaggeration at low values, the cutoff was set at -50%. The sensitivity analysis showing the effect of large changes in the cutoff value on the 2x2 table outcome values is presented in Table 14.

In addition, we looked at the change in the absolute number of inclusion/capillary. The median difference between the two pathologists' counts for the 43 phase 2 slides was 0.2. A cutoff of a reduction of at least 0.2 was selected for further analysis. The performance metrics were similar to those obtained using the % cutoff values (not shown).

**Table 14: Effect of changing the delta % change cutoff value for kidney capillary GL-3 inclusions on 2x2 table outcome measures.** Open box – Applicant cutoff, pale green box – adjusted cutoff

CT 11		Parameter Sensitivity Analysis					
Call on delta %		GLP HEK vs Kidney Capillary GL-3 inclusions					
ALL Patients		delta %					
	<0%	-5%	-10%	-25%	-50%	-75%	
Sensitivity	0.85	0.93	0.93	0.93	0.88	0.78	
Specificity	0.38	0.43	0.43	0.41	0.30	0.25	
PPV	0.65	0.63	0.63	0.60	0.35	0.16	
NPV	0.64	0.86	0.86	0.86	0.86	0.86	

*2 x 2 table evaluations – Kidney GL-3 inclusions*

The results comparing the calls for the GLP HEK-293 assay versus the kidney capillary GL3 inclusions for all patients in CT11 are shown in Table 15. Sensitivity and NPV are reasonable while specificity and PPV are not good. The same data set split for sex are shown in Table 16. No dramatic sex effect is observed for this biomarker.

**Table 15: GLP HEK-293 assay calls versus CT11 kidney capillary inclusion % change for all treated patients, adjusted cutoff set at least 50% decrease (-50%)**

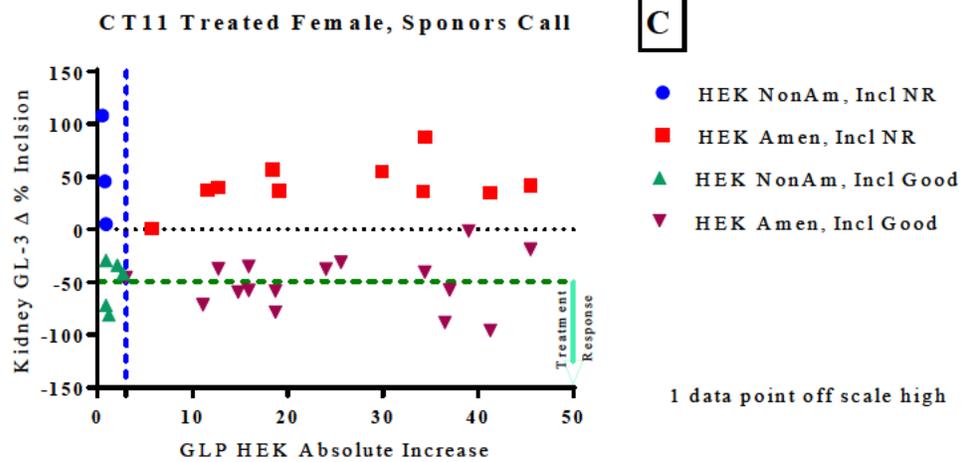
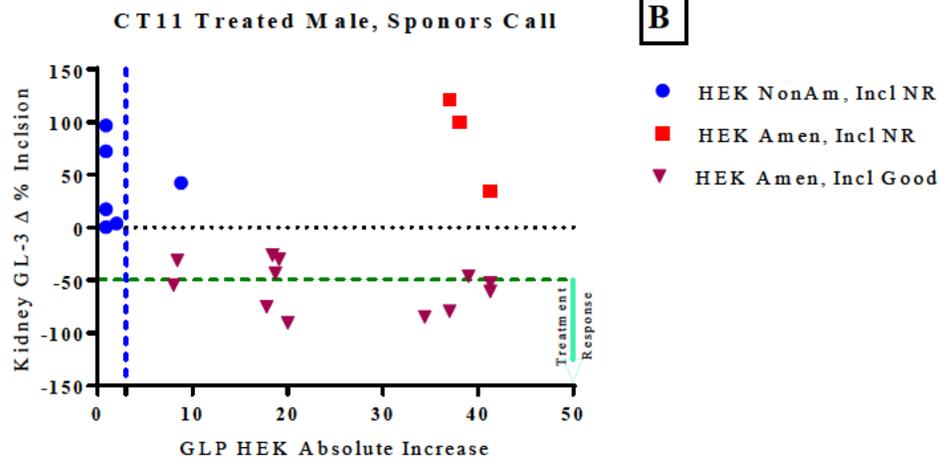
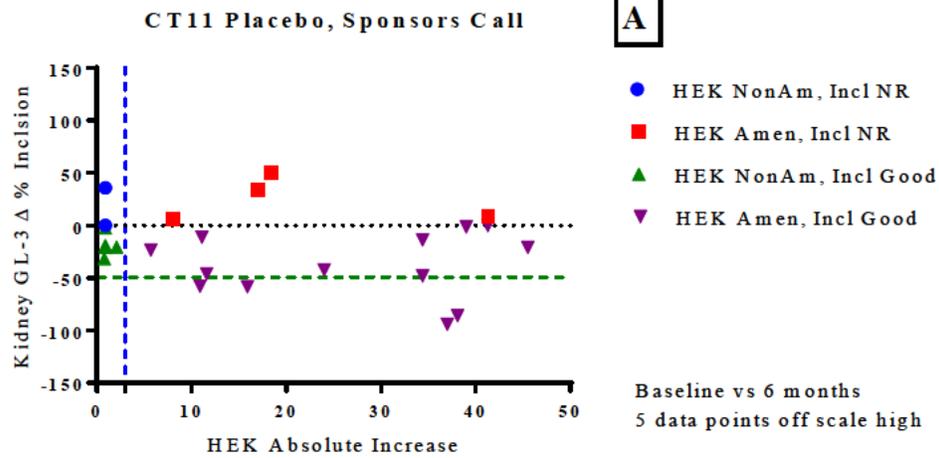
CT 11	Call on delta % at:	-50%	
GLP HEK vs Kidney Capillary GL-3 incl			
ALL Patients		Clinical Effect	
		Yes	No
In vitro effect	Yes	15	27
	No	2	12
	Sensitivity	0.88	
	Specificity	0.31	
	PPV	0.36	
	NPV	0.86	
	Total pts	56	
	Actual pts:	64	
	missing:	8	
	Total discordant:	29	
	% discordant:	51.8%	

**Table 16: GLP HEK-293 assay calls versus CT11 kidney capillary inclusion % change for treated patients grouped by sex, adjusted cutoff set at -50% change. A – Male treated patients, B – Female treated patients**

<b>A</b>				<b>B</b>			
CT 11	Call on delta % at:	-50%		CT 11	Call on delta % at:	-50%	
GLP HEK vs Kidney Capillary GL-3 incl				GLP HEK vs Kidney Capillary GL-3 incl			
Male Patients		Clinical Effect		Female Patients		Clinical Effect	
		Yes	No			Yes	No
In vitro effect	Yes	7	8	In vitro effect	Yes	8	19
	No	0	6		No	2	6
	Sensitivity	1.00			Sensitivity	0.80	
	Specificity	0.43			Specificity	0.24	
	PPV	0.47			PPV	0.30	
	NPV	1.00			NPV	0.75	
	Total pts	21			Total pts	35	
	Actual male pts:	24			Actual female pts:	40	
	missing:	3			missing:	5	
	Total discordant:	8			Total discordant:	21	
	% discordant:	38.1%			% discordant:	60.0%	

*Numerical correlations – Kidney capillary GL-3 inclusions*

The relationship between the two measures of changes in kidney capillary GL-3 inclusions and changes in HEK-293 assay absolute increase are shown in Figure 6. For both measures, there is no obvious relationship between GL-3 inclusions and HEK-293 absolute value.



caption next page

**Figure 6:** Relationship between % change in kidney GL-3 inclusions and GLP HEK-293 assay absolute activity. A: placebo for both sexes; B: migalastat treated male patients C: migalastat treated female patients. Color/shape coding for the symbols is per the Applicant's calls for each parameter. HEK NonAm = nonamenable; HEK Amen = amenable; Incl NR = GL-3 inclusion count not responsive; Incl Good = GL-3 inclusion count responsive. The adjusted cutoffs are shown by the horizontal green dashed lines. The vertical blue dashed line shows the Applicant's amenable versus nonamenable cutoff value.

### **Plasma lyso-GB3**

Lyso-GB3 is a breakdown product of globotriaosylceramides. This is measured in plasma using a conventional validated liquid chromatography/mass spectrometry assay. The level in healthy subjects is reported as  $0.53 \pm 0.09$  nmol/L (Sakuraba et al, 2017). Patient levels at baseline in CT11 ranged from 1.19 to 249.3 nmol/L.

#### *Applicant cutoffs for responsive vs. nonresponsive – plasma lyso-GB3*

For plasma lyso-GB3 concentration the Applicant called any reduction from baseline as a clinical response for this biomarker.

#### *Alternate cutoffs for responsive vs. nonresponsive – plasma lyso-GB3*

For plasma lyso-GB3, the Applicant used a validated liquid chromatography/mass spectrometry method for quantitation. Inter-assay precision was evaluated across four studies and ranged from 2.3% to 9.9%. A cutoff value of -10% was applied to this data set.

#### *2 x 2 table evaluations – plasma lyso-GB3*

The analysis for all patients is shown in Table 17 and split by sex in Table 18. The larger number of missing patients is reported by the Applicant to be due to issues with the patient consent form. The overall performance is similar to the other biomarkers. The sex specific data shows a larger tilt in favor of male patients, but the overall number of male patients is small.

**Table 17: GLP HEK-293 assay calls versus CT11 plasma lyso-GB3 % change for all treated patients, adjusted cutoff set at -10% change**

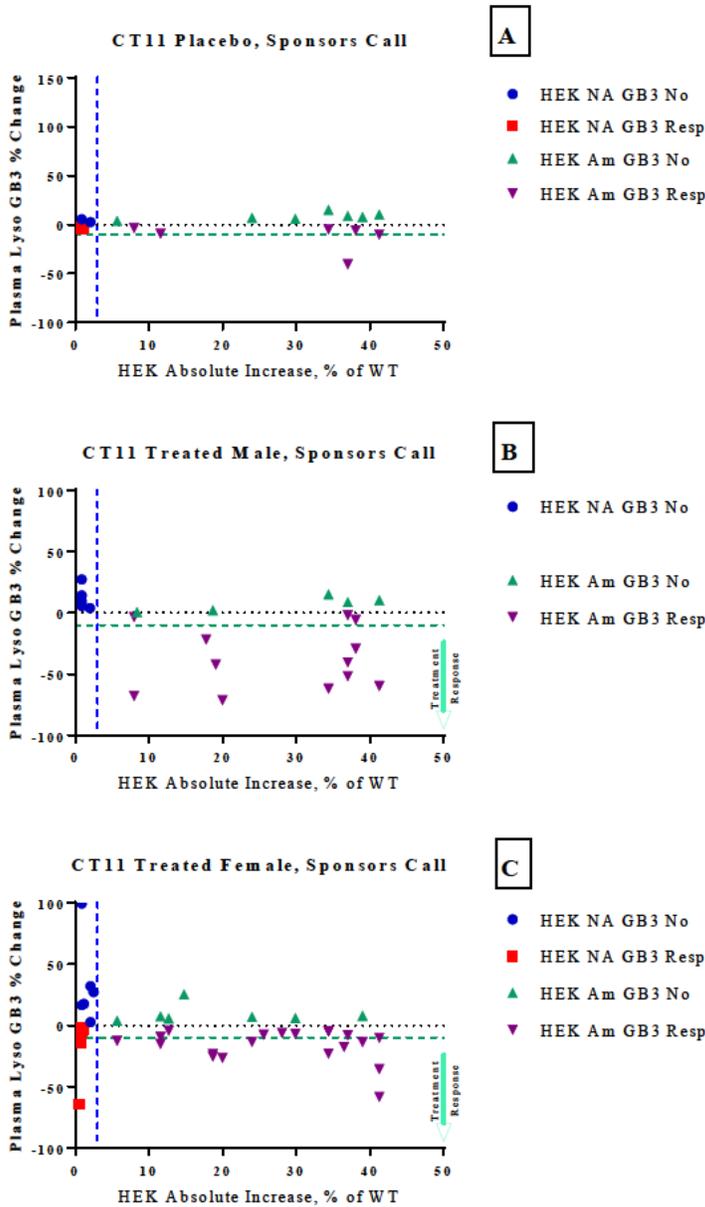
CT 11		10% cutoff	
GLP HEK vs Plasma Lyso-GB3			
ALL Patients		Clinical Effect	
		Yes	No
In vitro effect	Yes	21	23
	No	0	15
Sensitivity		1.00	
Specificity		0.39	
PPV		0.48	
NPV		1.00	
Total pts		59	
Actual pts:		61	
missing:		2	
Total discordant:		23	
% discordant:		39.0%	

**Table 18: GLP HEK-293 assay calls versus CT11 plasma lyso-GB3 % change for treated patients grouped by sex, adjusted cutoff set at -10% change. A – male patients, B – female patients**

<b>A</b>				<b>B</b>			
CT 11		10% cutoff		CT 11		10% cutoff	
GLP HEK vs Plasma Lyso-GB3				GLP HEK vs Plasma Lyso-GB3			
Male Patients		Clinical Effect		Female Patients		Clinical Effect	
		Yes	No			Yes	No
In vitro effect	Yes	9	8	In vitro effect	Yes	12	15
	No	0	5		No	0	10
	Sensitivity	1.00			Sensitivity	1.00	
	Specificity	0.38			Specificity	0.40	
	PPV	0.53			PPV	0.44	
	NPV	1.00			NPV	1.00	
	Total pts	22			Total pts	37	
	Actual pts	22			Actual pts	39	
	Total discordant:	8			Total discordant:	15	
	% discordant:	36.4%			% discordant:	40.5%	

*Numerical correlations – plasma lyso-GB3*

The relationship between % change in plasma lyso-GB3 and HEK absolute increase is shown in Figure 7. As seen with the other clinical biomarkers, there is no relationship between these two parameters.



**Figure 7:** Relationship between plasma lyso-GB3 and GLP HEK-293 assay absolute activity. A – placebo, both sexes; B – migalastat-treated male patients; C- migalastat-treated female patients. Color/shape coding for the symbols is per the Applicant’s calls for each parameter. HEK NA-nonamenable; HEK Am-amenable; GB3 No – GB3 value not responsive; GB3 Resp – GB3 value responsive. The adjusted cutoffs are shown by the horizontal green dashed lines. The vertical blue dashed line shows the Applicant’s amenable versus nonamenable cutoff value.

### Agreement among endpoints

At least one biomarker result was reported for 62 patients from CT11. Biomarker calls are based on adjusted cutoffs and not the Applicant cutoffs. Calls for all 3 biomarkers were available for 39 patients, 2 calls for 19 and only one call for 4 patients. For patients with two or three reported biomarker results the biomarkers agreed with each other for 23 of 62 patients (37%). The detailed categories are shown in Table 19. Agreement could be a biomarker response above adjusted threshold values (Good) or no response above the threshold (Non/Limited).

**Table 19: Agreement among clinical biomarker calls**

<b>Group</b>	<b>#</b>	<b>%</b>
Three Biomarkers Agree Good	3	4.7
Three Biomarkers Agree Non/Limited	7	10.9
Three Biomarkers Disagree	25	39.1
Two Biomarkers Agree Good	7	10.9
Two Biomarkers Agree Non/Limited	6	9.4
Two Biomarkers Disagree	12	18.8
Single Biomarker Report	4	6.3
<b>Total:</b>	<b>64</b>	<b>100.0</b>

### Agreement between endpoints and HEK-293 assay

The adjusted biomarker calls were compared with the Applicant call for the GLP HEK assay. The results are shown in Table 20. Total or majority agreement between the clinical biomarker call and the GLP HEK assay were seen for 30 of 64 patients (46.9%), including the single biomarker calls.

**Table 20: Agreement between HEK-293 and clinical biomarker calls**

<b>Group</b>	<b>#</b>	<b>%</b>
2 or 3 Biomarkers Perfect Agreement w/HEK + or -	10	15.6
2 of 3 Biomarkers Agree w/HEK	17	26.6
2 of 3 Biomarkers Disagree w/HEK	8	12.5
2 Biomarkers Disagree w/HEK	12	18.8
2 or 3 Biomarker Perfect Disagreement w/HEK	13	20.3
Single Biomarker Agrees w/HEK	3	4.7
Single Biomarker Disagrees w/HEK	1	1.6
<b>Total</b>	<b>64</b>	<b>100.0</b>

## Summary Results

The overall performance of the HEK-293 assays compared to all three clinical biomarkers are shown below for all patients in Table 21 and split by sex in Table 22. Overall the PPV is around 0.5 and the NPV around 0.75.

**Table 21: Consolidated 2x2 table outcome values for clinical biomarkers vs the GLP HEK-293 assay. Data for all patients, grouped by clinical biomarker**

	GLP HEK vs Biomarker		adjusted cutoffs		
	ALL Patients				
	WBC Gal A	Kidney GL3	Lyso GB3		Average
Sensitivity	0.77	0.88	1.00		0.88
Specificity	0.27	0.31	0.39		0.33
PPV	0.65	0.36	0.48		0.50
NPV	0.40	0.86	1.00		0.75

**Table 22: Consolidated 2x2 table outcome values for clinical biomarkers vs the GLP HEK-293 assay. Data grouped by sex for each clinical biomarker**

	GLP HEK vs Biomarker		adjusted cutoffs				
	Patients By Gender						
	WBC Gal A		Kidney GL3		Plasma Lyso GB3		
	Male	Female	Male	Female	Male	Female	Average
Sensitivity	0.89	0.73	1.00	0.80	1.00	1.00	0.90
Specificity	0.31	0.22	0.43	0.24	0.38	0.40	0.33
PPV	0.47	0.76	0.47	0.30	0.53	0.44	0.49
NPV	0.80	0.20	1.00	0.75	1.00	1.00	0.79

## Conclusions

The data and analysis of these studies leads to several conclusions and recommendations. The overall design of the HEK-293 assay is acceptable. The performance metrics of the assay show acceptable results.

Evaluation of the sequencing of *GLA* cDNA constructs indicated that bidirectional analysis of full length *GLA* was not completed by the Applicant. Using matched data from both strands of the cDNA or bidirectional sequencing is the standard sequencing approach and best practice. Further, 498 of 753 individual *GLA* mutations or recombinant mutant constructs (230 amenable and 268 nonamenable) were confirmed using data from only one single strand and not matched data from both strands (as indicated in the Applicant's criteria). We recommend that all 753 mutant *GLA* cDNA constructs (amenable and nonamenable) used to generate *in vitro* enzyme data in the HEK-293 system should be sequenced using additional internal bidirectional *GLA* primers to complete bidirectional sequencing of full length *GLA*.

The correlation of the HEK-293 assay with clinical biomarkers is moderate at best. Overall, a mutation classified as amenable by the HEK-293 assay had only around a 50% chance of showing a response for any one of the three clinical biomarkers evaluated. We suggest that the text of the label should be clear that an amenable mutation has only some chance of a clinical response. Because the assay does not have high positive or negative predictive value, a therapeutic trial period with measurement of WBC alpha-Gal A and plasma lyso GB3 should be considered.

## References

Durmowicz AG, Lim R, Rogers H, Rosebraugh CJ, Chowdhury BA. (2018) The U.S. Food and Drug Administration's experience with ivacaftor in cystic fibrosis. *Ann Am Thorac Soc* **15**, 1-2.

Garman SC. (2007) Structure-function relationships in alpha-galactosidase A. *Acta Paediatrica* **96**, 6-16.

Mayes JS, Scheerer JB, Sifers RN, Donaldson ML. (1981) Differential assay for lysosomal alpha-galactosidases in human tissues and its application to Fabry's disease. *Clin Chimca Acta* **112**, 247-251.

Sakuraba H, Togawa T, Tsukimura T, Kato H. (2017) Plasma lyso-GB3: a biomarker for monitoring fabry patients during enzyme replacement therapy. *Clin Exp Nephrol* **epub**, doi: 10.1007/s10157-017-1525-3

Yu Y, Mena-Barragan T, Higaki K, Johnson JL, Drury JE, Lieberman RL, Nakasone N, Ninomiya H, Tsukimura T, Sakuraba H, Suzuki Y, Nanba e, Mellet CO, Fernandez JM, Ohno K. (2014) Molecular basis of 1-deoxygalactonojirimycin arylthiourea binding to human alpha-galactosidase A: Pharmacological chaperoning efficacy on Fabry disease mutants. *ACS Chem Biol* **9**, 1460-1469.

## 6. Summary of Bioanalytical Method Validation and Performance

Brian Furmanski & Dilara Jappar

The Applicant developed several analytical methods to support the use of migalastat in the treatment of (b) (4) patients (b) (4) with Fabry disease who have amenable mutations. The FDA determined that the analytical methods for the quantitation of the drug product migalastat and biomarkers (iohexol, lyso-Gb3 and the C22:0 and C24:0 isoforms of GL-3) were adequately designed and executed. The below figures and tables were taken from there respective bioanalytical reports provided by the Applicant.

### 6.1. Migalastat

#### 6.1.1. Bioanalytical Method Validation for Migalastat:

The Office of Clinical Pharmacology review team has assessed the adequacy and acceptability of the following bioanalytical methods used to quantify migalastat in clinical studies AT1001-011, FAB-CL-201, FAB-CL-204 and FAB-CL-205 and phase 1 studies AT1001-016, AT1001-015, AT1001-013, AT1001-010, FAB-CL-101 and FAB-CL-102 for the quantation of migalastat.

The Sponsor developed a liquid chromatography tandem mass spectrometry (LC/MS/MS) method to quantify migalastat in plasma containing EDTA (AA05326-01). Briefly, analyte and internal standard were extracted using a solid phase extraction. The extracted samples were analyzed by an HPLC equipped with an AB/MDS Sciex API 4000 mass spectrometer. The method utilized an isotopically-labelled internal standard (<sup>13</sup>C-d2-AT1001) of migalastat. The assay was validated to a lower limit of quantitation (LLOQ) of 5.88 ng/mL using 0.150 mL of plasma. The method was sufficiently validated to support PK studies with adequate dynamic range.

During the method validation process, the Sponsor established that migalastat is stable in plasma matrix at -20°C for 1007 days. All PK plasma samples from phase 1, phase 2, and phase 3 studies were analyzed within 1007 days of collection.

Matrix	Human plasma with K3EDTA		
Test article purity Internal standard purity		<b>Analyte</b>	<b>Internal Standard (IS)</b>
	ID	AT-1001 HCl	d <sub>2</sub> -AT-1001 <sup>13</sup> C HCl
	Source	Amicus Therapeutics	(b) (4)
	Lot No.	398-35-1	CD-MDSPSRS-111-227
	Purity/Potency	47.5%	81.7%
	Expiry Date	01-May-2004	24-Jul-2004
	Storage Conditions	Ambient temperature	Ambient temperature
<b>Validated assay range</b>	5.88 (LLOQ) – 2940 (ULOQ) ng/mL in 100% Plasma		
<b>Regression model &amp; weighting</b>	Linear (1/x <sup>2</sup> )		
<b>Validation parameters</b>	Method validation summary		
<b>Standard curve performance during accuracy &amp; precision</b>	No of standard calibrators from LLOQ to ULOQ	10	
	Cumulative accuracy (% bias) from LLOQ to ULOQ	-5.4 to 3.7%	

	Cumulative precision (% CV) from LLOQ to ULOQ	1 to 6.2%				
<b>QCs performance during accuracy &amp; precision</b>	Inter-Batch Precision Range (% CV)	4.5 to 9.5%				
	Inter-Batch Accuracy Range (% Bias)	-2.7 to 8.5%				
	Intra-Batch Precision Range (% CV)	1.1 to 13.4%				
	Intra-Batch Accuracy Range (% Bias)	-10.0 to 11.3%				
<b>Selectivity &amp; matrix effect</b>	Matrix Effect for AT1001 in Human Plasma (EDTA)					
	<b>Batch</b>	<b>Lot#</b>	<b>LLOQ</b>		<b>High</b>	
		<b>5.88 ng/mL</b>	<b>% Dev.</b>	<b>2210 ng/mL</b>	<b>% Dev.</b>	
	110	1	5.27	-10.4	2010	-9.0
		2	5.04	-14.3	2010	-9.0
		3	6.54	+11.2	2220	+0.5
		4	5.90	+0.3	2300	+4.1
		5	5.94	+1.0	2250	+1.8
		6	6.21	+5.6	2130	-3.6
		7	6.16	+4.8	2190	-0.9
		8	6.41	+9.0	2200	-0.5
		9	5.95	+1.2	2260	+2.3
		10	4.80	-18.4	2260	+2.3
	Mean		5.82		2180	
	% CV		10.1		4.7	
	% Theoretical		99.0		98.6	
	n		10		10	
<b>Interference &amp; specificity</b>	QC samples were fortified with moxifloxacin at a concentration of 6.00 µg/mL					
	<b>Batch</b>	<b>QC A</b>	<b>QC C</b>			
		<b>20.1 ng/mL</b>	<b>2210 ng/mL</b>			
	122	20.3	2230			
		18.2	2230			
		19.9	2180			
		18.5	2200			
		18.4	2150			
		18.4	2110			
	Mean	19.0	2180			
	% CV	4.8	2.2			
	% Theoretical	94.5	98.6			
	n	6	6			
<b>Hemolysis effect</b>	Hemolyzed human plasma was fortified with AT1001 at the LLOQ and high QC sample concentrations. The samples were assessed against calibration standards in a non-hemolyzed control matrix. No significant interference from hemolyzed matrix was demonstrated in any of the six human plasma lots.					
	<b>Batch</b>	<b>Lot#</b>	<b>LLOQ</b>		<b>High</b>	
		<b>5.88 ng/mL</b>	<b>% Dev.</b>	<b>2210 ng/mL</b>	<b>% Dev.</b>	
	128	1	5.28	-10.2	2270	+2.7
		2	5.17	-12.1	2360	+6.8
		3	6.23	+6.0	2230	+0.9
		4	5.58	-5.1	2450	+10.9
		5	6.26	+6.5	2280	+3.2
		6	5.42	-7.8	2270	+2.7
	Mean		5.66		2310	
	% CV		8.4		3.5	
	% Theoretical		94.6		103.6	
	n		3		3	

<b>Lipemic (Turbidity) effect</b>	Lipemic human plasma (with EDTA) was fortified with AT1001 at the LLOQ and high QC sample concentrations. The samples were assessed against calibration standards in a non-lipemic control matrix. No significant interference from the turbid matrix was demonstrated in any of the three human plasma lots.																																																																		
	<table border="1"> <thead> <tr> <th rowspan="2">Batch</th> <th rowspan="2">Lot#</th> <th colspan="2">LLOQ</th> <th colspan="2">High</th> </tr> <tr> <th>5.88 ng/mL</th> <th>% Dev.</th> <th>2210 ng/mL</th> <th>% Dev.</th> </tr> </thead> <tbody> <tr> <td rowspan="3">123</td> <td>1</td> <td>5.94</td> <td>+1.0</td> <td>2060</td> <td>-6.8</td> </tr> <tr> <td>2</td> <td>5.66</td> <td>-3.7</td> <td>2110</td> <td>-4.5</td> </tr> <tr> <td>3</td> <td>5.71</td> <td>-2.9</td> <td>2030</td> <td>-8.1</td> </tr> <tr> <td>Mean</td> <td></td> <td>5.77</td> <td></td> <td>2070</td> <td></td> </tr> <tr> <td>% CV</td> <td></td> <td>2.6</td> <td></td> <td>2.0</td> <td></td> </tr> <tr> <td>% Theoretical</td> <td></td> <td>98.1</td> <td></td> <td>93.7</td> <td></td> </tr> <tr> <td>n</td> <td></td> <td>3</td> <td></td> <td>3</td> <td></td> </tr> </tbody> </table>	Batch	Lot#	LLOQ		High		5.88 ng/mL	% Dev.	2210 ng/mL	% Dev.	123	1	5.94	+1.0	2060	-6.8	2	5.66	-3.7	2110	-4.5	3	5.71	-2.9	2030	-8.1	Mean		5.77		2070		% CV		2.6		2.0		% Theoretical		98.1		93.7		n		3		3																	
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<b>Dilution linearity &amp; hook effect</b>	Samples with a concentration up to 14,700 ng/mL were quantified after the application with the below dilution factors.																																																																		
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<b>Bench-top/process stability</b>	Short-term Stability of AT1001 in Human Plasma (EDTA) at Ambient Temperature Under White Light Conditions Short-term stability period: 27 hours in polypropylene tubes																																																																		
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	17.2	184	2150	15200	14300
	17.2	182	2260	15100	*21300
	17.4	178	2210	15400	15300
	18.0	177	2200	15800	15000
Mean	17.2	179	2230	15300	15200
% CV	3.9	2.6	2.1	2.7	3.7
% Theoretical	97.2	101.1	100.9	104.1	103.4
n	6	6	6	6	5

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<b>Long-term storage</b>	Long-term Stability of AT1001 in Human Plasma (EDTA) at -20°C																																													
	1003 days at -20°C in polypropylene tubes (QC A)																																													
	1007 days at -20°C in polypropylene tubes (QC B and C)																																													
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n	6																																													
	<p>Extraction date: 11-May-2009</p> <p>Preparation date: 14-May-2007</p> <p>Long-term stability period: 728 days in polypropylene tubes</p>																																													



Standard curve performance	Assay Date	Batch Number	STD B 5.88 ng/mL	STD C 11.8 ng/mL	STD D 29.4 ng/mL	STD E 58.8 ng/mL	STD F 118 ng/mL	STD G 294 ng/mL	STD H 588 ng/mL	STD I 1180 ng/mL	STD J 2360 ng/mL	STD K 2940 ng/mL
	Mean		5.95	11.6	29.0	58.2	119	297	593	1190	2330	2970
	S.D.		0.170	0.503	1.62	1.78	2.17	8.95	38.5	28.3	64.9	141
	%CV		2.9	4.3	5.6	3.1	1.8	3.0	6.5	2.4	2.8	4.7
	%Bias		1.2	-1.7	-1.4	-1.0	0.8	1.0	0.9	0.8	-1.3	1.0
	n		11	11	12	12	11	12	12	12	11	12

QC performance	Assay Date	Batch Number	QC A 177 ng/mL	QC B 177 ng/mL	QC C 2210 ng/mL	QC C DF2 2210 ng/mL	QC E DF10 6000 ng/mL	QC D DF10 14700 ng/mL	
	Mean		16.3	170	2160	2200	5970	14300	
	S.D.		1.16	11.0	104	83.9	328	656	
	%CV		7.1	6.5	4.8	3.8	5.5	4.6	
	%Theoretical		92.1	96.0	97.7	99.5	99.5	97.3	
	%Bias		-7.9	-4.0	-2.3	-0.5	-0.5	-2.7	
		n		27	27	27	12	12	3

Study sample analysis/ stability	Maximum time between sample collection and sample analysis did not exceed 843 days. Study samples were stored at a nominal temperature of -20°C.
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### 6.1.3. Migalastat Analysis of Samples From Study FAB-CL-205 (Phase 2 trial)

(b) (4) performed the bioanalysis (b) (4). Study samples were collected between May 5, 2008 and Sept 8, 2012 and were received frozen on dry ice between Dec 16, 2008 and Oct 2, 2012.

Assay passing rate	21 of 102 samples were reanalyzed																																																																																																																								
	<table border="1"> <thead> <tr> <th>Batch Number</th> <th>Regression Status</th> <th>Extraction Date</th> <th>Assay Date</th> <th>Description*</th> <th>Comment</th> </tr> </thead> <tbody> <tr> <td>12</td> <td>Accepted</td> <td>12-Jan-2009</td> <td>13-Jan-2009</td> <td>SEE WORKLIST FOR SUBS AND TIMEPOINTS</td> <td>OK</td> </tr> <tr> <td>13</td> <td>Accepted</td> <td>14-Jan-2009</td> <td>14-Jan-2009</td> <td>SEE WORKLIST FOR SUBS AND TIMEPOINTS</td> <td>OK</td> </tr> <tr> <td>14</td> <td>Accepted</td> <td>15-Jan-2009</td> <td>15-Jan-2009</td> <td>REASSAYS</td> <td>OK</td> </tr> <tr> <td>16</td> <td>Accepted</td> <td>12-May-2009</td> <td>12-May-2009</td> <td>ISR REASSAYS</td> <td>OK</td> </tr> <tr> <td>17</td> <td>Accepted</td> <td>26-Oct-2012</td> <td>28-Oct-2012</td> <td>SUB 205-02-202 PD 1</td> <td>OK</td> </tr> </tbody> </table>	Batch Number	Regression Status	Extraction Date	Assay Date	Description*	Comment	12	Accepted	12-Jan-2009	13-Jan-2009	SEE WORKLIST FOR SUBS AND TIMEPOINTS	OK	13	Accepted	14-Jan-2009	14-Jan-2009	SEE WORKLIST FOR SUBS AND TIMEPOINTS	OK	14	Accepted	15-Jan-2009	15-Jan-2009	REASSAYS	OK	16	Accepted	12-May-2009	12-May-2009	ISR REASSAYS	OK	17	Accepted	26-Oct-2012	28-Oct-2012	SUB 205-02-202 PD 1	OK																																																																																				
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16	Accepted	12-May-2009	12-May-2009	ISR REASSAYS	OK																																																																																																																				
17	Accepted	26-Oct-2012	28-Oct-2012	SUB 205-02-202 PD 1	OK																																																																																																																				
ISR passing rate	13 clinical samples were reassayed for incurred sample reproducibility and results demonstrate that 92.3% of the pairs matched																																																																																																																								
Standard curve performance	No sample was recovered for STD points G and H.																																																																																																																								
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Assay Date	Batch Number	STD B 5.88 ng/mL	STD C 11.8 ng/mL	STD D 29.4 ng/mL	STD E 58.8 ng/mL	STD F 118 ng/mL	STD G 294 ng/mL	STD H 588 ng/mL	STD I 1180 ng/mL	STD J 2360 ng/mL	STD K 2940 ng/mL																																																																																																														
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QC performance	<b>Assay Date</b>	<b>Batch Number</b>	<b>QC A 17.7 ng/mL</b>	<b>QC B 177 ng/mL</b>	<b>QC C 2210 ng/mL</b>	<b>QC D DF 10 14700 ng/mL</b>
	13-Jan-2009	12	19.4	179	2020	
			16.5	155	2030	
	14-Jan-2009	13	15.3	164	2040	
			16.4	171	~1850	
	15-Jan-2009	14	17.4	185	1970	13800
			15.8	181	1930	14100
						14000
	28-Oct-2012	17	17.5	174	2350	
			~21.5	159	2290	
	Mean		17.5	171	2060	14000
	S.D.		2.05	10.8	173	153
	%CV		11.7	6.3	8.4	1.1
%Theoretical		98.9	96.6	93.2	95.2	
%Bias		-1.1	-3.4	-6.8	-4.8	
n		8	8	8	3	
Study sample analysis/ stability	The maximum time between sample collection and sample analysis did not exceed 255 days. Study samples were stored at a nominal temperature of -20°C.					

#### 6.1.4. Migalastat Analysis of Samples From Study FAB-CL-204 (Phase 2 trial)

(b) (4) performed the bioanalysis (b) (4). Study samples were collected between Jun 8, 2006 and Aug 10, 2007 and were received frozen on dry ice between June 13, 2006 and Aug 16, 2007.

Assay passing rate	16 of 554 samples were reanalyzed											
	<b>Batch Number</b>	<b>Regression Status</b>	<b>Extraction Date</b>	<b>Assay Date</b>	<b>Description</b>					<b>Comment</b>		
	1	Accepted	26-Jul-2007	26-Jul-2007	SUB 201-02-04 PD 1					OK		
	2	Accepted	26-Jul-2007	26-Jul-2007	SUB 201-02-05 PD 1					OK		
	3	Accepted	27-Jul-2007	28-Jul-2007	SUB 204-0101, 204-0102, 204-0203 PD 1					OK		
	4	Accepted	30-Jul-2007	31-Jul-2007	SUB 204-0201, 204-0301 PD 1					OK		
	5	Accepted	30-Jul-2007	31-Jul-2007	SUB 204-0302, 204-0305 PD 1					OK		
	6	Accepted	31-Jul-2007	31-Jul-2007	SUB 204-0402, 204-0603 PD1					OK		
	7	Accepted	31-Jul-2007	31-Jul-2007	SUB 201-01-06 Day 1 to 42 PD 1					OK		
	8	Accepted	02-Aug-2007	02-Aug-2007	SUB 201-03-05 Day 1 to 42 PD 1					OK		
	9	Accepted	02-Aug-2007	03-Aug-2007	SUB 201-01-06,201-03-05 Day 168 PD 1 + REASSAYS					OK		
	10	Accepted	07-Aug-2007	07-Aug-2007	REASSAYS AND VRC SAMPLE					OK		
	11	Accepted	20-Aug-2007	20-Aug-2007	SEE WORKLIST FOR SUBS AND TIMEPOINTS					OK		
ISR passing rate	Not included in the study report. ISR white paper was published in 2007											
Standard curve performance	<b>Assay Date</b>	<b>Batch Number</b>	<b>STD B 5.88 ng/mL</b>	<b>STD C 11.8 ng/mL</b>	<b>STD D 29.4 ng/mL</b>	<b>STD E 58.8 ng/mL</b>	<b>STD F 118 ng/mL</b>	<b>STD G 294 ng/mL</b>	<b>STD H 588 ng/mL</b>	<b>STD I 1180 ng/mL</b>	<b>STD J 2360 ng/mL</b>	<b>STD K 2940 ng/mL</b>
	Mean		5.90	11.7	29.3	59.4	115	292	596	1180	2360	2970
	S.D.		0.127	0.538	0.802	1.36	2.32	9.16	14.6	22.9	51.5	144
	%CV		2.2	4.6	2.7	2.3	2.0	3.1	2.4	1.9	2.2	4.8
	%Bias		0.3	-0.8	-0.3	1.0	-2.5	-0.7	1.4	0.0	0.0	1.0
	n		11	10	11	11	11	11	11	11	11	11

QC performance	<p>The sponsor reports 30.9 %CV for the 177 ng/ml mid QC level, this was due to only one errant sample. Per Applicant, the assignable cause was indeterminate and was an isolated event. Removal of the single sample results in a %CV of 2.5%.</p> <table border="1"> <thead> <tr> <th>Assay Date</th> <th>Batch Number</th> <th>QC A 17.7 ng/mL</th> <th>QC B 177 ng/mL</th> <th>QC C 2210 ng/mL</th> <th>QC D DF 10 14700 ng/mL</th> </tr> </thead> <tbody> <tr><td>26-Jul-2007</td><td>1</td><td>17.6</td><td>176</td><td>2160</td><td></td></tr> <tr><td></td><td></td><td>18.0</td><td>180</td><td>2130</td><td></td></tr> <tr><td>26-Jul-2007</td><td>2</td><td>18.4</td><td>183</td><td>2160</td><td></td></tr> <tr><td></td><td></td><td>16.8</td><td>179</td><td>2160</td><td></td></tr> <tr><td>28-Jul-2007</td><td>3</td><td>17.2</td><td>174</td><td>2160</td><td></td></tr> <tr><td></td><td></td><td>17.3</td><td>175</td><td>2140</td><td></td></tr> <tr><td>31-Jul-2007</td><td>4</td><td>16.9</td><td>178</td><td>2090</td><td></td></tr> <tr><td></td><td></td><td>16.7</td><td>174</td><td>2080</td><td></td></tr> <tr><td>31-Jul-2007</td><td>5</td><td>17.7</td><td>182</td><td>2080</td><td></td></tr> <tr><td></td><td></td><td>17.9</td><td>184</td><td>2160</td><td></td></tr> <tr><td>31-Jul-2007</td><td>6</td><td>16.3</td><td>180</td><td>2150</td><td></td></tr> <tr><td></td><td></td><td>16.9</td><td>171</td><td>2180</td><td></td></tr> <tr><td>31-Jul-2007</td><td>7</td><td>16.3</td><td>176</td><td>2120</td><td></td></tr> <tr><td></td><td></td><td>17.1</td><td>179</td><td>2170</td><td></td></tr> <tr><td>02-Aug-2007</td><td>8</td><td>17.6</td><td>173</td><td>2190</td><td></td></tr> <tr><td></td><td></td><td>16.9</td><td>180</td><td>2120</td><td></td></tr> <tr><td>03-Aug-2007</td><td>9</td><td>17.0</td><td>~452</td><td>2160</td><td>15500</td></tr> <tr><td></td><td></td><td>17.9</td><td>174</td><td>2210</td><td>14900</td></tr> <tr><td></td><td></td><td></td><td></td><td></td><td>15800</td></tr> <tr><td>07-Aug-2007</td><td>10</td><td>17.1</td><td>173</td><td>2140</td><td>15400</td></tr> <tr><td></td><td></td><td>16.8</td><td>182</td><td>2150</td><td>15700</td></tr> <tr><td></td><td></td><td></td><td></td><td></td><td>14900</td></tr> <tr><td>20-Aug-2007</td><td>11</td><td>17.5</td><td>167</td><td>2130</td><td></td></tr> <tr><td></td><td></td><td>16.9</td><td>182</td><td>2200</td><td></td></tr> <tr><td>Mean</td><td></td><td>17.2</td><td>190</td><td>2150</td><td>15400</td></tr> <tr><td>S.D.</td><td></td><td>0.549</td><td>58.7</td><td>34.9</td><td>388</td></tr> <tr><td>%CV</td><td></td><td>3.2</td><td>30.9</td><td>1.6</td><td>2.5</td></tr> <tr><td>%Theoretical</td><td></td><td>97.2</td><td>107.3</td><td>97.3</td><td>104.8</td></tr> <tr><td>%Bias</td><td></td><td>-2.8</td><td>7.3</td><td>-2.7</td><td>4.8</td></tr> <tr><td>n</td><td></td><td>22</td><td>22</td><td>22</td><td>6</td></tr> </tbody> </table>	Assay Date	Batch Number	QC A 17.7 ng/mL	QC B 177 ng/mL	QC C 2210 ng/mL	QC D DF 10 14700 ng/mL	26-Jul-2007	1	17.6	176	2160				18.0	180	2130		26-Jul-2007	2	18.4	183	2160				16.8	179	2160		28-Jul-2007	3	17.2	174	2160				17.3	175	2140		31-Jul-2007	4	16.9	178	2090				16.7	174	2080		31-Jul-2007	5	17.7	182	2080				17.9	184	2160		31-Jul-2007	6	16.3	180	2150				16.9	171	2180		31-Jul-2007	7	16.3	176	2120				17.1	179	2170		02-Aug-2007	8	17.6	173	2190				16.9	180	2120		03-Aug-2007	9	17.0	~452	2160	15500			17.9	174	2210	14900						15800	07-Aug-2007	10	17.1	173	2140	15400			16.8	182	2150	15700						14900	20-Aug-2007	11	17.5	167	2130				16.9	182	2200		Mean		17.2	190	2150	15400	S.D.		0.549	58.7	34.9	388	%CV		3.2	30.9	1.6	2.5	%Theoretical		97.2	107.3	97.3	104.8	%Bias		-2.8	7.3	-2.7	4.8	n		22	22	22	6
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S.D.		0.549	58.7	34.9	388																																																																																																																																																																																						
%CV		3.2	30.9	1.6	2.5																																																																																																																																																																																						
%Theoretical		97.2	107.3	97.3	104.8																																																																																																																																																																																						
%Bias		-2.8	7.3	-2.7	4.8																																																																																																																																																																																						
n		22	22	22	6																																																																																																																																																																																						
Study sample analysis/ stability	<p>Study samples were stored from sample collection to the end of sample analysis at a nominal temperature of -20°C for a duration not exceeding 439 days. Samples from protocol FAB-CL-204 were stored at -80°C for 8 days between 14-Dec-2006 and 22-Dec-2006.</p>																																																																																																																																																																																										

### 6.1.5. Migalstat Analysis of Samples From Study FAB-CL-201 (Phase 2 trial)

(b) (4) performed the bioanalysis (b) (4). Study samples received frozen on dry ice between Jan 10, 2006 and Feb 14, 2007 were analyzed between May 26, 2006 through Jun 07, 2006. Performance of this sample analysis were shown below. Sample received after Mar 07, 2007 were analyzed with the samples from Study FAB-CL-204. The performance of this analysis with study FAB-CL-204 are shown in section 4.1.4.

Assay passing rate	261 samples were reanalyzed in 7 batches					
	Batch Number	Regression Status	Extraction Date	Assay Date	Description	Comment
	1	Accepted	26-May-2006	27-May-2006	SUB 01-01 PD 1	OK
	6	Accepted	02-Jun-2006	05-Jun-2006	SUB 01-02 PD 1	OK
	7	Accepted	02-Jun-2006	06-Jun-2006	SUB 01-03 PD 1	OK
	8	Accepted	05-Jun-2006	06-Jun-2006	SUB 01-04 PD 1	OK
	9	Accepted	05-Jun-2006	06-Jun-2006	SUB 01-05 PD 1	OK
	10	Accepted	07-Jun-2006	07-Jun-2006	REASSAYS	OK
ISR passing rate	Not included in the study report. ISR white paper was published in 2007					

Standard curve performance	Assay Date	Batch Number	STD B 5.88 ng/mL	STD C 11.8 ng/mL	STD D 29.4 ng/mL	STD E 58.8 ng/mL	STD F 118 ng/mL	STD G 294 ng/mL	STD H 588 ng/mL	STD I 1180 ng/mL	STD J 2360 ng/mL	STD K 2940 ng/mL
	27-May-2006	1	5.74	12.1	30.7	60.9	116	287	596	1190	2150	3040
05-Jun-2006	6	5.95	11.3	29.9	61.4	122	295	558	1140	2350	3010	
06-Jun-2006	7	5.98	11.7	28.8	52.8	117	319	575	1210	2360	3040	
06-Jun-2006	8	5.95	11.4	30.0	55.0	129	305	659	1070	2250	2790	
06-Jun-2006	9	5.77	12.5	26.8	63.7	110	294	564	1270	2180	3130	
07-Jun-2006	10	6.08	11.0	28.9	57.9	125	290	590	1270	2290	2860	
Mean		5.91	11.7	29.2	58.6	120	298	590	1190	2260	2980	
S.D.		0.131	0.554	1.37	4.15	6.85	11.8	36.7	77.6	86.6	127	
%CV		2.2	4.7	4.7	7.1	5.7	4.0	6.2	6.5	3.8	4.3	
%Bias		0.5	-0.8	-0.7	-0.3	1.7	1.4	0.3	0.8	-4.2	1.4	
n		6	6	6	6	6	6	6	6	6	6	

QC performance	Assay Date	Batch Number	QC A 17.7 ng/mL	QC B 177 ng/mL	QC C 2210 ng/mL
	27-May-2006	1	16.6	178	2190
05-Jun-2006	6	17.4	184	2320	
		19.6	186	2130	
06-Jun-2006	7	18.3	178	2080	
		17.1	189	2290	
06-Jun-2006	8	19.4	175	2360	
		18.7	177	2100	
06-Jun-2006	9	17.3	187	2070	
		17.0	176	2220	
07-Jun-2006	10	18.2	173	1950	
		17.3	172	-1840	
Mean		17.8	180	2140	
S.D.		1.00	5.69	152	
%CV		5.6	3.2	7.1	
%Theoretical		100.6	101.7	96.8	
%Bias		0.6	1.7	-3.2	
n		12	12	12	

Study sample analysis/ stability	Study samples were stored from sample receipt at (b) (4) to the end of sample analysis at a nominal temperature of -20°C for a duration not exceeding 439 days.
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### 6.1.6. Migalastat Analysis of Samples From Phase 1 Studies

Clinical Study	QC's Inter-run Precision	QC's Inter-run Accuracy	% of samples for ISR	% ISR passed	Maximum Storage Time
AT1001-016	4.1 to 7.1%	-8.6 to 9.5%	10% (114/1159)	97%	101 days
AT1001-015	5.3 to 11.9%	-6.2 to 1.7%	10% (54/544)	71%	225 days
AT1001-013	6.8 to 10.8%	-1.4 to 8.8%	12% (48/417)	92%	575 days
AT1001-010	3.5 to 7.4%	-2.7 to 6.2%	9% (120/1339)	97%	57 days
AT1001-018	5.2 to 7.3%	-0.6 to 7%	11% (60/560)	98%	103 days
FAB-CL-102	1.7 to 14.9%	-6.2 to 0%	NA; Report Date: 24 May, 2005		61 days

## 6.2. Lyso-Gb3

### 6.2.1. Bioanalytical Method Validation for Lyso-Gb3

The Office of Clinical Pharmacology review team has assessed the adequacy and acceptability of the following bioanalytical methods used in clinical studies AT1001-011 and AT1001-012 for the quantitation of lyso-Gb3.

The sponsor developed a liquid chromatography tandem mass spectrometry (LC/MS/MS) method to quantify lyso-Gb3 in human plasma containing EDTA. No major issues were identified in validation report (No. RR1001-57). Briefly, lyso-Gb3 and the internal standard were extracted from human plasma potassium containing K2 EDTA using protein precipitation followed by strong cation exchange solid phase extraction. The extracted samples were analyzed by an HPLC equipped with an AB/MDS Sciex API 4000 mass spectrometer. The method utilized an isotopically-labelled internal standard (<sup>13</sup>C6-lyso-Gb3) of lyso-Gb3. Solvent based calibrators and solvent and plasma QCs were used in the quantitation of lyso-Gb3 due to endogenous Lyso-Gb3 levels. The assay was validated to a lower limit of quantitation (LLOQ) of 0.2 ng/mL. The method was sufficiently validated to support the quantitation of lyso-Gb3 in human plasma.

The long-term stability of lyso-Gb3 in plasma with anticoagulant K2-EDTA was established for 666 days at -80°C and for 947 days at -70°C, and with anticoagulant ACD for 1694 days at -80°C. Anticoagulants K2-EDTA and ACD had similar performance in regards to QC CV%. In both phase 3 trials AT1001-011 and AT1001-012, K2-EDTA was used as anticoagulant in analyzing plasma lyso-Gb3, and plasma samples were stored at -80°C until bioanalysis. The potential maximum storage time (from the first day of sample collection to the last day of sample analysis) were 1561 days for study AT1001-011 and 1022 days for study AT1001-012. The longest actual storage time was 1439 days in trial AT1001-011 and 1021 days in trial AT1001-012. These times exceed the established stability of 666 days for lyso-Gb3 in plasma with K2-EDTA. However, the performance of the anticoagulants K2-EDTA and ACD were similar in terms of QC CV%, and the stability of lyso-Gb3 was longer (1694 days) with ACD. The similarity of the anticoagulants and the longer stability of plasma with ACD supports the analysis of plasma stored longer than 666 days with K2-EDTA.

<b>Matrix</b>	Human Plasma EDTA; Solvent (1:1) Methanol and Dimethyl sulfoxide	
<b>Test article purity</b>	Lyso-Gb3 (also referred to as lyso-ceramide trihexoside or globotriaosylsphingosine)	
<b>Internal standard purity</b>	was purchased (b) (4) <sup>13</sup> C6-lyso-Gb3 was synthesized at Amicus Therapeutics, Cranbury, NJ	
<b>Validated assay range</b>	Solvent (0.200 to 200 ng/mL); Plasma (1.6 to 201 ng/ml)	
<b>Regression model &amp; weighting</b>	Linear (1/x <sup>2</sup> )	
<b>Validation parameters</b>	Method validation summary	
<b>Solvent standard curve performance during accuracy &amp; precision</b>	No of standard calibrators from LLOQ to ULOQ	10
	Cumulative accuracy (%bias) from LLOQ to ULOQ	-4.4 to 5.6%
	Cumulative precision (%CV) from LLOQ to ULOQ	1.4 to 13.4%
<b>Plasma standard curve performance during accuracy &amp; precision</b>	No of standard calibrators from LLOQ to ULOQ	8
	Cumulative accuracy (%bias) from LLOQ to ULOQ	-3.2 to 3.8%
	Cumulative precision (%CV) from LLOQ to ULOQ	2.3 to 5.4
<b>Solvent QCs using solvent curve performance during accuracy &amp; precision</b>	Inter-Batch Precision Range (% CV)	5.7 to 16.1
	Inter-Batch Accuracy Range (% Bias)	-13.4 to -0.7
	Intra-Batch Precision Range (% CV)	2.4 to 21.8
	Intra-Batch Accuracy Range (% Bias)	-19.0 to 6.5
<b>Solvent QCs using plasma curve performance</b>	Inter-Batch Precision Range (% CV)	2.7 to 9.7
	Inter-Batch Accuracy Range (% Bias)	-11.3 to -4.3

<b>during accuracy &amp; precision</b>	Intra-Batch Precision Range (% CV)	2.3 to 12.4		
	Intra-Batch Accuracy Range (% Bias)	-15.3 to 2.5		
<b>Plasma QCs using solvent curve performance during accuracy &amp; precision</b>	Inter-Batch Precision Range (% CV)	4.4 to 8.4		
	Inter-Batch Accuracy Range (% Bias)	1.9 to 4.2		
	Intra-Batch Precision Range (% CV)	1.3 to 7.1		
	Intra-Batch Accuracy Range (% Bias)	-3.1 to 11.1		
<b>Plasma QCs using plasma curve performance during accuracy &amp; precision</b>	Inter-Batch Precision Range (% CV)	3.0 to 6.7		
	Inter-Batch Accuracy Range (% Bias)	-5.7 to 0.7		
	Intra-Batch Precision Range (% CV)	1.1 to 7.3		
	Intra-Batch Accuracy Range (% Bias)	-8.8 to 3.7		
<b>Interference &amp; specificity</b>	Lyso-Gb3 in Human Plasma Samples Spiked with iohexol (200µM) and/or AT1001 (4000 µg/mL) Incubated at 37 °C for 4 Hours (Using Solvent Calibration Curves)			
	<b>Nominal Concentration (ng/mL)</b>	<b>Lyso-Gb3 Alone</b>	<b>Lyso-Gb3 + AT1001 + Iohexol</b>	<b>Lyso-Gb3 + AT1001</b>
	<b>3.64</b>	3.85	4.05	4.12
		4.10	4.00	4.17
		3.60	3.97	4.40
		3.96	3.80	3.74
	<b>Mean</b>	<b>3.88</b>	<b>3.96</b>	<b>4.11</b>
	<b>SD</b>	<b>0.211</b>	<b>0.108</b>	<b>0.274</b>
	<b>%CV</b>	<b>5.4</b>	<b>2.7</b>	<b>6.7</b>
	<b>% Difference from Nominal</b>	<b>6.6</b>	<b>8.8</b>	<b>12.9</b>
	<b>n</b>	<b>4</b>	<b>4</b>	<b>4</b>
	<b>40.6</b>	44.8	45.0	42.4
		47.2	43.5	45.4
		44.8	43.8	46.1
		44.7	41.8	45.0
	<b>Mean</b>	<b>45.4</b>	<b>43.5</b>	<b>44.7</b>
	<b>SD</b>	<b>1.22</b>	<b>1.32</b>	<b>1.62</b>
	<b>%CV</b>	<b>2.7</b>	<b>3.0</b>	<b>3.6</b>
	<b>% Difference from Nominal</b>	<b>11.8</b>	<b>7.1</b>	<b>10.1</b>
	<b>n</b>	<b>4</b>	<b>4</b>	<b>4</b>
	<b>161</b>	186	185	170
		177	179	180
		187	184	187
		171	178	178
	<b>Mean</b>	<b>180</b>	<b>182</b>	<b>179</b>
	<b>SD</b>	<b>7.63</b>	<b>3.51</b>	<b>6.99</b>
	<b>%CV</b>	<b>4.2</b>	<b>1.9</b>	<b>3.9</b>
	<b>% Difference from Nominal</b>	<b>11.8</b>	<b>13.0</b>	<b>11.2</b>
	<b>n</b>	<b>4</b>	<b>4</b>	<b>4</b>

(continued)

**Interference & specificity  
(continued)**

Stability of Lyso-Gb3 in Human Plasma Samples Spiked with Iohexol (200µM) and/or AT1001 (4000 µg/mL) Incubated at 37°C for 4 Hours (Using Plasma Calibration Curves)

Nominal Concentration (ng/mL)	Lyso-Gb <sub>3</sub> Alone	Lyso-Gb <sub>3</sub> + AT1001 + Iohexol	Lyso-Gb <sub>3</sub> + AT1001
<b>3.64</b>	3.71	3.91	3.98
	3.96	3.86	4.03
	3.47	3.83	4.25
	3.82	3.66	3.60
<b>Mean</b>	<b>3.74</b>	<b>3.82</b>	<b>3.97</b>
<b>SD</b>	<b>0.207</b>	<b>0.108</b>	<b>0.270</b>
<b>%CV</b>	<b>5.5</b>	<b>2.8</b>	<b>6.8</b>
<b>% Diff</b>	<b>2.7</b>	<b>4.9</b>	<b>9.1</b>
<b>n</b>	<b>4</b>	<b>4</b>	<b>4</b>
<b>40.6</b>	43.5	43.7	41.1
	45.8	42.2	44.1
	43.5	42.5	44.7
	43.4	40.6	43.7
<b>Mean</b>	<b>44.1</b>	<b>42.3</b>	<b>43.4</b>
<b>SD</b>	<b>1.17</b>	<b>1.28</b>	<b>1.59</b>
<b>%CV</b>	<b>2.7</b>	<b>3.0</b>	<b>3.7</b>
<b>% Diff</b>	<b>8.6</b>	<b>4.2</b>	<b>6.9</b>
<b>n</b>	<b>4</b>	<b>4</b>	<b>4</b>
<b>161</b>	180	179	165
	172	173	175
	182	179	182
	166	173	173
<b>Mean</b>	<b>175</b>	<b>176</b>	<b>174</b>
<b>SD</b>	<b>7.39</b>	<b>3.46</b>	<b>6.99</b>
<b>%CV</b>	<b>4.2</b>	<b>2.0</b>	<b>4.0</b>
<b>% Diff</b>	<b>8.7</b>	<b>9.3</b>	<b>8.1</b>
<b>n</b>	<b>4</b>	<b>4</b>	<b>4</b>

**Parallelism**

Parallelism tests were conducted using six human plasma samples from a previously conducted Amicus phase 2 clinical study. The results were acceptable for 2-fold, 5-fold and 10-fold dilutions.

Incurred Human Plasma Sample Parallelism for Lyso-Gb3 (Diluted with Surrogate Matrix [DMSO/MeOH: 1/1 (v/v)] Using Solvent Calibration Curves

Dilution Factor	Undiluted	2-fold	5-fold	10-fold
Incurred Sample 1	85.6	85.0	88.8	92.0
% Difference [from undiluted]		-0.7	3.7	7.5
Incurred Sample 2	104	99.9	99.0	102
% Difference [from undiluted]		-3.9	-4.8	-1.9
Incurred Sample 3	96.9	92.8	101	102
% Difference [from undiluted]		-4.2	4.2	5.3
Incurred Sample 4	181	189	181	184
% Difference [from undiluted]		4.4	0.0	1.7
Incurred Sample 5	81.2	86.2	86.5	78.2
% Difference [from undiluted]		6.2	6.5	-3.7
Incurred Sample 6	99.2	103	92.9	89.5
% Difference [from undiluted]		3.8	-6.4	-9.8

Incurred Human Plasma Sample Parallelism for Lyso-Gb3 (Diluted with Surrogate Matrix [DMSO/MeOH: 1/1 (v/v)] Using Plasma Calibration Curves

Dilution Factor	Undiluted	2-fold	5-fold	10-fold
Incurred Sample 1	74.9	74.3	77.5	80.2
% Difference [from undiluted]		-0.8	3.4	7.1
Incurred Sample 2	90.6	87.4	86.5	89.1
% Difference [from undiluted]		-3.5	-4.5	-1.7
Incurred Sample 3	84.7	81.1	88.6	88.9
% Difference [from undiluted]		-4.3	4.6	5.0
Incurred Sample 4	158	166	159	161
% Difference [from undiluted]		5.1	0.6	1.9
Incurred Sample 5	71.1	75.4	75.5	68.1
% Difference [from undiluted]		6.0	6.2	-4.2
Incurred Sample 6	86.8	90.3	81.1	78.0
% Difference [from undiluted]		4.0	-6.6	-10.1

**Human Plasma Matrix**

Human plasma matrix effect for both lyso-Gb3 and <sup>13</sup>C6-lyso-Gb3 were assessed, and were found to be +129% and +124%, respectively.

	Lyso-Gb <sub>3</sub> [ng/mL]						<sup>13</sup> C <sub>6</sub> -Lyso-Gb <sub>3</sub> [ng/mL]	
	3.00		40.0		160		75.0	75.0
	Post-extraction			External Solution			Post-extraction	External Solution
Mean	4037	48102	186689	2947	37422	153509	105221	84611
SD	153	679	3541	23.8	2090	4318	3558	3183
% CV	3.79	1.41	1.90	0.808	5.58	2.81	3.38	3.76
Matrix Effects (%)	-	-	-	137	129	122	-	-
Overall Matrix Effects (%)	-	-	-	-	-	129	-	124
n	4	4	4	4	4	4	12	12

**Recovery**

Recoveries for both lyso-Gb3 and <sup>13</sup>C6-lyso-Gb3 were assessed, and were found to be 71.0% and 67.2%, respectively.

**Recovery of Lyso-Gb3 and <sup>13</sup>C6-Lyso-Gb3 from Solvent [MeOH/DMSO: 1/1 (v/v)]**

	Lyso-Gb <sub>3</sub> [ng/mL]						<sup>13</sup> C <sub>6</sub> -Lyso-Gb <sub>3</sub> [ng/mL]	
	3.00	40.0	160	3.00	40.0	160	75.0	75.0
	Post-extraction			Extracted Solvent			Post-extraction	Extracted Solvent
Mean	2947	37422	153509	1571	17883	74616	84611	41976
SD	24	2090	4318	181	1200	5193	3183	3794
% CV	0.81	5.59	2.81	11.53	6.71	6.96	3.76	9.04
Recovery (%)	-	-	-	53.3	47.8	48.6	-	-
Overall Recovery (%)	-	-	-	-	-	49.9	-	49.6
n	4	4	4	4	4	4	12	12

**Recovery of Lyso-Gb3 and <sup>13</sup>C6-Lyso-Gb3 from Human Plasma**

	Lyso-Gb <sub>3</sub> [ng/mL]						<sup>13</sup> C <sub>6</sub> -Lyso-Gb <sub>3</sub> [ng/mL]	
	3.00	40.0	160	3.00	40.0	160	75.0	75.0
	Post-extraction			Extracted Plasma			Post-extraction	Extracted Plasma
Mean	4037	48102	186689	2940	33771	130806	105221	70724
SD	153	679	3541	134	1801	7876	3558	2486
% CV	3.8	1.4	1.9	4.6	5.3	6.0	3.4	3.5
Recovery (%)	-	-	-	72.8	70.2	70.1	-	-
Overall Recovery (%)	-	-	-	-	-	71.0	-	67.2
n	4	4	4	4	4	4	12	12

**Dilution linearity**

Dilution linearity tests were conducted and results were acceptable for 5-fold and 10-fold dilutions of a plasma dilution sample prepared at a lyso-Gb3 concentration of 500 ng/mL.

**Dilution Linearity for Lyso-Gb3 (500 ng/mL) Using Solvent Calibration Curves**

Nominal Concentration (ng/mL)	5-fold Dilution	10-fold Dilution
500	568	540
	529	525
	541	521
	570	520
	549	541
Mean	551	529
SD	17.6	10.3
% CV	3.2	1.9
% Diff	10.2	5.8
n	5	5

**Dilution Linearity for Lyso-Gb3 (500 ng/mL) Using Plasma Calibration Curves**

Nominal Concentration (ng/mL)	5-fold Dilution	10-fold Dilution
500	497	473
	463	459
	473	455
	499	455
	481	473
Mean	483	463
SD	15.5	9.27
% CV	3.2	2.0
% Diff	-3.4	-7.4
n	5	5

**Bench-top/process stability****Stability of Lyso-Gb3 in Human Plasma at Room Temperature for 7 Hours (Using Solvent Calibration Curves)**

Nominal Concentration (ng/mL)	3.59	40.6	161
Set 1	3.46	44.1	176
Set 2	3.86	46.4	165
Set 3	4.04	42.8	185
Set 4	3.38	42.4	162
<b>Mean</b>	<b>3.69</b>	<b>43.9</b>	<b>172</b>
<b>SD</b>	<b>0.316</b>	<b>1.80</b>	<b>10.6</b>
<b>% CV</b>	<b>8.6</b>	<b>4.1</b>	<b>6.2</b>
<b>% Diff</b>	<b>2.8</b>	<b>8.1</b>	<b>6.8</b>
<b>n</b>	<b>4</b>	<b>4</b>	<b>4</b>

**Stability of Lyso-Gb3 in Human Plasma at Room Temperature for 7 Hours (Using Human Plasma Calibration Curves)**

Nominal Concentration (ng/mL)	3.59	40.6	161
Set 1	3.55	45.2	180
Set 2	3.96	47.5	169
Set 3	4.15	43.9	189
Set 4	3.47	43.4	166
<b>Mean</b>	<b>3.78</b>	<b>45.0</b>	<b>176</b>
<b>SD</b>	<b>0.326</b>	<b>1.83</b>	<b>10.6</b>
<b>% CV</b>	<b>8.6</b>	<b>4.1</b>	<b>6.0</b>
<b>% Diff</b>	<b>5.3</b>	<b>10.8</b>	<b>9.3</b>
<b>n</b>	<b>4</b>	<b>4</b>	<b>4</b>

**Standard Solution Stability****Long-Term Frozen Stability of Lyso-Gb3 in Stock Solution [chloroform/MeOH: 2/1 (v/v)] at 1.00 mg/mL for 808 Days at -20 °C**

Stock Solution		Stored at -20 °C	Freshly Prepared
Nominal Concentration (mg/mL)		1.00	1.00
Peak Area Ratio	Rep 1	0.844	0.935
	Rep 2	0.910	0.916
	Rep 3	0.880	0.956
	Rep 4	0.877	0.887
	Rep 5	0.843	0.979
	Rep 6	0.822	0.928
<b>Mean</b>		<b>0.863</b>	<b>0.934</b>
<b>% CV</b>		<b>0.0371</b>	<b>0.0341</b>
<b>% Difference</b>		<b>-</b>	<b>7.6</b>
<b>n</b>		<b>6</b>	<b>6</b>

(continued)

**Standard Solution Stability (continued)**

Long-Term Frozen Stability of Lyso-Gb3 in Stock Solution [DMSO/MeOH: 1/1 (v/v)] at 10.0 µg/mL for 717 Days at -20 °C

Stock Solution		Stored at -20 °C	Freshly Prepared
Nominal Concentration (µg/mL)		10.0	10.0
Peak Area Ratio	Rep 1	0.981	0.935
	Rep 2	1.04	0.916
	Rep 3	0.988	0.956
	Rep 4	1.01	0.887
	Rep 5	1.01	0.979
	Rep 6	0.967	0.928
<b>Mean</b>		<b>0.999</b>	<b>0.934</b>
<b>% CV</b>		<b>0.0260</b>	<b>0.0341</b>
<b>% Difference</b>		<b>-</b>	<b>-7.0</b>
<b>n</b>		<b>6</b>	<b>6</b>

**Freeze-Thaw stability**

Stability of Lyso-Gb3 in Human Plasma Following Four Freeze/Thaw Cycles (Using Solvent Calibration Curves)

Nominal Concentration (ng/mL)	3.59	40.6	161
Set 1	3.67	42.1	158
Set 2	4.04	43.2	173
Set 3	3.80	41.0	176
Set 4	3.59	41.1	169
<b>Mean</b>	<b>3.78</b>	<b>41.9</b>	<b>169</b>
<b>SD</b>	<b>0.197</b>	<b>1.03</b>	<b>7.87</b>
<b>% CV</b>	<b>5.2</b>	<b>2.5</b>	<b>4.7</b>
<b>% Diff</b>	<b>5.3</b>	<b>3.2</b>	<b>5.0</b>
<b>n</b>	<b>4</b>	<b>4</b>	<b>4</b>

Stability of Lyso-Gb3 in Human Plasma Following Four Freeze/Thaw Cycles (Using Human Plasma Calibration Curves)

Nominal Concentration (ng/mL)	3.59	40.6	161
Set 1	3.77	43.1	162
Set 2	4.14	44.2	177
Set 3	3.90	42.0	180
Set 4	3.68	42.1	174
<b>Mean</b>	<b>3.87</b>	<b>42.9</b>	<b>173</b>
<b>SD</b>	<b>0.200</b>	<b>1.03</b>	<b>7.89</b>
<b>% CV</b>	<b>5.2</b>	<b>2.4</b>	<b>4.6</b>
<b>% Diff</b>	<b>7.8</b>	<b>5.7</b>	<b>7.5</b>
<b>n</b>	<b>4</b>	<b>4</b>	<b>4</b>

**Long-term storage**

Long-Term Frozen Stability of lyso-Gb3 in Human Plasma with K2 EDTA as Anticoagulant at -80 °C for 666 Days

Nominal Concentration (ng/mL) <sup>a</sup>	3.56	40.6	161
Set 1	2.90	34.0	124
Set 2	2.92	33.7	130
Set 3	2.68	33.0	131
Set 4	3.60	34.0	131
<b>Mean</b>	<b>3.03</b>	<b>33.7</b>	<b>129</b>
<b>SD</b>	<b>0.398</b>	<b>0.472</b>	<b>3.37</b>
<b>% CV</b>	<b>13.2</b>	<b>1.4</b>	<b>2.6</b>
<b>% Diff</b>	<b>-15.0</b>	<b>-17.1</b>	<b>-19.9</b>
<b>n</b>	<b>4</b>	<b>4</b>	<b>4</b>

Additional Long-Term Frozen Stability of lyso-Gb3 in Human Plasma with K2 EDTA as Anticoagulant at -70 °C was established for 947 Days.

Nominal	Quality Control Concentrations (ng/mL)					
	NA		8.00		160	
Mean Initial <sup>a</sup>	0.402		7.58		144	
Run # (Days Stored)	Amount Found (ng/mL)	Dev. (%)	Amount Found (ng/mL)	Dev. (%)	Amount Found (ng/mL)	Dev. (%)
33228_006 (947 days)	0.485	20.6	8.73	9.13	161	0.625
	0.455	13.2	8.68	8.50	163	1.88
	0.477	18.7	8.62	7.75	164	2.50
Mean	0.472		8.68		163	
sd	0.0155		0.0551		1.53	
%CV	3.29		0.635		0.939	
%Dev	NA		8.46		1.67	
%Dev <sub>0</sub>	17.5		14.5		13.0	

Long-Term Frozen Stability of lyso-Gb3 in Human Plasma with ACD as Anticoagulant at -80 °C for 1694 Days.

Anticoagulants K2-EDTA and ACD had similar performance in regards to QC CV%.

Nominal Concentration (ng/mL) <sup>a</sup>	10.6	40.6	161
Set 1	12.0	45.4	181
Set 2	12.7	46.7	177
Set 3	11.6	44.4	172
Set 4	11.2	46.6	175
<b>Mean</b>	<b>11.9</b>	<b>45.8</b>	<b>176</b>
<b>SD</b>	<b>0.640</b>	<b>1.09</b>	<b>3.77</b>
<b>% CV</b>	<b>5.4</b>	<b>2.4</b>	<b>2.1</b>
<b>% Diff</b>	<b>12.0</b>	<b>12.7</b>	<b>9.5</b>
<b>n</b>	<b>4</b>	<b>4</b>	<b>4</b>

**Carry-over**

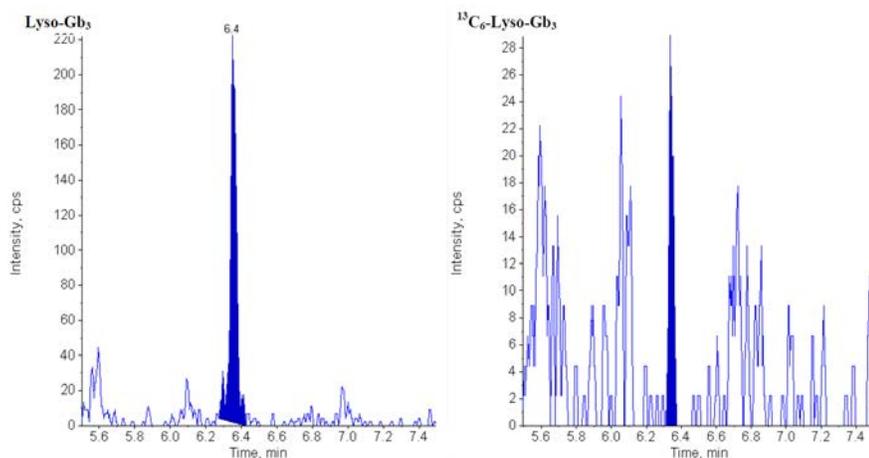
Carryover was significant for all runs in the full method qualification, ranging from 30-90% of the solvent LLOQ standards in the respective runs. For each run of the full method qualification, the impact of carryover on the data was assessed. Carryover did not impact any of the reported data for the full method qualification.

## Endogenous Lyso-Gb3

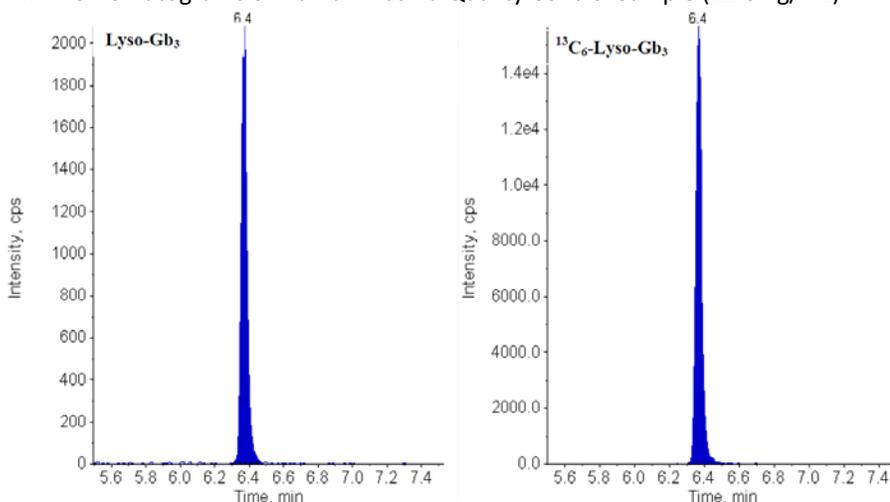
The nominal lyso-Gb3 concentration of the pooled endogenous quality control sample (normal pooled plasma) was determined in the three core runs. This value was found to be 0.587 ng/mL, see table below. The sponsor added 0.587 ng/mL to the plasma calibration standard concentrations. The accuracy and precision of plasma calibration standards were assessed in three separate core runs. The inter-assay accuracy ranged from -3.2 to 3.8%. The inter-assay precision ranged from 2.3 to 5.4%.

Sample Number	Calculated Concentration (ng/mL)		
	Core-run #1	Core-run #2	Core-run #3
PooledPBLK-001	0.541	0.581	0.599
PooledPBLK-002	0.530	0.615	0.629
PooledPBLK-003	0.502	0.666	0.553
PooledPBLK-004	0.510	0.636	0.617
PooledPBLK-005	0.506	0.658	0.651
PooledPBLK-006	0.562	0.670	0.545
<b>Intra-assay Mean</b>	<b>0.525</b>	<b>0.638</b>	<b>0.599</b>
<b>Intra-assay SD</b>	<b>0.0235</b>	<b>0.0346</b>	<b>0.0423</b>
<b>Intra-assay % CV</b>	<b>4.5</b>	<b>5.4</b>	<b>7.1</b>
<b>n</b>	<b>6</b>	<b>6</b>	<b>6</b>
<b>Inter-assay Mean</b>	-	-	<b>0.587</b>
<b>Inter-assay SD</b>	-	-	<b>0.0579</b>
<b>Inter-assay % CV</b>	-	-	<b>9.9</b>
<b>n</b>	-	-	<b>18</b>

Endogenous Lyso-Gb3 can also be observed in the human plasma double blank MRM chromatograms, see below.



**Example Chromatogram** MRM Chromatograms of Human Plasma Quality Control Sample (12.6 ng/mL)



**6.2.2. Lyso-Gb3 Analysis of Samples From Study AT1001-011**

All procedures associated with this bioanalysis were conducted by Amicus Therapeutics, Molecular and Bioanalytical Pharmacology Department between March 6, 2014 and April 21, 2014. Study sample aliquots were analyzed for lyso-Gb3 in three separate analytical runs to obtain three independent measurements of plasma lyso-Gb3 from each study sample.

**Assay passing rate** A total of 531 study samples were analyzed in a total of 14 analytical runs ( no analytical runs failed). A total of 30 study samples were reanalyzed (5.7% of total number of samples analyzed). No study samples required reassay as a result of carryover.

**ISR passing rate** Incurred sample reanalysis for lyso Gb3 in plasma was not conducted. However, the sponsor reanalyzed 18 of 531 (3.4%) samples with all 17 reanalyzed concentration within 30% of the original report concentration. Additionally, parallelism was demonstrated in six human plasma samples from a previously conducted Amicus Phase 2 clinical study were analyzed in singlet with no dilution, 2-fold dilution, 5-fold dilution and 10-fold dilution.

	Run ID	Concentration (ng/mL)					
		0.500	1.50	4.00	12.0	40.0	160
<b>Solvent QC performance</b>	Mean	0.487	1.48	4.01	12.0	39.9	156
	SD	0.0683	0.101	0.216	0.526	2.13	9.10
	%CV	14.0	6.8	5.4	4.4	5.3	5.8
	%Diff	-2.6	-1.3	0.2	0.0	-0.3	-2.5
	n	39	40	40	40	40	40

	Run ID	Concentration (ng/mL)				
		0.590	3.59	12.6	40.6	161
Plasma QC performance	Mean	0.548	3.86	13.4	44.1	175
	SD	0.0760	0.202	0.572	1.81	9.51
	%CV	13.9	5.2	4.3	4.1	5.4
	%Diff	-7.1	7.5	6.3	8.6	8.7
	n	39	40	36	40	40

	Run ID	Lyso-Gb <sub>3</sub> Concentration (ng/mL)									
		0.200	0.500	1.00	2.50	5.00	10.0	25.0	50.0	100	200
Solvent standard curve performance	Mean	0.198	0.506	1.01	2.57	5.09	10.0	25.5	49.2	98.3	192
	SD <sup>b</sup>	0.0245	0.0464	0.0817	0.179	0.206	0.342	1.05	2.15	3.79	6.92
	%CV	12.4	9.2	8.1	7.0	4.0	3.4	4.1	4.4	3.9	3.6
	%Diff	-1.0	1.2	1.0	2.8	1.8	0.0	2.0	-1.6	-1.7	-4.0
	n	39	35	39	36	40	40	40	40	40	40

Reference standards	Lyso-Gb <sub>3</sub> (also referred to as lyso-ceramide trihexoside or globotriaosylsphingosine) was purchased (b) (4)
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<sup>13</sup>C6-lyso-Gb<sub>3</sub> was synthesized at Amicus Therapeutics, Cranbury, NJ

### 6.2.3. Lyso-Gb<sub>3</sub> Analysis of Samples From Study AT1001-012

All procedures associated with this bioanalysis were conducted by Amicus Therapeutics, Molecular and Bioanalytical Pharmacology Department. AT1001-012 plasma study samples were stored at -80 °C for no more than 1022 days prior to analysis. This is the number of days from the first AT1001-012 sample collection (September 8, 2011) to the last day of sample analysis (June 25, 2014).

Assay passing rate	A total of 624 study samples were analyzed in a total of 23 analytical runs (1 analytical run failed). A total of 73 study samples were reanalyzed (5.7% of total number of samples analyzed). 33 study samples required reassay as a result of carryover. Also run 13 was truncated (range 5.00 to 200 ng/mL) due to operator error. Samples that were below the limit of detection in run 13 due to truncation were reassayed in run 18.
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<b>Carry over</b>	<p>In all 23 runs conducted in this study, carryover ranged from 50 to 180% of the solvent LLOQ standards in the respective runs, failing to meet acceptance criteria. The sponsor indicated that the investigation of the raw data in all runs revealed that the carryover did not impact any of the reported data for the calibration standards or quality control samples. This was because runs were always arranged in order of increasing lyso-Gb3 concentration and solvent double blanks were always placed after high concentration samples. The placement of 2 to 3 solvent double blanks after the plasma double blanks injected after the upper limit of quantitation (ULOQ - 200 ng/mL) calibration standard to assess carryover, and 2 to 3 solvent double blanks after injection of the high concentration quality control samples, eliminated the impact of carryover on the next set of samples injected in all runs.</p> <p>To determine if any study samples in that run were impacted by carryover, each study sample lyso-Gb3 concentration was multiplied by the Carryover % and if that value was &gt; 20% of the lyso-Gb3 concentration of the next, subsequently injected study sample, that subsequently injected study sample was reassayed in singlet, and that value reported. In this study thirty three samples were reassayed because the lyso-Gb3 concentration was impacted by carryover.</p>																																																	
<b>ISR passing rate</b>	<p>Incurred sample reanalysis for Lyso Gb3 in plasma was not conducted. However, the sponsor reanalyzed 12 of 624 (2%) samples with all 12 reanalyzed concentration within 30% of the original report concentration. Additionally, parallelism was demonstrated in six human plasma samples from a previously conducted Amicus Phase 2 clinical study were analyzed in singlet with no dilution, 2-fold dilution, 5-fold dilution and 10-fold dilution.</p>																																																	
<b>Solvent QC performance</b>	<p>Inter-Run Accuracy and Precision for Lyso-Gb3 Solvent QCs</p> <table border="1" data-bbox="394 911 1409 1255"> <thead> <tr> <th data-bbox="467 919 540 945">Run ID</th> <th colspan="6" data-bbox="889 919 1133 945">Concentration (ng/mL)</th> </tr> <tr> <td></td> <th data-bbox="643 968 711 993">0.500</th> <th data-bbox="786 968 834 993">1.50</th> <th data-bbox="922 968 974 993">4.00</th> <th data-bbox="1062 968 1110 993">12.0</th> <th data-bbox="1192 968 1260 993">40.0</th> <th data-bbox="1321 968 1370 993">160</th> </tr> </thead> <tbody> <tr> <td data-bbox="477 1031 540 1056">Mean</td> <td data-bbox="643 1031 711 1056">0.543</td> <td data-bbox="786 1031 834 1056">1.50</td> <td data-bbox="922 1031 974 1056">3.80</td> <td data-bbox="1062 1031 1110 1056">12.0</td> <td data-bbox="1192 1031 1260 1056">40.0</td> <td data-bbox="1321 1031 1370 1056">149</td> </tr> <tr> <td data-bbox="477 1077 509 1102">SD</td> <td data-bbox="643 1077 711 1102">0.0758</td> <td data-bbox="786 1077 854 1102">0.0934</td> <td data-bbox="922 1077 974 1102">0.198</td> <td data-bbox="1062 1077 1130 1102">0.557</td> <td data-bbox="1192 1077 1240 1102">1.40</td> <td data-bbox="1321 1077 1370 1102">5.56</td> </tr> <tr> <td data-bbox="477 1123 540 1148">%CV</td> <td data-bbox="643 1123 711 1148">14.0</td> <td data-bbox="786 1123 834 1148">6.2</td> <td data-bbox="922 1123 974 1148">5.2</td> <td data-bbox="1062 1123 1110 1148">4.6</td> <td data-bbox="1192 1123 1240 1148">3.5</td> <td data-bbox="1321 1123 1370 1148">3.7</td> </tr> <tr> <td data-bbox="477 1169 540 1194">%Diff</td> <td data-bbox="643 1169 711 1194">8.6</td> <td data-bbox="786 1169 834 1194">0.0</td> <td data-bbox="922 1169 974 1194">-5.0</td> <td data-bbox="1062 1169 1110 1194">0.0</td> <td data-bbox="1192 1169 1240 1194">0.0</td> <td data-bbox="1321 1169 1370 1194">-6.9</td> </tr> <tr> <td data-bbox="493 1215 509 1241">n</td> <td data-bbox="643 1215 711 1241">44</td> <td data-bbox="786 1215 834 1241">44</td> <td data-bbox="922 1215 974 1241">44</td> <td data-bbox="1062 1215 1110 1241">46</td> <td data-bbox="1192 1215 1240 1241">46</td> <td data-bbox="1321 1215 1370 1241">46</td> </tr> </tbody> </table>	Run ID	Concentration (ng/mL)							0.500	1.50	4.00	12.0	40.0	160	Mean	0.543	1.50	3.80	12.0	40.0	149	SD	0.0758	0.0934	0.198	0.557	1.40	5.56	%CV	14.0	6.2	5.2	4.6	3.5	3.7	%Diff	8.6	0.0	-5.0	0.0	0.0	-6.9	n	44	44	44	46	46	46
Run ID	Concentration (ng/mL)																																																	
	0.500	1.50	4.00	12.0	40.0	160																																												
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n	44	44	46	46	46																																													

Solvent standard curve performance	Back-calculated Calibration Standards for Lyso-Gb3 in Solvent									
	Run ID	Lyso-Gb <sub>3</sub> Concentration (ng/mL)								
		0.200	0.500	1.00	2.50	5.00	10.0	25.0	50.0	100
Mean	0.198	0.509	1.01	2.50	4.89	10.0	24.9	50.3	100	198
SD	0.0168	0.0343	0.0792	0.182	0.206	0.416	0.850	1.48	4.55	7.10
%CV	8.5	6.7	7.8	7.3	4.2	4.2	3.4	2.9	4.6	3.6
% Bias	-1.0	1.8	1.0	0.0	-2.2	0.0	-0.4	0.6	0.0	-1.0
n	42	44	43	44	45	45	45	45	46	46

The assay range for Run13 was truncated and was therefore excluded from the mean calculation and statistics.

Reference standards	Lyso-Gb3 (also referred to as lyso-ceramide trihexoside or globotriaosylsphingosine) was purchased (b) (4)
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<sup>13</sup>C6-lyso-Gb3 was synthesized at Amicus Therapeutics, Cranbury, NJ

### 6.3. $\alpha$ -Galactosidase A Activity ( $\alpha$ -Gal A) in Human White Blood Cells (WBC)

#### 6.3.1. Bioanalytical Method Validation for $\alpha$ -Gal A Activity in human WBC

The Office of Clinical Pharmacology review team has assessed the adequacy and acceptability of the following bioanalytical methods used in clinical studies AT1001-011 for the quantitation of  $\alpha$  Gal –A activity in human WBC.

(b) (4) developed a fluorometric assay for the determination of  $\alpha$  Gal –A activity in human white blood cell lysate (AA19779-01). Briefly, an aliquot of human white blood cell lysate was analyzed for alpha-galactosidase A activity, with 4-methylumbelliferone (4MU) as a reference, using a fluorometric assay method. The reaction product 4MU is detected. The assay was performed in a 96-well microtiter plate format. The signal (relative fluorescence unit) was read on a spectrofluorometer. Alpha-galactosidase A activity was reported as 4-methylumbelliferone concentration. 4-methylumbelliferone calibration curves and quality controls were prepared in 0.5% BSA in 1X WBC lysis buffer and assayed to establish linearity, precision and accuracy of the enzyme reaction product. Enzyme activity quality controls were prepared from isolated human whole blood cell lysates and assayed to establish precision and biological stability.

Matrix	Human White Blood Cell Lysate	
Analyte	4-Methylumbelliferone	(b) (4)
Assay Method	Fluorometric	
Validated assay range	0.050 – 20.0 $\mu$ M	
Regression model & weighting	four parameter logistic (4 PL) method, 1/y <sub>2</sub> weighting	
Validation parameters	Method validation summary	
Standard curve performance during accuracy & precision	No of standard calibrators from LLOQ to ULOQ	10
	Cumulative accuracy (% bias) from LLOQ to ULOQ	-2.1 to 2.0%
	Cumulative precision (% CV) from LLOQ to ULOQ	0.8 to 4.4%

<b>QCs performance during accuracy &amp; precision</b>	Inter-Batch Precision Range (% CV)	2.0 to 12.9%	
	Inter-Batch Accuracy Range (% Bias)	-3.3 to 6.4%	
	Intra-Batch Precision Range (% CV)	0.4 to 15.0%	
	Intra-Batch Accuracy Range (% Bias)	-11.5 to 17.8%	
<b>Dilution linearity &amp; hook effect</b>	40-fold		
	Dilution Linearity of 4-Methylumbelliferone in 0.5% BSA in 1X WBC Lysis Buffer		
		<b>4MU QC D</b>	
	<b>Batch</b>	<b>Dilution Factor 50 <math>\mu</math>M 4MU</b>	
	22	3	50.3
		5	51.2
		10	51.5
		20	47.7
		40	47.4
	Mean		49.6
% CV		3.9	
% Theoretical		99.2	
n		5	
	Dilution Linearity of 4-Methylumbelliferone Generation in WBC Lysate		
	<b>WBC Lysate QC D</b>		
<b>Batch</b>	<b>Dilution Factor</b>	<b>44.9 <math>\mu</math>M 4MU</b>	
22	3	48.1	
	5	46.0	
	10	48.1	
	20	46.4	
	40	45.7	
Mean		46.9	
% CV		2.5	
% Theoretical		104.5	
n		5	

**Bench-top/process stability**

Buffer samples 24 hours at ambient temperature,  
 WBC lysate samples 16 hours in an ice water bath  
 1124 hours (46 days) in microcentrifuge tubes at 5°C under white light

Short-term Stability of 4-Methylumbelliferone in 0.5% BSA in 1X WBC Lysis Buffer at Ambient Temperature Under White Light (24 hours)

Batch	4MU STS A	4MU STS C	4 MU STS D DF = 5
	0.150 µM	15.0 µM	50.0 µM
21	0.142	15.2	51.1
	0.142	15.4	50.7
	0.144	15.7	52.3
Mean	0.143	15.4	51.4
% CV	0.81	1.6	1.6
% Theoretical	95.3	102.7	102.8
n	3	3	3

Short-term Stability of 4-Methylumbelliferone Generation in Human WBC Lysate in an Ice Water Bath under White Light (16 hours)

Batch	WBC Lysate STS D DF = 5
	44.9 µM 4MU
23	47.0
	45.7
	45.6
Mean	46.1
% CV	1.7
% Theoretical	102.7
n	3

Short-Term Stability of 4-Methylumbelliferone Generation in Human WBC Lysate at 5°C Under White Light Conditions (1124 hours)

Short-term stability period: 1124 hours (46 days) in microcentrifuge tubes

Batch	DF = 5 STS D 46.1 uM/mL
	127
49.9	
50.5	
49.7	
54.6	
50.2	
Mean	52.2
% CV	6.7
% Theoretical	113.2
n	6

**Freeze-Thaw stability**

Buffer samples 6 cycles at -20°C,  
 WBC lysate samples 6 cycles at -80°C and ice water bath

Stability of 4-Methylumbelliferone in 0.5% BSA in 1X WBC Lysis Buffer  
 Following Freeze (-20°C) and Thaw Cycles (6 Cycles)

Batch	FT A	FT C	FT D DF = 5
	0.150 µM	15.0 µM	50.0 µM
25	0.148	15.4	52.1
	0.158	15.4	52.0
	0.166	15.6	53.2
Mean	0.157	15.5	52.4
% CV	5.7	0.7	1.3
% Theoretical	104.7	103.3	104.8
n	3	3	3

Stability of 4-Methylumbelliferone Generation in Human WBC Lysate Following  
 Freeze (-80°C) and Thaw Cycles (in an Ice Water Bath) (6 Cycles)

Batch	WBC Lysate FT D DF = 5
	44.9 µM 4MU
26	42.3
	43.7
	43.7
Mean	43.2
% CV	1.9
% Theoretical	96.2
n	3

**Long-term storage**

Buffer samples 329 days in a polypropylene container at -20°C

WBC lysate samples 368 days in a polypropylene container at -20°C

WBC lysate samples 532 days in a polypropylene container at -80°C

WBC lysate samples 1035 days in a polypropylene container at -80°C

**Long-term Stability of 4-Methylumbelliferone in 0.5% BSA in 1X WBC Lysis Buffer at -20°C**

Extraction date: 10-Aug-2005

Preparation date: 15-Sep-2004

Long-term stability period: 329 days in a polypropylene container

Batch	4 MU QC A	4 MU QC C	4 MU QC D
	0.150 µM	15.0 µM	50.0 µM
52	0.158	15.6	50.5
	0.158	15.5	50.1
	0.160	15.0	49.3
Mean	0.159	15.4	50.0
% CV	0.7	2.1	1.2
% Theoretical	106.0	102.7	100.0
n	3	3	3

**Long-Term Stability of 4-Methylumbelliferone Generation in Human WBC Lysate at -20°C**

Extraction date: 26-Aug-2008

Preparation date: 24-Aug-2007

Long-term stability period: 368 days in polypropylene tubes

Batch	DF = 5 LTS WBC QC D 68.3 µM
	102
Mean	75.5
% CV	1.5
% Theoretical	110.5
n	3

**Long-term Stability of 4-Methylumbelliferone Generation in Human WBC Lysate at -80°C**

Extraction date: 17-Aug-2007

Preparation date: 03-Mar-2006

Long-term stability period: 532 days

Batch	DF = 5 WBC QC D 61.6 µM
	95
Mean	62.0
% CV	2.6
% Theoretical	100.6
n	3

**Long-term Stability of 4-Methylumbelliferone Generation in Human WBC Lysate at -80°C**

Extraction date: 24-Nov-2014

Preparation date: 24-Jan-2012

Long-term stability period: 1035 days

Batch	DF = 5 WBC QC D 46.1 µM
	174
Mean	51.1
% CV	1.8
% Theoretical	110.8
n	6

<b>Stock Solution Stability</b>	<p>134 days in diluent at -20°C</p> <p>Stock Solution Stability of 4-Methylumbelliferone in Methanol at -20°C</p> <p>Theoretical concentration: 10.0 mM  Fresh stock preparation date: 13-Jan-2005  Stability stock preparation date: 01-Sep-2004  Stock solution stability period: 134 days in a polypropylene container</p> <table border="1" data-bbox="760 422 1117 676"> <thead> <tr> <th rowspan="2">Batch</th> <th colspan="2">Stability Stock</th> </tr> <tr> <th>50.0 µM</th> <th>0.150 µM</th> </tr> </thead> <tbody> <tr> <td rowspan="3">39</td> <td>47.6</td> <td>0.140</td> </tr> <tr> <td>48.8</td> <td>0.150</td> </tr> <tr> <td>48.5</td> <td>0.153</td> </tr> <tr> <td>Mean</td> <td>48.3</td> <td>0.148</td> </tr> <tr> <td>% CV</td> <td>1.3</td> <td>4.6</td> </tr> <tr> <td>% Theoretical</td> <td>96.6</td> <td>98.7</td> </tr> <tr> <td>n</td> <td>3</td> <td>3</td> </tr> </tbody> </table>	Batch	Stability Stock		50.0 µM	0.150 µM	39	47.6	0.140	48.8	0.150	48.5	0.153	Mean	48.3	0.148	% CV	1.3	4.6	% Theoretical	96.6	98.7	n	3	3
Batch	Stability Stock																								
	50.0 µM	0.150 µM																							
39	47.6	0.140																							
	48.8	0.150																							
	48.5	0.153																							
Mean	48.3	0.148																							
% CV	1.3	4.6																							
% Theoretical	96.6	98.7																							
n	3	3																							
<b>Substock Solution Stability</b>	<p>162 days at 0.0500 µM in 0.5% BSA in 1X WBC lysis buffer at -20°C</p> <p>Substock Solution Stability of 4-Methylumbelliferone in 0.5% BSA in 1X WBC Lysis Buffer at -20°C</p> <p>Theoretical concentration: 0.0500 µM  Fresh stock preparation date: 17-Aug-2007  Stability stock preparation date: 08-Mar-2007  Stock solution stability period: 162 days in a polypropylene container</p> <table border="1" data-bbox="865 978 1179 1260"> <thead> <tr> <th>Batch</th> <th>0.0500 µM</th> </tr> </thead> <tbody> <tr> <td rowspan="3">95</td> <td>0.0526</td> </tr> <tr> <td>0.0501</td> </tr> <tr> <td>0.0537</td> </tr> <tr> <td>Mean</td> <td>0.0521</td> </tr> <tr> <td>% CV</td> <td>3.5</td> </tr> <tr> <td>% Theoretical</td> <td>104.2</td> </tr> <tr> <td>n</td> <td>3</td> </tr> </tbody> </table>	Batch	0.0500 µM	95	0.0526	0.0501	0.0537	Mean	0.0521	% CV	3.5	% Theoretical	104.2	n	3										
Batch	0.0500 µM																								
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% CV	3.5																								
% Theoretical	104.2																								
n	3																								

### 6.3.2. α-Gal A Activity in Human WBC lysate Sample Analysis from Study AT1001-011

(b) (4) has determined the concentrations of 4-methylumbelliferone (4MU) in human white blood cell (WBC) lysate using a fluorometric assay. The WBC lysate samples are assayed using both the α-galactosidase A assay and the total protein assay.

<b>Parameters</b>	Study summary
<b>Analyzed Samples</b>	A total of 551 study samples were analyzed in a total of 60 analytical runs (7 analytical runs failed).
<b>ISR passing rate</b>	Due to the nature of the method, incurred sample reproducibility was not performed
<b>Standard curve performance</b>	%CV: 1.7% to 6.8% % Bias: -3.1% to 1.6%
<b>QC performance</b>	Inter-Assay % CV: 0.1% to 16.9% Intera Assay % Bias: -3.2% to 6.6%

<b>Study sample analysis/ stability</b>	Study samples were collected between Oct 22, 2009 and Jan 06, 2014 and were received frozen on dry ice as WBC pallets between March 11, 2011 and Jan 10, 2014. Samples were stored at a nominal temperature of -80°C until the analysis. Samples were analyzed in multiple batches throughout the study between Jan 06, 2012 through Jan 24, 2014. Samples were assayed in multiple batches throughout the study. The maximum time between sample collection and sample analysis did not exceed 975 days
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## 6.4. Total Protein in Human WBC

### 6.4.1. Bioanalytical Method Validation for Total Protein in human WBC

The Office of Clinical Pharmacology review team has assessed the adequacy and acceptability of the following bioanalytical methods used in clinical studies AT1001-011 for the quantitation of total protein in human white blood cell lysate.

(b) (4) developed a colorimetric assay for the determination of total protein in human white blood cell lysate (validation report AA19779-02). A colorimetric kit (BCA Protein Assay Kit) from (b) (4) was used. The assay was performed in a 96-well microtiter plate format. The signal (absorbance) was read on a spectrophotometer. The assay was

developed for use in conjunction with enzyme activity assays (e.g.  $\alpha$ -galactosidase A) so that the specific activity of an enzyme can be determined by normalizing the results based on the amount of protein.

Albumin, bovine serum (BSA) was used as the reference standard.

<b>Matrix</b>	Human White Blood Cell Lysate	
<b>Analyte</b>	Total protein	
<b>Assay Method</b>	Colorimetric	
<b>Validated assay range</b>	25.0 – 2000 $\mu$ g/mL	
<b>Regression model &amp; weighting</b>	four parameter logistic (4 PL) method, 1/y weighting	
<b>Validation parameters</b>	Method validation summary	
<b>Standard curve performance during accuracy &amp; precision</b>	No of standard calibrators from LLOQ to ULOQ	10
	Cumulative accuracy (% bias) from LLOQ to ULOQ	-3.8 to 4.8%
	Cumulative precision (% CV) from LLOQ to ULOQ	1.2 to 4.3%
<b>QCs performance during accuracy &amp; precision</b>	Inter-Batch Precision Range (% CV)	3.5 to 12.2%
	Inter-Batch Accuracy Range (% Bias)	-4.0 to 4.0 %
	Intra-Batch Precision Range (% CV)	1.1 to 15.9%
	Intra-Batch Accuracy Range (% Bias)	-8.4 to 13.0%

**Dilution linearity & hook effect**

40-fold

**Dilution Linearity of BSA in WBC Lysate Buffer**

<b>Batch</b>	<b>Dilution Factor</b>	<b>BSA QC D 4000 µg/mL</b>
7	3	4000
	5	3720
	10	3590
	20	3920
	40	4140
Mean		3870
% CV		5.7
% Theoretical		96.8
n		5

**Dilution Linearity of Total Protein in WBC Lysate**

<b>Batch</b>	<b>Dilution Factor</b>	<b>WBC LYS QC D 2010 µg/mL</b>
9	3	2100
	5	1990
	10	1970
	20	1950
	25	2130
Mean		2030
% CV		4.0
% Theoretical		101.0
n		5

**Bench-top/process stability**

22 hours at ambient temperature under white light  
 837 hours (34 days) in microcentrifuge tubes at 5°C under white light

**Short-term Stability of BSA in WBC Lysate Buffer Under White Light at Ambient Temperature (22 hours)**

Batch	BSA STS D DF = 5			
	75.0 µg/mL	300 µg/mL	1500 µg/mL	4000 µg/mL
6	78.9	303	1710	3630
	111*	307	1460	3760
	75.7	295	1390	3830
Mean	77.3	302	1520	3740
% CV	2.9	2.0	11.1	2.7
% Theoretical	103.1	100.7	101.3	93.5
n	2	3	3	3

**Short-term Stability of Total Protein in WBC Lysate Under White Light at Ambient Temperature (22 hour)**

Batch	WBC LYS STS D DF = 5
	2010 µg/mL
6	2070
	2070
	2220
Mean	2120
% CV	4.1
% Theoretical	105.5
n	3

**Short-Term Stability of Total Protein in WBC Lysate at 5°C Under White Light Conditions**

Batch	DF = 5
	STS D
68	1170 µg/mL
	962
	970
	1040
	999
	1300
1090	
Mean	1060
% CV	12.0
% Theoretical	90.6
n	6

**Freeze-Thaw stability**

BSA samples 6 cycles at -20°C,  
WBC Lysate 6 cycles at -80°C

Stability of BSA in WBC Lysate Buffer Following Freeze (-20°C) and Thaw Cycles (6 cycles)

	BSA FT A	BSA FT B	BSA FT C	BSA FT D	DF = 5
Batch <sup>3</sup>	75.0 µg/mL	300 µg/mL	1500 µg/mL	4000 µg/mL	
8, 10	86.8	302	1510	3740	
	83.6	308	1420	3890	
	78.0	319	1450	3890	
Mean	82.8	310	1460	3840	
% CV	5.4	2.8	3.1	2.3	
% Theoretical	110.4	103.3	97.3	96.0	
n	3	3	3	3	

Stability of Total Protein in Human WBC Lysate Following Freeze (-80°C) and Thaw Cycles (6 cycles)

Batch	WBC LYS FT	DF = 5
	2010 µg/mL	
8	1770	
	1810	
	1840	
Mean	1810	
% CV	1.9	
% Theoretical	90.0	
n	3	

**Long-term storage**

BSA samples 531 days at -20°C,  
 WBC Lysate 356 days at -20°C,  
 WBC Lysate 539 days at -80°C  
 Long-term stability of WBCs (white blood cells) cannot be determined due to the nature of the samples.

**Long-term Stability of BSA in WBC Lysate Buffer at -20°C**

Extraction date: 13-Mar-2006  
 Preparation date: 28-Sep-2004  
 Long-term stability period: 531 days in polypropylene

Batch	BSA QC A	BSA QC C	DF=5 BSA QC D
	75.0 µg/mL	1500 µg/mL	4000 µg/mL
31	74.8	1510	3920
	71.1	1450	3940
	71.8	1520	3810
Mean	72.6	1490	3890
% CV	2.7	2.5	1.8
% Theoretical	96.8	99.3	97.3
n	3	3	3

**Long-term Stability of Total Protein in Human WBC Lysate at -20°C**

Extraction date: 14-Aug-2008  
 Preparation date: 24-Aug-2007  
 Long-term stability period: 356 days in polypropylene tubes

Batch	DF = 5 LTS WBC QC D
	2640 µg/mL
49	2460
	2060
	2370
Mean	2300
% CV	9.1
% Theoretical	87.1
n	3

**Long-term Stability of Total Protein in Human WBC Lysate at -80°C**

Extraction date: 13-Mar-2006  
 Preparation date: 20-Sep-2004  
 Long-term stability period: 539 days in polypropylene

Batch	DF=5 WBC QC D
	2010 µg/mL
31	2040
	2080
	2010
Mean	2040
% CV	1.7
% Theoretical	101.5
n	3

<b>Stock Solution Stability</b>	161 days at ~10000 µg/mL in antiseptic saline in polypropylene tubes at 5°C																					
	<p>Stock Solution Stability of BSA in Antiseptic Saline at 5°C</p> <p>Theoretical concentration: approximately 10000 µg/mL  Fresh stock preparation date: 27-Apr-2007  Stability stock preparation date: 17-Nov-2006  Stock solution stability period: 161 days in a polypropylene tube</p> <table border="1"> <thead> <tr> <th>Batch</th> <th>75.0 µg/mL</th> <th>1500 µg/mL</th> </tr> </thead> <tbody> <tr> <td rowspan="3">42</td> <td>75.5</td> <td>1430</td> </tr> <tr> <td>75.7</td> <td>1440</td> </tr> <tr> <td>79.9</td> <td>1610</td> </tr> <tr> <td>Mean</td> <td>77.0</td> <td>1490</td> </tr> <tr> <td>% CV</td> <td>3.2</td> <td>6.8</td> </tr> <tr> <td>% Theoretical</td> <td>102.7</td> <td>99.3</td> </tr> <tr> <td>n</td> <td>3</td> <td>3</td> </tr> </tbody> </table>	Batch	75.0 µg/mL	1500 µg/mL	42	75.5	1430	75.7	1440	79.9	1610	Mean	77.0	1490	% CV	3.2	6.8	% Theoretical	102.7	99.3	n	3
Batch	75.0 µg/mL	1500 µg/mL																				
42	75.5	1430																				
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Mean	77.0	1490																				
% CV	3.2	6.8																				
% Theoretical	102.7	99.3																				
n	3	3																				
<b>Substock Solution Stability</b>	131 days at 2000 µg/mL in antiseptic saline in polypropylene tubes at 5°C																					
	<p>Substock Solution Stability of BSA in Antiseptic Saline at 5°C</p> <p>Theoretical concentration: 2000 µg/mL  Fresh substock preparation date: 19-Nov-2006  Stability substock preparation date: 11-Jul-2006  Substock solution stability period: 131 days in a polypropylene tube</p> <table border="1"> <thead> <tr> <th>Batch</th> <th>75.0 µg/mL</th> <th>1500 µg/mL</th> </tr> </thead> <tbody> <tr> <td rowspan="3">39</td> <td>88.8</td> <td>1510</td> </tr> <tr> <td>89.8</td> <td>1510</td> </tr> <tr> <td>87.6</td> <td>1580</td> </tr> <tr> <td>Mean</td> <td>88.7</td> <td>1530</td> </tr> <tr> <td>% CV</td> <td>1.2</td> <td>2.6</td> </tr> <tr> <td>% Theoretical</td> <td>118.3</td> <td>102.0</td> </tr> <tr> <td>n</td> <td>3</td> <td>3</td> </tr> </tbody> </table>	Batch	75.0 µg/mL	1500 µg/mL	39	88.8	1510	89.8	1510	87.6	1580	Mean	88.7	1530	% CV	1.2	2.6	% Theoretical	118.3	102.0	n	3
Batch	75.0 µg/mL	1500 µg/mL																				
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	87.6	1580																				
Mean	88.7	1530																				
% CV	1.2	2.6																				
% Theoretical	118.3	102.0																				
n	3	3																				

#### 6.4.2. Total Protein in Human WBC Lysate Analysis from Study AT1001-011

(b) (4) has determined the concentrations of total protein in human white blood cell (WBC) lysate using a colorimetric assay.

The WBC pellets were lysed prior to analysis. The WBC lysate samples are assayed using both the α-galactosidase A assay and the total protein assay.

<b>Parameters</b>	Study summary
<b>Analyzed Samples</b>	A total of 551 study samples were analyzed in a total of 33 analytical runs (2 analytical runs failed).
<b>ISR passing rate</b>	Due to the nature of the method, incurred sample reproducibility was not performed
<b>Standard curve performance</b>	%CV: 1.3% to 7.2% -% Bias: 4.4% to 3.2%
<b>QC performance</b>	Inter-Assay % CV: 3.2% to 14.9% Intera Assay % Bias: -10.3% to 15.3%

<b>Study sample analysis/ stability</b>	Study samples were collected between Oct 22, 2009 and Jan 06, 2014 and were received frozen on dry ice as WBC pallets between March 11, 2011 and Jan 10, 2014. Samples were stored at a nominal temperature of -80°C until the analysis. Samples were analyzed in multiple batches throughout the study between Jan 09, 2012 through Jan 23, 2014. The maximum time between sample collection and sample analysis did not exceed 975 days
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## **6.5. C22:0 and C24:0 isoforms of GL-3**

### **6.5.1. Bioanalytical Method Validation for C22:0 and C24:0 isoforms of GL-3**

The Office of Clinical Pharmacology review team has assessed the adequacy and acceptability of the following bioanalytical methods used in clinical studies AT1001-011 and AT1001-012 for the quantitation of C22:0 and C24:0 isoforms of GL-3, simply referred to as GL-3 hereinafter.

The sponsor developed a liquid chromatography tandem mass spectrometry (LC/MS/MS) method to quantify GL-3 in human urine containing 0.01% Tween 80. No major issues were identified in validation report (No. AFGS2). Briefly, GL-3 and the internal standard were extracted from human plasma potassium containing K2 EDTA using liquid-liquid extraction. The extracted samples were analyzed by an HPLC equipped with an AB/MDS Sciex API 4000 mass spectrometer. The method utilized derivative of GL-3 as the internal standard (C17-CTH). Charcoal-stripped human urine with Tween 80 based calibrators and QCs were used in the quantitation of GL-3 due to endogenous GL-3 levels. The method was sufficiently validated to support the quantitation of lyso-Gb3 in human plasma.

<b>Matrix</b>	Human Urine with 0.01% Tween 80 as the additive (10:1 human urine : Tween 80) Surrogate Matrix: Charcoal-stripped human urine with Tween 80	
<b>Test article purity</b>	C22:0 (Synonymous with C22-CTH) Amicus Therapeutics 372-ZC-1-20D	
<b>Internal standard purity</b>	C24:0 (Synonymous with C24-CTH) Amicus Therapeutics 372-ZC-1-19D C17-CTH Amicus Therapeutics 372-ZC-1-5/GL-3	
<b>Validated assay range</b>	Stripped urine 1.0 to 200 ng/mL for C22:0 and C24:0	
<b>Regression model &amp; weighting</b>	$1/x^2$	
<b>Validation parameters</b>	Method validation summary	
<b>Stripped urine standard curve performance during accuracy &amp; precision for C22:0</b>	No of standard calibrators from LLOQ to ULOQ	8
	Cumulative accuracy (%bias) from LLOQ to ULOQ	-1.51 to 2.51
	Cumulative precision (%CV) from LLOQ to ULOQ	3.56 to 7.08
<b>Stripped urine standard curve performance during accuracy &amp; precision for C24:0</b>	No of standard calibrators from LLOQ to ULOQ	8
	Cumulative accuracy (%bias) from LLOQ to ULOQ	-3.28 to 3.55
	Cumulative precision (%CV) from LLOQ to ULOQ	4.02 to 6.49
<b>Stripped urine QCs performance during accuracy &amp; precision for C22:0</b>	Intra-Batch Precision Range (% CV)	1.48 to 7.31
	Intra-Batch Accuracy Range (% Bias)	-13.4 to 5.01
	Inter-Batch Precision Range (% CV)	4.47 to 15.1
	Inter-Batch Accuracy Range (% Bias)	-0.92 to -4.9
<b>Un-stripped urine QCs performance during accuracy &amp; precision for C22:0</b>	Intra-Batch Precision Range (% CV)	1.89 to 4.82
	Intra-Batch Accuracy Range (% Bias)	0.172 to 2.34
	Inter-Batch Precision Range (% CV)	4.26 to 5.21
	Inter-Batch Accuracy Range (% Bias)	-2.4 to -4.01
<b>Stripped urine QCs performance during accuracy &amp; precision for C24:0</b>	Intra-Batch Precision Range (% CV)	2.64 to 11.1
	Intra-Batch Accuracy Range (% Bias)	-5.65 to 2.01
	Inter-Batch Precision Range (% CV)	5.59 to 7.05
	Inter-Batch Accuracy Range (% Bias)	-11.6 to -3.52
<b>Un-stripped urine QCs performance during accuracy &amp; precision for C24:0</b>	Intra-Batch Precision Range (% CV)	2.94 to 3.87
	Intra-Batch Accuracy Range (% Bias)	-5.79 to -1.39
	Inter-Batch Precision Range (% CV)	4.9 to 5.0
	Inter-Batch Accuracy Range (% Bias)	-6.82 to -8.33

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**Interference and Specificity**

Aliquots of blank human urine were fortified with only one analyte at the ULOQ level or one internal standard at the level of use and analyzed in triplicate.

Cross analyte interference did not meet the 20% acceptance criteria because there appears to be contribution from C22:0 to C24:0 and from C24:0 to C22:0. The contribution is approximately 74% of the LLOQ of C24:0 from the C22:0 ULOQ and 500% of the LLOQ of C22:0 from the C24:0 ULOQ. Part of this contribution is due to the impurities of the other isoforms in each standard, as stated in the

Certificates of Analysis. Although the concentrations of the isoforms vary between individuals, the two isoforms tend to have similar concentrations within each individual. Therefore, this cross analyte contribution is unlikely to effect the analysis of clinical data.

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**Human urine matrix**

Matrix-related ionization effects were also evaluated by comparing analyte responses of the post-extraction spiked matrix samples to those of external standards prepared at the same analyte concentrations in neat solution. Matrix-related ionization effects for C22:0 and internal standards ranged from 93 to 110% of the nominal concentration.

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**Recovery**

General extraction recovery of the analytes from human urine was evaluated by comparing the analyte responses of pre-extraction spiked samples to those of post-extraction spiked samples, representing 100% recovery. General extraction recovery for C22:0 and internal standard was ~78%.

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**Dilution linearity**

The ability to analyze samples with insufficient volume for a full aliquot was validated by analyzing six replicate QCs, containing 19.5 ng/mL C22:0 and 18.4 ng/mL C24:0, as five-fold dilutions in Run 12AFGS2. The ability to dilute samples originally above the upper limit of the calibration range was validated by analyzing six replicate QCs, containing 5000 ng/mL C22:0 and 2000 ng/mL C24:0, as 50-fold for C22:0 and 20-fold for C24:0 dilutions.

C:22

	QC 13 Dil 5 (ng/mL)	QC 6 Dil 50 (ng/mL)
N	6	6
Theoretical Concentration	19.5	5000
Mean	20.4	4380
S.D.	0.894	208
%C.V.	4.38	4.74
% Difference from Theoretical	4.65	-12.3

(continued)

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**Dilution linearity****(continued)**

C:24

Run ID	QC 13 Dil 5 (ng/mL)	QC 8 Dil 20 (ng/mL)
Theoretical Concentration	18.4	2000
Mean	18.4	2090
S.D.	0.774	110
%C.V.	4.22	5.27
% Difference from Theoretical	-0.227	4.65

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**Standard Solution Stability**

Analyte stability in solution under nominal storage and bench-top stress conditions has been demonstrated as outlined in table below.

Analyte	Concentration	Solvent	Storage Temperature	Storage Period
C22:0	5000 ng/mL	Dimethylsulfoxide	Room Temperature	722 Days
C22:0	500 µg/mL	2:1:3 Chloroform:MeOH:DMSO	Room Temperature	841 Days
C24:0	5000 ng/mL	Dimethylsulfoxide	Room Temperature	270 Days
C24:0	500 µg/mL	2:1:3 Chloroform:MeOH:DMSO	Room Temperature	873 Days

**Internal Standard solution stability**

C17-CTH	500 µg/mL	2:1:3 Chloroform:Methanol:DMSO	Room Temperature	61 Days
C17-CTH	10 µg/mL	DMSO	Room Temperature	13 Days

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**Bench-top/process stability**

Quality controls were allowed to remain at room temperature for 24.5 hours prior to extraction.

Run ID	Charcoal-Stripped Urine		Un-Stripped Urine	
	TM 1 (ng/mL)	TM 5 (ng/mL)	TM 13 (ng/mL)	TM 15 (ng/mL)
N	6	6	6	6
Theoretical Concentration	2.00	150	19.5	154
Mean	2.00	142	18.1	138
S.D.	0.134	3.90	0.925	2.25
%C.V.	6.70	2.74	5.12	1.63
% Difference from Theoretical	0.115	-5.07	-7.30	-10.1

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**-20 °C Freeze-Thaw  
stability for C22:0 (Five  
Cycles)**

Run ID	<u>Charcoal-Stripped Urine</u>		<u>Un-Stripped Urine</u>	
	5FTF 1 (ng/mL)	5FTF 5 (ng/mL)	5FTF 13 (ng/mL)	5FTF 15 (ng/mL)
Theoretical Concentration	2.00	150	19.5	154
Mean	1.94	138	18.3	140
S.D.	0.0722	3.72	1.61	6.55
%C.V.	3.71	2.69	8.78	4.68
% Difference from Theoretical	-2.77	-7.89	-6.12	-9.19

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**-70 °C Freeze-Thaw  
stability for C24:0 (Five  
Cycles)**

Run ID	<u>Charcoal-Stripped Urine</u>		<u>Un-Stripped Urine</u>	
	5FTC 1 (ng/mL)	5FTC 5 (ng/mL)	5FTC 13 (ng/mL)	5FTC 15 (ng/mL)
Theoretical Concentration	2.00	150	19.5	154
Mean	1.98	145	17.7	142
S.D.	0.148	8.75	0.785	8.17
%C.V.	7.48	6.03	4.44	5.75
% Difference from Theoretical	-0.771	-3.18	-9.36	-7.61

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**Long-term storage**

Analyte stability in frozen stripped and un-stripped matrix was evaluated by analyzing samples which had been stored for 975 days at -70 °C versus freshly prepared calibration standards.

Run ID	<u>Charcoal-Stripped Urine</u>		<u>Un-Stripped Urine</u>	
	STABC 1 (ng/mL)	STABC 5 (ng/mL)	STABC 13 (ng/mL)	STABC 15 (ng/mL)
Theoretical Concentration	2.00	150	19.5	154
Mean	1.80	143	17.4	141
S.D.	0.0978	4.22	1.11	4.58
%C.V.	5.42	2.95	6.37	3.25
% Difference from Theoretical	-9.83	-4.47	-10.9	-8.33

(continued)

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**Long-term storage**

**(continued)**

Analyte stability in frozen stripped and un-stripped matrix was evaluated in by analyzing samples which had been stored for 975 days at -70 °C versus freshly prepared calibration standards.

Run ID	<u>Charcoal-Stripped Urine</u>		<u>Un-Stripped Urine</u>	
	STABC 1 (ng/mL)	STABC 5 (ng/mL)	STABC 13 (ng/mL)	STABC 15 (ng/mL)
Theoretical Concentration	2.00	150	18.4	153
Mean	1.83	139	17.0	137
S.D.	0.0430	2.94	0.844	2.84
%C.V.	2.35	2.12	4.97	2.07
% Difference from Theoretical	-8.56	-7.28	-7.73	-10.4

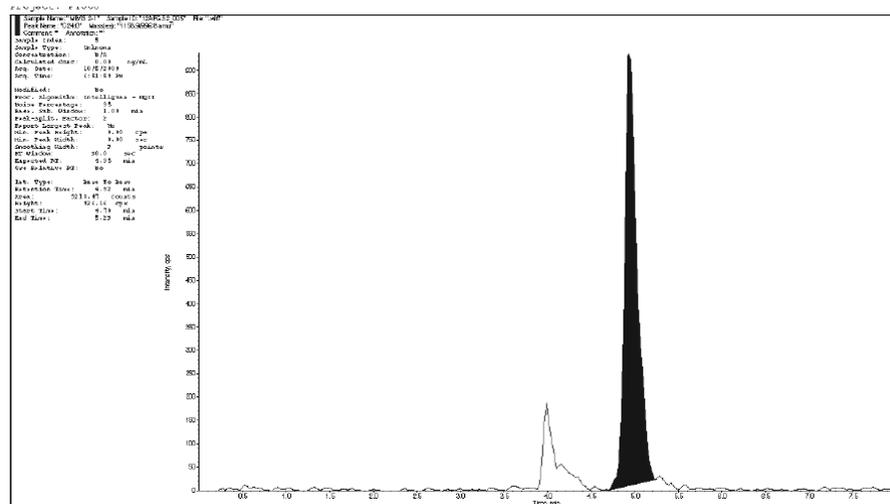
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<b>Carry-over</b>	The potential for carryover from a sample containing a high concentration of analyte to the following sample in an injection sequence was evaluated by injecting duplicate extracted matrix blanks immediately after the ULOQ calibration standards in each validation run. There were no contributions from chromatographic peaks, at the expected retention time of the analyte in the blank samples, greater than 20% of the mean analyte response for the LLOQ calibration standards and quality controls in the validation run.		
<b>Endogenous GL3 for 22:0</b>	Pooled un-stripped urine was assessed from 38 healthy individuals to determine baseline levels of GL-3 for C22:0 over three analytical runs: Mean 8.7 ng/ml %CV 10.6		
<b>Endogenous GL3 for 24:0</b>	Pooled un-stripped urine was assessed from 38 healthy individuals to determine baseline levels of GL-3 for C24:0 over three analytical runs: Mean 6.13 ng/ml %CV 13.9		
<b>C22:0 and C24:0 Urine Values for 38 Individual Healthy Donors Normalized to Creatinine</b>	The normal level of GL-3 was calculated by analyzing 38 individual urine samples. Inclusion in the study required subjects to have four times the upper limit of normal. The upper limit of normal was calculated by determining the average C22:0 and C24:0 value normalized to creatinine and adding two standard deviations to this average value, see table below.		
	The inclusion criteria for subject samples is that their values normalized to creatinine exceed the 4 x mean + 2 x standard deviation value of 135.05 ng/mg which was determined by the 38 normal healthy donors described in this table.	Mean S.D. 2 x S.D. (Mean + 2 x S.D.) 4 x (Mean + 2 x S.D.)	17.09 8.337 16.674 33.674 135.05

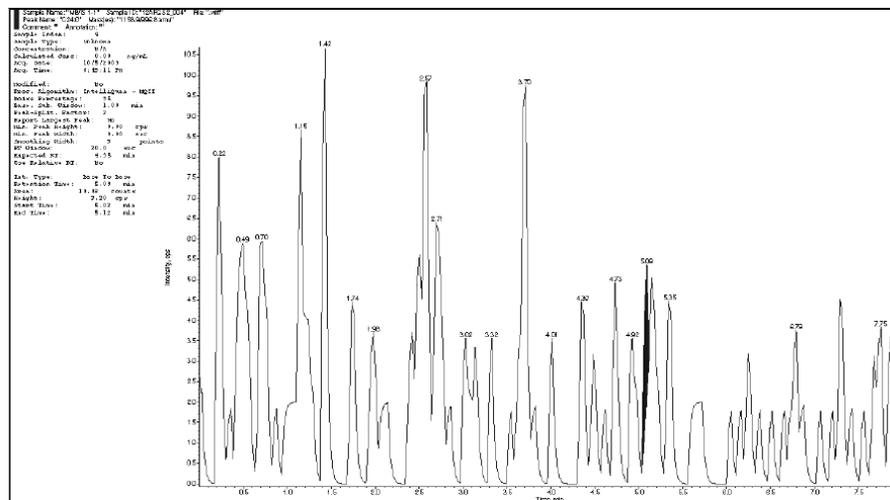


**Example Chromatogram C24:0**

Un-Stripped Blank Human Urine with Internal Standard (C24:0)



Charcoal-Stripped Blank Human Urine with Internal Standard (C24:0)



**6.5.2. GL-3 Analysis of Samples From Study AT1001-011**

All procedures associated with this bioanalysis were conducted by   (b) (4).

<b>Assay passing rate</b>	534 original and 38 duplicate human urine samples, containing 0.01% Tween 80, were received frozen from various clinical sites between Nov 27, 2009 and May 23, 2013. There were two run failures out of 29 analytical runs and 76 (14%) samples reassayed. Majority of the reassayed samples were due to samples above the limit of quantitation.
<b>ISR passing rate</b>	Not reported

Standard curve performance	Average Back-calculated Calibration Standards for C22:0										
	Run ID	CAL 1 (ng/mL)	CAL 2 (ng/mL)	CAL 3 (ng/mL)	CAL 4 (ng/mL)	CAL 5 (ng/mL)	CAL 6 (ng/mL)	CAL 7 (ng/mL)	CAL 8 (ng/mL)		
N	52	53	51	52	54	53	52	52			
Theoretical Concentration	1.00	1.60	3.00	9.00	25.0	75.0	160	200			
Mean	1.01	1.59	2.99	8.86	25.5	76.2	160	196			
S.D.	0.0834	0.102	0.211	0.436	1.47	4.64	7.39	11.1			
%C.V.	8.30	6.43	7.05	4.92	5.76	6.08	4.63	5.63			
% Difference from Theoretical	0.500	-0.429	-0.490	-1.53	2.19	1.61	-0.221	-1.75			
Average Back-calculated Calibration Standards for C24:0											
Run ID	CAL 1 (ng/mL)	CAL 2 (ng/mL)	CAL 3 (ng/mL)	CAL 4 (ng/mL)	CAL 5 (ng/mL)	CAL 6 (ng/mL)	CAL 7 (ng/mL)	CAL 8 (ng/mL)			
N	46	43	42	44	43	46	46	44			
Theoretical Concentration	1.00	1.60	3.00	9.00	25.0	75.0	160	200			
Mean	0.996	1.60	3.04	9.01	25.8	76.2	157	193			
S.D.	0.0663	0.110	0.179	0.488	1.31	4.35	7.76	9.93			
%C.V.	6.65	6.88	5.88	5.42	5.07	5.71	4.95	5.14			
% Difference from Theoretical	-0.406	-0.141	1.20	0.146	3.01	1.60	-1.88	-3.39			
QC performance	Inter-assay Precision and Accuracy for C22:0										
	Run ID	QC 1* (ng/mL)	QC 2* (ng/mL)	QC 3* (ng/mL)	QC 4* (ng/mL)	QC 5* (ng/mL)	QC 23** (ng/mL)	QC 25** (ng/mL)	QC 53** (ng/mL)	QC 55** (ng/mL)	QC 63** (ng/mL)
N	54	54	54	54	54	8	8	20	46	20	6
Theoretical Concentration	2.00	5.00	15.0	40.0	150	27.2	162	24.5	159	26.1	21.4
Mean	1.97	4.85	14.4	38.7	144	29.2	164	24.1	150	27.3	20.3
S.D.	0.195	0.347	1.17	2.72	9.11	1.59	10.6	1.31	9.87	2.16	0.776
%C.V.	9.88	7.15	8.13	7.04	6.30	5.44	6.48	5.43	6.57	7.92	3.83
% Difference from Theoretical	-1.46	-2.98	-3.74	-3.33	-3.70	7.44	1.17	-1.46	-5.58	4.51	-5.21
Inter-assay Precision and Accuracy for C24:0											
Run ID	QC 1* (ng/mL)	QC 2* (ng/mL)	QC 3* (ng/mL)	QC 4* (ng/mL)	QC 5* (ng/mL)	QC 23** (ng/mL)	QC 25** (ng/mL)	QC 53** (ng/mL)	QC 55** (ng/mL)	QC 63** (ng/mL)	QC 73** (ng/mL)
N	46	46	46	46	46	6	6	18	40	16	6
Theoretical Concentration	2.00	5.00	15.0	40.0	150	22.3	157	20.7	156	22.2	19.5
Mean	2.08	5.20	15.4	41.2	150	24.2	158	21.6	154	23.0	19.4
S.D.	0.158	0.308	0.940	2.76	8.78	0.810	5.05	1.17	8.55	1.80	0.755
%C.V.	7.57	5.91	6.09	6.69	5.86	3.35	3.20	5.41	5.57	7.82	3.88
% Difference from Theoretical	4.06	4.09	2.83	3.01	-0.0509	8.46	0.616	4.30	-1.51	3.53	-0.258

Diluted Quality Controls	Diluted Quality Controls for C22:0					
	Run ID	QC 5 Dil 5 (ng/mL)	QC 5 Dil 10 (ng/mL)	QC 5 Dil 20 (ng/mL)	QC 5 Dil 40 (ng/mL)	QC 6 Dil 100 (ng/mL)
N	54	18	9	3	3	
Theoretical Concentration	150	150	150	150	150	10000
Mean	147	154	142	162	162	9820
S.D.	10.0	8.40	7.92	5.83	5.83	471
%C.V.	6.81	5.44	5.58	3.60	3.60	4.80
% Difference from Theoretical	-1.78	2.87	-5.40	8.00	8.00	-1.84
	Diluted Quality Controls for C24:0					
Run ID	QC 5 Dil 5 (ng/mL)	QC 5 Dil 10 (ng/mL)	QC 5 Dil 20 (ng/mL)	QC 5 Dil 40 (ng/mL)	QC 6 Dil 100 (ng/mL)	
N	48	15	12	0	3	
Theoretical Concentration	150	150	150	150	150	10000
Mean	154	164	154	---	---	10300
S.D.	8.65	9.33	15.2	---	---	583
%C.V.	5.61	5.70	9.87	---	---	5.69
% Difference from Theoretical	2.83	9.10	2.48	---	---	2.54

Reference standards	Analyte	Source	Lot	Purity	Correction Factor	Expiration Date
	C22:0		Amicus	372-ZC-1-20D	92.9%	0.929
372-ZC-1-43				96.5%	0.913	27-DEC-2012
				88.6%	0.886	06-MAY-2011
C24:0 (C24-CTH)		Amicus	372-ZC-41-19D	86.1%	0.861	06-MAY-2010
			297-GL-1-11	91.2%	0.912	31-MAR-2012
C17-CTH (IS)		Amicus	372-ZC-1-45	97.5%	N/A <sup>a</sup>	16-NOV-2016
			372-ZC-1-5/GL-3	98.2%		26-JUN-2014

<sup>a</sup> Not applicable; correction factors are not used for internal standards.

### 6.5.3. GL-3 Analysis of Samples From Study AT1001-012

All procedures associated with this bioanalysis were conducted by (b) (4).

<b>Assay passing rate</b>	Analysis of human urine samples began on Mar 28, 2012 and was completed on Mar 14, 2013. Sample analysis was halted by the Sponsor prior to completion and analysis was not completed for all of the samples received. Out of the 334 samples received, only 276 samples had results reported. All runs passed for C24:0. However, 3 of 15 (20%) failed for C22:0.
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**ISR passing rate** Incurred Sample Reproducibility Results for C22:0  
Total % ISR Samples Pass: 76.7%

Incurred Sample Reproducibility Results for C24:0  
Total % ISR Samples Pass: 70.0%

**Plasma QC performance**

Plasma QC performance for C22:0

Run ID	QC 1* (ng/mL)	QC 2* (ng/mL)	QC 3* (ng/mL)	QC 4* (ng/mL)	QC 5* (ng/mL)	QC 53** (ng/mL)	QC 55** (ng/mL)	QC 63** (ng/mL)	QC 73** (ng/mL)
N	22	22	22	22	22	4	22	10	8
Theoretical Concentration	2.00	5.00	15.0	40.0	150	24.5	159	26.1	21.4
Mean	1.96	4.88	14.2	39.0	146	24.8	151	25.7	19.8
S.D.	0.159	0.308	0.628	1.87	6.20	0.956	6.96	1.63	1.05
%C.V.	8.11	6.31	4.41	4.78	4.23	3.86	4.62	6.32	5.32
% Difference from Theoretical	-2.03	-2.48	-5.07	-2.40	-2.34	1.07	-5.32	-1.36	-7.60

Plasma QC performance for C24:0

Run ID	QC 1* (ng/mL)	QC 2* (ng/mL)	QC 3* (ng/mL)	QC 4* (ng/mL)	QC 5* (ng/mL)	QC 53** (ng/mL)	QC 55** (ng/mL)	QC 63** (ng/mL)	QC 73** (ng/mL)
N	28	28	28	28	28	4	28	16	8
Theoretical Concentration	2.00	5.00	15.0	40.0	150	20.7	156	22.2	19.5
Mean	2.03	5.21	16.8	44.2	154	22.8	150	21.6	19.0
S.D.	0.214	0.250	6.51	11.6	5.96	0.660	20.6	1.35	0.471
%C.V.	10.5	4.79	38.9	26.1	3.87	2.89	13.8	6.26	2.48
% Difference from Theoretical	1.46	4.21	11.7	10.6	2.57	10.1	-4.07	-2.66	-2.58

**Dilution QC performance**

Diluted Quality Controls for C22:0

Run ID	QC 5 Dil 5 (ng/mL)	QC 5 Dil 10 (ng/mL)	QC 5 Dil 20 (ng/mL)
N	12	3	3
Theoretical Concentration	150	150	150
Mean	149	152	127
S.D.	7.96	8.69	12.3
%C.V.	5.34	5.73	9.74
% Difference from Theoretical	-0.677	1.09	-15.5

(continued)

Dilution QC performance (continued)	Diluted Quality Controls for C24:0			
	Run ID	QC 5 Dil 5 (ng/mL)	QC 5 Dil 10 (ng/mL)	QC 5 Dil 20 (ng/mL)
N	15	3	3	
Theoretical Concentration	150	150	150	
Mean	157	166	138	
S.D.	8.94	5.33	4.70	
%C.V.	5.70	3.21	3.40	
% Difference from Theoretical	4.47	10.9	-7.81	

Standard curve performance	Standard curve performance for C22:0								
	Run ID	CAL 1 (ng/mL)	CAL 2 (ng/mL)	CAL 3 (ng/mL)	CAL 4 (ng/mL)	CAL 5 (ng/mL)	CAL 6 (ng/mL)	CAL 7 (ng/mL)	CAL 8 (ng/mL)
N	22	20	22	22	22	22	22	22	22
Theoretical Concentration	1.00	1.60	3.00	9.00	25.0	75.0	160	200	
Mean	1.00	1.60	2.99	8.91	25.4	76.7	158	197	
S.D.	0.0746	0.0979	0.171	0.379	0.784	3.15	5.60	6.47	
%C.V.	7.45	6.11	5.74	4.26	3.09	4.11	3.54	3.28	
% Difference from Theoretical	0.114	0.0457	-0.329	-1.01	1.68	2.22	-1.18	-1.55	

Standard curve performance	Standard curve performance for C24:0								
	Run ID	CAL 1 (ng/mL)	CAL 2 (ng/mL)	CAL 3 (ng/mL)	CAL 4 (ng/mL)	CAL 5 (ng/mL)	CAL 6 (ng/mL)	CAL 7 (ng/mL)	CAL 8 (ng/mL)
N	27	27	27	27	28	28	28	28	
Theoretical Concentration	1.00	1.60	3.00	9.00	25.0	75.0	160	200	
Mean	0.998	1.59	3.02	9.17	25.8	76.2	156	193	
S.D.	0.0580	0.0854	0.171	0.367	1.10	3.14	5.30	7.36	
%C.V.	5.81	5.37	5.65	4.01	4.27	4.12	3.41	3.82	
% Difference from Theoretical	-0.153	-0.624	0.661	1.84	3.23	1.58	-2.74	-3.73	

Reference standards	Analyte	Source	Lot	Purity	Correction Factor	Expiration Date
	C22:0	Amicus	372-ZC-1-43	88.6%	0.886	06-MAY-2011
				96.5%	0.913	27-DEC-2012
	C24:0 (C24-CTH)	Amicus	297-GL-1-11	91.2%	0.912	31-MAR-2012
C17-CTH (IS)	Amicus	372-ZC-1-45	97.5%	N/A <sup>a</sup>	16-NOV-2016	

<sup>a</sup> Not applicable; correction factors are not used for internal standards.

## 6.6. Iohexol

### 6.6.1. Bioanalytical Method Validation for Iohexol

The Sponsor developed a liquid chromatography tandem mass spectrometry (LC/MS/MS) method to quantify iohexol. The method was sufficiently validated to support the quantitation of iohexol in human



Interference & specificity	Analysis Group	Blank Source	Theoretical Concentration ( $\mu\text{g/mL}$ )	
			1.00	DEV (%)
	002	19082	1.03	3.0
		19784	1.09	9.0
		19796	1.10	10.0
		21238	1.10	10.0
		21399	1.10	10.0
		21400	1.12	12.0
	n		6	
	Mean		1.09	
	SD		0.0310	
	RSD (%)		2.8	
	Accuracy (%)		109.0	

#### Matrix Effect Data for Iohexol and the Internal Standard in Human Plasma

Analysis Group	Theoretical Concentration ( $\mu\text{g/mL}$ )	Iohexol		Internal Standard	
		Post Extraction Spike	Pure QC Sample	Post Extraction Spike	Pure QC Sample
		QC Sample Peak Area	QC Sample Peak Area	QC Sample Peak Area	QC Sample Peak Area
002	1.00	5587.2	5963.8	1858892.5	1814492.3
		5747.8	5834.2	1770777.1	1832858.7
		5904.7	5895.5	1775287.4	1832388.9
		5775.6		1796723.8	
		6202.0		1823913.5	
		6080.4		1870089.7	
n		6	3	6	3
Mean		5883.0	5897.8	1815947.3	1826580.0
SD		227.4	64.8	42195.4	10470.9
RSD (%)		3.9	1.1	2.3	0.6
Matrix Effect (%)			-0.3		-0.6

#### Hemolysis effect

Run ID	QC-3-HE 3.00 $\mu\text{g/mL}$	%Bias	Mean (%Bias)	RSD (%)
3	3.28	9.3	3.23 (7.7)	3.4
	3.19	6.3		
	3.18	6.0		
	3.43	14.3		
	3.15	5.0		
	3.15	5.0		

#### Dilution linearity

Analysis Group	Theoretical Concentration 2500 $\mu\text{g/mL}$ (10X Dilution)	
		DEV (%)
001	2260	-9.6
	2420	-3.2
	2480	-0.8
	2490	-0.4
	2500	0.0
	2300	-8.0
n	6	
Mean	2410	
SD	104	
RSD (%)	4.3	
Accuracy (%)	96.4	

**Bench-top/process stability**

**Solution stability for Iohexol at room temperature for 7 hours**

	S01-26898-1	S01-26898-1
Stock Solution ID	S01-26898-1	S01-26898-1
Preparation Date	14-Feb-06	14-Feb-06
Intermediate Solution ID	SC1-26905-1	SC1-26905-2
	Hour 0	Hour 7
Peak Area	1755503.5	1791118.1
Peak Area	1772279.1	1782522.6
Peak Area	1757499.9	1783922.1
n	3	3
Mean Peak Area	1761760.8	1785854.3
% Difference	1.4	

**Internal standard solution stability for Iohexol related compound b stored at room temperature for 7 hours**

	I01-26899-1	I01-26899-1
Stock Solution ID	I01-26899-1	I01-26899-1
Preparation Date	14-Feb-06	14-Feb-06
Intermediate Solution ID	SC1-26905-1	SC1-26905-2
	Hour 0	Hour 7
Peak Area	1685496.5	1735672.1
Peak Area	1701759.2	1708337.6
Peak Area	1723323.1	1707171.1
n	3	3
Mean Peak Area	1703526.3	1717060.3
% Difference	0.8	

**Standard Solution Stability for Iohexol Stored Refrigerated (2 to 8°C) for 41 days**

	S01-26924-1	S01-26924-2	S01-26898-2
Stock Solution ID	S01-26924-1	S01-26924-2	S01-26898-2
Preparation Date	27-Mar-06	27-Mar-06	14-Feb-06
Analyte Peak Area	1711741.7	1730532.3	1732315.8
Analyte Peak Area	1735681.7	1735256.3	1732186.6
Analyte Peak Area	1751312.9	1760191.1	1739403.4
Analyte Peak Area	1783781.0	1767157.9	1775957.3
Analyte Peak Area	1788051.0	1779079.6	1779517.9
n	5	5	5
Mean Peak Area	1754113.7	1754443.4	1751876.2
Combined Mean (1 and 2)	1754278.6		
% Difference (1 vs 2)	0.0		
% Difference (combined vs 3)	0.1		

**Freeze-Thaw stability**

**Four cycles at -10 to -30°C and Five cycles at -60 to -80°C**

Analysis Group	Theoretical Concentration (µg/mL)					
	3.00	DEV (%)	400	DEV (%)	2500	DEV (%)
<b>Four Freeze-Thaw Cycles</b>						
003	3.00	0.0	388	-3.0	2370	-5.2
	3.19	6.3	398	-0.5	2380	-4.8
	3.04	1.3	390	-2.5	2350	-6.0
	3.10	3.3	392	-2.0	2440	-2.4
	3.05	1.7	391	-2.3	2420	-3.2
	2.97	-1.0	388	-3.0	2420	-3.2
n	6		6		6	
Mean	3.06		391		2400	
SD	0.0783		3.71		35.0	
RSD (%)	2.6		0.9		1.5	
Accuracy (%)	102.0		97.8		96.0	

**Long-term storage**

Long-term frozen matrix stability over 1260 days at -60 to -80°C

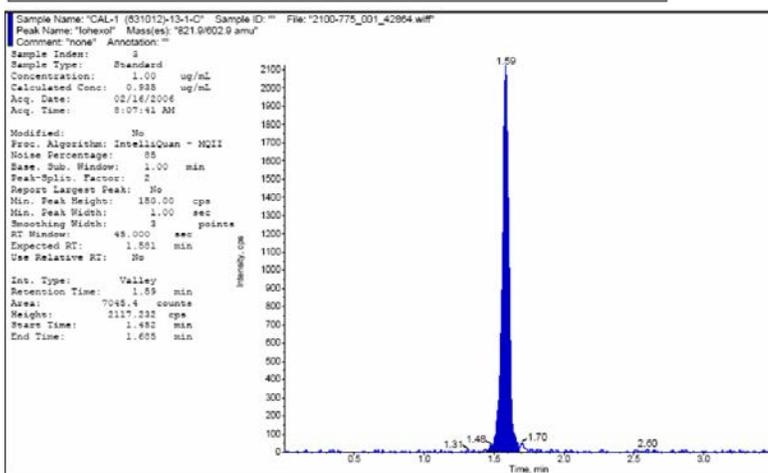
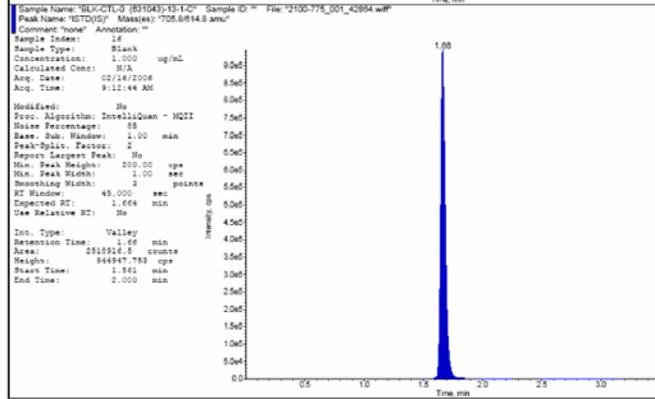
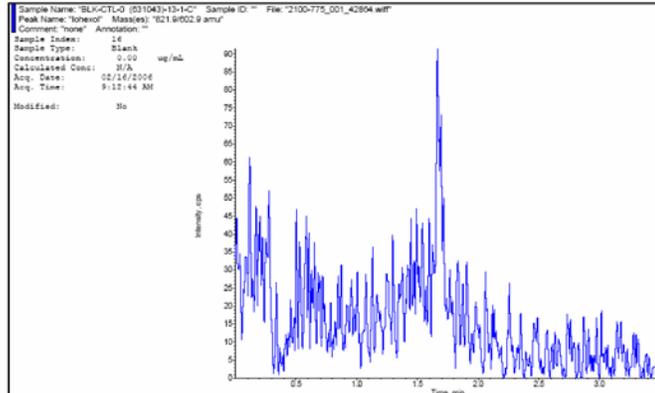
Run ID	LTS (-70) 3.00 µg/mL	%Bias	Mean (%Bias)	LTS (-70) 400 µg/mL	%Bias	Mean (%Bias)
14	3.15	5.0	3.12 (4.0)	409	2.3	402 (0.5)
	3.13	4.3		396	-1.0	
	3.10	3.3		400	0.0	
	3.16	5.3		408	2.0	
	3.15	5.0		402	0.5	
	3.03	1.0		397	-0.8	

**Frozen Matrix Stability**

**Data for Iohexol in  
Human Plasma  
Stored at -10 to -30°C for  
42 Days**

Analysis Group	Theoretical Concentration (µg/mL)								
	3.00	DEV (%)	225	DEV (%)	400	DEV (%)	2500 (10x)	DEV (%)	
008*	a	3.04	1.3	218	-3.1	382	-4.5	2460	-1.6
		2.99	-0.3	218	-3.1	391	-2.3	2440	-2.4
		3.04	1.3	218	-3.1	380	-5.0	2390	-4.4
		3.01	0.3	217	-3.6	377	-5.8	2430	-2.8
		2.92	-2.7	218	-3.1	376	-6.0	2430	-2.8
		2.95	-1.7	218	-3.1	378	-5.5	2400	-4.0
	n	6		6		6		6	
Mean	2.99		218		381		2430		
SD	0.0488		0.408		5.50		25.9		
RSD (%)	1.6		0.2		1.4		1.1		
Accuracy (%)	99.7		96.9		95.3		97.2		
	b	3.06	2.0	228	1.3	403	0.8	NA	NA
		3.06	2.0	217	-3.6	386	-3.5	NA	NA

Example Chromatogram



For studies AT1001-011 iohexol concentrations were determined with modified method stated in the table below. Modification and cross-validation result were described in the table below.

<b>Cross-validation review summary</b>	Cross-validation was adequate and no major issues were identified
<b>Changes in method description</b>	Cross validation of an internal standard from iohexol related compound B to d5-iohexol.
<b>New validated assay range if any</b>	Single replicates of calibration standards at concentrations of 1.00 to 500 µg/mL of iohexol in human plasma.

<b>Test article purity</b> <b>Internal standard purity</b>	Iohexol			
	<b>Lot No.:</b>	G0F254		
		(b) (4)		
	<b>Supplier:</b>	(b) (4)		
	<b>Receipt Date:</b>	31 December 2009		
	<b>Storage Conditions:</b>	Protected from light, desiccated, room temperature		
	<b>Stock Prep Date:</b>	<b>Correction Factor:</b>	<b>Purity:</b>	<b>Material Expiration Date:</b>
	07 January 2010	1.000	Not provided	This is the current (b) (4) lot
	d <sub>5</sub> -Iohexol			
	<b>Lot No.:</b>	1-RNP-141-2		
		(b) (4)		
	<b>Supplier:</b>	(b) (4)		
	<b>Receipt Date:</b>	06 January 2010		
<b>Storage Conditions:</b>	Protected from light, desiccated, refrigerated (2 to 8°C)			
<b>Stock Prep Date:</b>	<b>Correction Factor:</b>	<b>Purity:</b>	<b>Material Retest Date:</b>	
07 January 2010	0.980	98%	25 November 2011	
<b>Validation parameters</b>	<b>Cross-validation performance</b>			
<b>Standard curve performance during accuracy &amp; precision</b>	Cumulative accuracy (%bias) in standard calibrators from LLOQ to ULOQ	-7 to 12.5%		
	Cumulative precision (%CV) from LLOQ to ULOQ	0.3 to 3.9%		
<b>QCs performance during accuracy &amp; precision</b>	Cumulative accuracy (%bias) in 3 QCs	10.3 to 15.3%		
	3 µg/ml	-0.5 to 3.3%		
	40 µg/ml	-2.5 to 0.5%		
	400 µg/ml			
	Inter-batch %CV	2.1 to 4.8		
	3 µg/ml	1.8 to 3		
40 µg/ml	1.3 to 2.5			
400 µg/ml				
<b>Sensitivity</b>				
	<b>Analysis Group</b>	<b>Theoretical Concentration (µg/mL)</b>	<b>Calculated Concentration (µg/mL)</b>	<b>DEV (%)</b>
	<b>Multiplexing Batches</b>			
	AD*	1.00	0.985	-1.5
			0.878	-12.2
			0.798	-20.2
			0.874	-12.6
			0.917	-8.3
			0.864	-13.6
	n		6	
	Mean		0.886	
	SD		0.0620	
	RSD (%)		7.0	
	Accuracy (%)		88.6	
	AE*	1.00	0.885	-11.5
			0.884	-11.6
			0.893	-10.7
			0.820	-18.0
			0.920	-8.0
			0.896	-10.4
	n		6	
	Mean		0.883	
	SD		0.0335	
	RSD (%)		3.8	
	Accuracy (%)		88.3	
	* Multiplexing batch			

**Reinjection Reproducibility  
Data for Iohexol in Human  
Plasma Stored in an  
Injector (5°C) for 77 Hours**

Theoretical Concentration (µg/mL)	Calculated Concentration (µg/mL)	DEV (%)
<u>Calibration Standards</u>		
1.00	0.968	-3.2
2.00	2.10	5.0
5.00	5.25	5.0
20.0	18.8	-6.0
100	100	0.0
250	241	-3.6
425	420	-1.2
500	518	3.6
<u>Quality Control Samples</u>		
3.00	2.99	-0.3
	3.02	0.7
	3.13	4.3
	2.88	-4.0
	3.16	5.3
	2.95	-1.7
Mean	3.02	
Accuracy (%)	100.7	
40.0	41.2	3.0
	42.3	5.7
	41.0	2.5
	42.3	5.7
	41.3	3.2
	42.0	5.0
Mean	41.7	
Accuracy (%)	104.3	
400	420	5.0
	413	3.3
	412	3.0
	409	2.3
	412	3.0
	413	3.3
Mean	413	
Accuracy (%)	103.3	
Analysis Group:	AG	
Extraction Date:	14-Jan-10	
Analysis Date:	17-Jan-10	
Slope:	3.52E-02	
y-Intercept:	1.24E-02	
Correlation Coefficient:	0.9989	

**Dilution**

Analysis Group	Theoretical Concentration (µg/mL)	
	2500 (10X Dilution)	DEV (%)
AC	2330	-6.8
	2260	-9.6
	2300	-8.0
	2290	-8.4
	2330	-6.8
	2300	-8.0
n	6	
Mean	2300	
SD	26.4	
RSD (%)	1.1	
Accuracy (%)	92.0	

**6.6.2. Iohexol Analysis of Samples From Study AT1001-011**

All procedures associated with this bioanalysis were conducted (b) (4). Study samples were received (b) (4) from Mar 11, 2011

through Jan 15, 2014. Study samples were received in good condition and stored in a freezer set to maintain -60 to -80°C.

<b>Assay passing rate</b>	A total of 1459 study samples were analyzed in a total of 34 analytical runs (1 analytical run failed). A total of 57 study samples were reanalyzed (~4% of total number of samples analyzed).																																																											
<b>ISR passing rate</b>	124 samples were randomly chosen for ISR and 87% of samples were confirmed within 20.0%																																																											
<b>QC performance</b>	(3.00 µg/mL)	(40.0 µg/mL)	(225 µg/mL)	(400 µg/mL)	(2500 µg/mL)																																																							
	Dilution=1		Dilution=1		Dilution=1		Dilution=1		Dilution=10																																																			
Mean			3.00	40.3	224	395	2490																																																					
S.D.			0.200	2.30	11.9	19.9	126																																																					
RSD (%)			6.7	5.7	5.3	5.0	5.1																																																					
Accuracy (%)			100.0	100.8	99.6	98.8	99.6																																																					
%Bias			0.0	0.8	-0.4	-1.2	-0.4																																																					
n			66	30	36	66	14																																																					
<b>Standard curve performance</b>	1.00	2.00	5.00	20.0	100	250	425	500																																																				
	(µg/mL)	(µg/mL)	(µg/mL)	(µg/mL)	(µg/mL)	(µg/mL)	(µg/mL)	(µg/mL)																																																				
Mean			0.984	2.05	5.06	20.5	100	248	416	493																																																		
S.D.			0.0327	0.119	0.292	1.19	3.65	9.81	18.3	19.3																																																		
RSD (%)			3.3	5.8	5.8	5.8	3.7	4.0	4.4	3.9																																																		
Accuracy			98.4	102.5	101.2	102.5	100.0	99.2	97.9	98.6																																																		
%Bias			-1.6	2.5	1.2	2.5	0.0	-0.8	-2.1	-1.4																																																		
n			33	32	31	31	33	33	33	33																																																		
<b>Reference standards</b>	<b>Iohexol</b>																																																											
	<table border="1"> <tr> <td>Lot Number:</td> <td colspan="9">H0J211 (b) (4)</td> </tr> <tr> <td>Supplier:</td> <td colspan="9">(b) (4)</td> </tr> <tr> <td>Material Expiration Date:</td> <td colspan="9">Per (b) (4)</td> </tr> <tr> <td>Purity/Correction Factor:</td> <td colspan="9">100% / 1.00</td> </tr> <tr> <td>Storage Conditions:</td> <td colspan="9">Room Temperature/Desiccator/Protect from light</td> </tr> </table>										Lot Number:	H0J211 (b) (4)									Supplier:	(b) (4)									Material Expiration Date:	Per (b) (4)									Purity/Correction Factor:	100% / 1.00									Storage Conditions:	Room Temperature/Desiccator/Protect from light								
Lot Number:	H0J211 (b) (4)																																																											
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	<b>Iohexol</b>																																																											
	<table border="1"> <tr> <td>Lot Number:</td> <td colspan="9">BCBG2193V (b) (4)</td> </tr> <tr> <td>Supplier:</td> <td colspan="9">(b) (4)</td> </tr> <tr> <td>Material Expiration Date:</td> <td colspan="9">26Jan2013*</td> </tr> <tr> <td>Purity/Correction Factor:</td> <td colspan="9">99.3% / 0.993</td> </tr> <tr> <td>Storage Conditions:</td> <td colspan="9">Room Temperature/Desiccator/Protect from light</td> </tr> </table>										Lot Number:	BCBG2193V (b) (4)									Supplier:	(b) (4)									Material Expiration Date:	26Jan2013*									Purity/Correction Factor:	99.3% / 0.993									Storage Conditions:	Room Temperature/Desiccator/Protect from light								
Lot Number:	BCBG2193V (b) (4)																																																											
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Purity/Correction Factor:	99.3% / 0.993																																																											
Storage Conditions:	Room Temperature/Desiccator/Protect from light																																																											
	<b>Iohexol-d5</b>																																																											
	<table border="1"> <tr> <td>Lot Number:</td> <td colspan="9">10-THT-155-3 (b) (4)</td> </tr> <tr> <td>Supplier:</td> <td colspan="9">(b) (4)</td> </tr> <tr> <td>Material Retest Date:</td> <td colspan="9">14Jan2014</td> </tr> <tr> <td>Purity/Correction Factor:</td> <td colspan="9">95% / 0.950</td> </tr> <tr> <td>Storage Conditions:</td> <td colspan="9">Refrigerated/ Desiccator/ Protect from light</td> </tr> </table>										Lot Number:	10-THT-155-3 (b) (4)									Supplier:	(b) (4)									Material Retest Date:	14Jan2014									Purity/Correction Factor:	95% / 0.950									Storage Conditions:	Refrigerated/ Desiccator/ Protect from light								
Lot Number:	10-THT-155-3 (b) (4)																																																											
Supplier:	(b) (4)																																																											
Material Retest Date:	14Jan2014																																																											
Purity/Correction Factor:	95% / 0.950																																																											
Storage Conditions:	Refrigerated/ Desiccator/ Protect from light																																																											

### 6.6.3. Iohexol Analysis of Samples From Study AT1001-012

All procedures associated with this bioanalysis were conducted (b) (4). Study samples were received (b) (4) from Nov 11, 2011 through May 28, 2015. Samples included in the interim report finalized on Dec 9, 2014 were received from Nov 11, 2011 through May 16, 2014. Samples were received in good condition and stored in a freezer set to maintain -60 to -80°C. All samples were analyzed within the known stability period of 1260 days when stored at -60 to -80°C.

<b>Assay passing rate</b>	A total of 1304 samples were analyzed in a total of 31 analytical runs (all analytical runs met acceptance criteria), with 1113 samples analyzed in 24 analytical runs included in the interim report provided Dec 9, 2014.									
<b>ISR passing rate</b>	There were 116 samples reanalyzed to test the reproducibility of the method. It was observed that 98% of the repeat results and original results were within 20.0% of each other; this is within the acceptance criteria.									
<b>QC performance</b>	Run Date	Curve Number	(3.00 µg/mL) Dilution=1	(40.0 µg/mL) Dilution=1	(400 µg/mL) Dilution=1	(2500 µg/mL) Dilution=50	(2500 µg/mL) Dilution=20	(2500 µg/mL) Dilution=10		
	Mean		3.04	39.9	394	2630	2690	2510		
	S.D.		0.157	1.73	14.7	62.4	59.0	47.1		
	RSD (%)		5.2	4.3	3.7	2.4	2.2	1.9		
	Accuracy (%)		101.3	99.8	98.5	105.2	107.6	100.4		
	%Bias		1.3	-0.3	-1.5	5.2	7.6	0.4		
	n		62	62	62	6	6	6		
<b>Standard curve performance</b>	Assay Date	Analytical Run Number	1.00 (µg/mL)	2.00 (µg/mL)	5.00 (µg/mL)	20.0 (µg/mL)	100 (µg/mL)	250 (µg/mL)	425 (µg/mL)	500 (µg/mL)
	Mean		0.990	2.02	5.07	20.3	100	250	419	489
	S.D.		0.0247	0.0848	0.247	0.860	3.98	8.02	11.3	15.1
	RSD (%)		2.5	4.2	4.9	4.2	4.0	3.2	2.7	3.1
	%Bias		-1.0	1.0	1.4	1.5	0.0	0.0	-1.4	-2.2
	n		31	31	31	31	31	31	31	31

**Reference standards****Iohexol (also known as Histodenz)**

Lot Number:	BCBG2193V (b) (4)
Supplier:	(b) (4)
Retest Date:	(b) (4)
Purity/Correction Factor:	99.3% / 0.993
Storage Conditions:	Protected from light, desiccated, room temperature

\* Assigned by (b) (4)

**Iohexol**

Lot Number:	H0J211 (b) (4)
Supplier:	(b) (4)
Expiration Date:	Current (b) (4) lot
Purity/Correction Factor:	100% / 1.000*
Storage Conditions:	Protected from light, desiccated, room temperature

\* Assigned by Covance

**Iohexol-d5**

Lot Number:	10-THT-155-3 (b) (4)
Supplier:	(b) (4)
Retest Date:	(b) (4)
Purity/Correction Factor:	95.0% / 0.950
Storage Conditions:	Protected from light, desiccated, refrigerated (2 to 8°C)

\* Most recent retest date assigned by (b) (4) A blank was fortified with internal standard, extracted, and analyzed to demonstrate that it was still valid for the intended use.

**Iohexol-d5**

Lot Number:	10-MIC-43-1 (b) (4)
Supplier:	(b) (4)
Retest Date:	(b) (4)
Purity/Correction Factor:	95.0% / 0.950
Storage Conditions:	Protected from light, desiccated, refrigerated (2 to 8°C)

**Migalastat (other names: GR181413A; AT1001)**

Lot Number:	R323358 (b) (4)
Supplier:	Amicus Therapeutics
Retest Date:	(b) (4)
Purity/Correction Factor:	99.9% / 0.817
Storage Conditions:	Protected from light, desiccated, room temperature

\* Assigned by (b) (4)

## 7. Appendix 7: Pharmacometric Review

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By: Justin Earp and Lian Ma

### 7.1. Applicant's Population PK Analysis:

The Applicant's final model for their exposure-response analyses was generated in three stages. In the first, the (b) (4) model was developed based on a dataset finalized prior to database lock. The second model ( (b) (4) model) was defined after the previous model was reevaluated by (b) (4) (b) (4) using the data generated after database lock, with the following revisions:

The NONMEM dataset was updated by (b) (4) to ensure inclusion of the final dates and times of dosing and sampling collection, as well as demographics and other covariates. The (b) (4) NONMEM model for the pre-database lock Stage 2 analysis was rerun with the original Stage 2 dataset from (b) (4) and with the new (b) (4) dataset. An abbreviated forward addition and backward elimination covariate selection process was conducted to optimize the model and minimize bias, which resulted in the following minor changes to the model: (i) updating the effect of estimated glomerular filtration rate (eGFR) on the apparent oral clearance (CLT/F) of migalastat to be described using a power function between 0 and 120 mL/min/1.73 m<sup>2</sup> and using a CLT/F for those with eGFR > 120 mL/min/1.73 m<sup>2</sup> (instead of using a single power function between 0 and 200 mL/min/1.73 m<sup>2</sup>); (ii) the effect of Fabry disease on CLT/F; and (iii) eliminating the inter-individual variability on lag time. Bootstrapping and visual predictive checks were conducted using the final (b) (4) model and showed good model performance. The following pharmacokinetic parameters were predicted for subjects in Study AT1001-011 based on the final (b) (4) model: the area under the curve during a dosing interval at steady-state (AUC<sub>0-48</sub>), the maximum plasma concentrations (C<sub>max</sub>), time to C<sub>max</sub> (t<sub>max</sub>), half-life (t<sub>1/2</sub>) and trough concentrations (C<sub>48</sub>) in Fabry patients receiving migalastat 150 mg once every other day at steady-state. The AUC<sub>0-48</sub> and C<sub>max</sub> were within an average of 5% of those estimated using the (b) (4) model.

The Applicant's conclusions based on the (b) (4) modeling are:

- A two-compartment population pharmacokinetic model with linear time-dependent absorption sufficiently characterizes the pharmacokinetics of migalastat in plasma after oral administration.
- Renal function is the most important determinant of variability in the exposure of migalastat, with an average 3-fold range occurring for eGFR values between 30 and 120 mL/min/1.73 m<sup>2</sup>. Subject weight is the second largest determinant of variability in exposure of migalastat, with an average < 2-fold difference for body weights between 50 and 170 kg. These average differences are not clinically relevant.
- The predicted exposures in Fabry disease remain similar to those reported for healthy volunteers.

The third stage updated the (b) (4) model to include allometric exponents to expand the ability to predict PK in the pediatric population. The results for this model are described further down.

(b) (4) **Population PK Model:**

The studies, doses therein, and time course of PK samples included in the analysis are outlined in Table 1. The summary of patient demographic and disease characteristics are indicated in Table 2.

**Table 1. Studies Included in Population PK Analysis**

Study	Phase	Study Description	Dose and Administration <sup>a</sup>	Pharmacokinetic Population <sup>b</sup>	Migalastat Pharmacokinetic Sampling
FAB-CL-101	I	Single dose escalation	Single dose of 25, 75, 225, or 675 mg (solution)	24 HVT <sup>d</sup> (24 M <sup>e</sup> )	Blood samples were collected at Hour 0 (pre-dose), and 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 16 and 24 hours post-dose.
FAB-CL-102	I	Multiple dose study	BID <sup>c</sup> doses of 50 mg and 150 mg of (solution) for 7 days	12 HVT (12 M)	Blood samples were collected before the morning doses on Days 1, 5, 6 and 7, and at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 9, 10 and 12 hours after the morning doses on Day 1 and Day 7, respectively.
FAB-CL-103	I	Randomized, single dose cross-over fasting bioavailability study	Single dose of either 100 mg (four 25 mg capsules) or 100 mg (solution) on fasting	15 HVT (15 M)	Blood samples were collected before dosing (0 hour) and at 0.5, 1, 1.5, 2, 2.5, 3, .3.5, 4, 5, 6, 8, 10, 12, 16 and 24 post-dose.
FAB-CL-104	I	Double-blinded, placebo-controlled single dose safety and tolerability study	Single dose of 500 mg (solution)	6 HVT (4 M, 2 F <sup>e</sup> )	Blood samples were collected before dosing (0 hour) and at 0.5, 1, 1.5, 2, 2.5, 3, .3.5, 4, 5, 6, 8, 10, 12, 16 and 24 post-dose.
AT1001-010	I	Four-way crossover Thorough QT study	Single doses of 150 mg (solution)	51 HVT (26 M, 25 F)	Blood samples were collected on Study Day +1 at pre-dose (0 hour), 0.5, 1, 2, 2.5, 3, 3.5, 4, 6, 8, 10, 12 and 22.5 hours post-dose.
AT1001-014 (MGM116435)	I	ADME study	Single dose of 150 mg [ <sup>14</sup> C] AT1001 (solution) containing 1 μCi AT1001	6 HVT (6 M)	Blood samples were collected at pre-dose (0 hour) and 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 12, 16, 24, 36, 48, 72, 96, 120, 144, 168, 192, 216 and 240 hours post-dose.
AT1001-015 (MGM116431)	I	Renal Impairment study	Single dose of 150 mg (150 mg capsule)	32 <sup>f</sup> (8 normal, 8 mild, 8 moderate, 8 severe)	Blood samples were collected at pre-dose (0 hour) and 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 15, 24, 48, 72, 96 and 120 hours post-dose.
AT1001-016 (MGM116050)	I	Cross-over study on food effects	Single dose of 150 mg (150 mg capsule) on fasting	20 HVT (12 M, 8F)	Blood samples were collected at pre-dose (0 hour), and 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 16 and 24 hours post-dosing.
MGM115806	I	Cross-over, dose-escalation study in healthy Japanese population	Single dose of 50 mg (10 mg/mL solution), 150 mg (150 mg capsule), and 450 mg (150 mg capsule) on separate days at least 7 days apart	14 Japanese HVT (14 M)	Blood samples were collected at pre-dose (0 hour) and 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10, 12, 16 and 24 hours post-dose.
Study	Phase	Study Description	Dose and Administration <sup>a</sup>	Pharmacokinetic Population <sup>b</sup>	Migalastat Pharmacokinetic Sampling
FAB-CL-201	II	Dose-escalation study	BID dosing (25 mg capsules) at dosages of 25, 100, then 250 mg for 2 weeks, respectively, separated by 24-hour washout; A daily dose of 50 mg during extensions (Day 43 to Week 96).	9 Patients <sup>d</sup> (9 M)	Blood samples were collected on Days 1, 14, 15, 28, 29, and 42 before the morning dose and at 0.5, 1, 2, 3, 4, 5, 6, 8, and 10 hours post-dose. Blood samples were also collected 14 to 12 hours before the morning dose on Days 14, 28, and 42. During the treatment extension, blood samples were collected at Week 24.
FAB-CL-204	II	Parallel-group, dose comparison	QOD <sup>c</sup> doses of 50, 150, or 250 mg (25 mg capsule)	9 Patients (9 F)	Blood samples were collected on Days 1, 14, and 84 at pre-dose and 0.5, 1, 2, 3, 4, 5, 6, 8, and 10 hours post-dose. Blood samples were also collected at 12 hours before the doses on Days 14 and 84.
FAB-CL-205	II	Dose escalation and extension	One patient (2-0202) was switched to 150 mg (150 mg capsule) Q4D <sup>c</sup> after Visit 18.	1 Patient (1 M)	Roughly 10 weeks and 26 weeks after the start of the 150 mg Q4D regimen, blood samples were collected at pre-dose, and 3, 48, 72 and 96 hours post-dose.
AT1001-011 (MGM116016)	III	Randomized, double-blind, placebo-controlled	Stage 1 (6 months): 150 mg (150 mg capsule) QOD Stage 2 (6 months): Open-label 150 mg (150 mg capsule) QOD	62 Patients (24 M, 38 F)	Blood samples were collected during visits in Month 1 (Stage 1) and Month 7 (Stage 2) at pre-dose and 2, 3, and 4 hours post-dose. A fourth post-dose blood sample was taken between 6 and 8 hours post-dose.

- a. Only the dosing and regimens within the scope of population pharmacokinetic analysis (oral dosing of AT1001, dosage ≤ 675 mg, fasting condition) are described here.  
b. Number of subjects described here are only those who received dosing and regimens that were within the scope of population pharmacokinetic analysis.  
c. BID: twice daily; QOD = once every other day; Q4D = once every four days  
d. HVT = healthy volunteer; Patient = Fabry disease patient who is either ERT-naïve or at least 1 month (Phase II) or 6 months (Phase III) since last ERT dose  
e. M = Male; F = Female  
f. Renal impairment status: Normal if creatinine clearance (CRCL, calculated by Cockcroft-Gault formula) at screening ≥ 90 mL/min; Mild if 60 mL/min ≤ CRCL < 90 mL/min; Moderate if 30 mL/min ≤ CRCL < 60 mL/min; and Severe if 15 mL/min ≤ CRCL < 30 mL/min.

(Source: Applicant's Final Population PK Report MGM116016, Table 1)

**Table 2. Summary of Subject Characteristics (Demography, Disease-Related Covariates and Baseline Lab Tests).**

Study	GSK Dataset		(b) (4) Dataset	
	AT1001-011	Total	AT1001-011	Total
N	62	260	62	260
Age in Years, Median (Range)	45 (16, 68)	36 (16, 74)	45 (16, 68)	36 (16, 74)
Sex, n (%)				
Female	38 (61.3)	91 (35.0)	38 (61.3)	91 (35.0)
Male	24 (38.7)	169 (65.0)	24 (38.7)	169 (65.0)
Race, n (%)				
White	58 (93.5)	204 (78.5)	58 (93.5)	204 (78.5)
Black	-	25 (9.62)	-	25 (9.6)
Asian	-	24 (9.23)	-	24 (9.2)
Other	4 (6.45)	7 (2.69)	4 (6.45)	7 (2.7)
Ethnicity, n (%)				
Hispanic	5 (8.06)	44 (16.9)	5 (8.06)	44 (16.9)
Non-Hispanic	32 (51.6)	191 (73.5)	32 (51.6)	191 (73.5)
Unknown	25 (40.3)	25 (9.62)	25 (40.3)	25 (9.6)
Weight in kg, Median (Range)	70.0 (38.0, 108)	74.0 (38.0, 141)	70.0 (38.0, 108.4)	74 (38, 141)
BSA <sup>a</sup> in m <sup>2</sup> , Median (Range)	1.82 (1.26, 2.38)	1.89 (1.26, 2.63)	1.82 (1.26, 2.38)	1.89 (1.26, 2.63)
BMI <sup>a</sup> in kg/m <sup>2</sup> , Median (Range)	25.2 (16.3, 37.9)	25 (16.3, 51.8)	25.2 (16.3, 37.9)	25 (16.3, 51.8)
Healthy Volunteer	-	179 (68.8)	-	179 (68.8)
Fabry Patient	62 (100)	81 (31.2)	62 (100)	81 (31.2)
ALB <sup>b</sup> in g/L, Median (Range)	44.0 (35.0, 50.0)	43.0 (35.0, 52.0)	44.0 (35.0, 50.0)	43 (35, 52)
Missing in ALB, n	0	38	0	36
ALK <sup>b</sup> in IU/L, Median (Range)	66.5 (39.0, 230)	65.0 (18.0, 230)	66.5 (39.0, 230)	65.0 (18, 230)
Missing in ALK, n	0	2	0	2
ALT <sup>b</sup> in IU/L, Median (Range)	17.0 (7.00, 53.0)	19.0 (7.00, 169)	17.0 (7.00, 53.0)	19.0 (7, 169)
Missing in ALT, n	0	2	0	2
AST <sup>b</sup> in IU/L, Median (Range)	20.5 (10.0, 37.0)	21.0 (9.0, 76.0)	20.5 (10.0, 37.0)	21.0 (9, 76)
Missing in AST, n	0	2	0	2
TBIL <sup>b</sup> in μmol/L, Median (Range)	7.00 (2.00, 17.0)	10.3 (1.71, 35.9)	7.00 (2.00, 17.0)	10.3 (1.71, 35.9)
Missing in TBIL, n	0	2	0	2
SCr <sup>b</sup> in μmol/L, Median (Range)	72.9 (42.0, 178)	79.0 (42.0, 575)	72.9 (42.0, 178)	79 (42, 575)
CRCL <sup>b</sup> in mL/min, Median (Range)	88.6 (34.0, 182)	100 (9.50, 182)	88.6 (34.0, 182)	100 (9.5, 182)
eGFR <sup>b</sup> in mL/min/1.73 m <sup>2</sup> , Median (Range)	83.9 (39.9, 236)	89.8 (7.10, 236)	83.9 (39.9, 236)	89.8 (7.1, 236)

- a. BSA = body surface area (m<sup>2</sup>); BMI = body mass index (kg/m<sup>2</sup>);
- b. ALB = albumin (g/L); ALK = alkaline phosphatase (IU/L); ALT = alanine aminotransferase (IU/L); AST = aspartate aminotransferase (IU/L); TBIL = total bilirubin (μmol/L); SCr = serum creatinine (μmol/L); CRCL = creatinine clearance (mL/min); eGFR = estimated glomerular filtration rate (mL/min/1.73 m<sup>2</sup>)
- c. Albumin was not measured in study FAB-CL-101 and study FAB-CL-102

(Source: Applicant's Final Population PK Report MGM116016, Table 2)

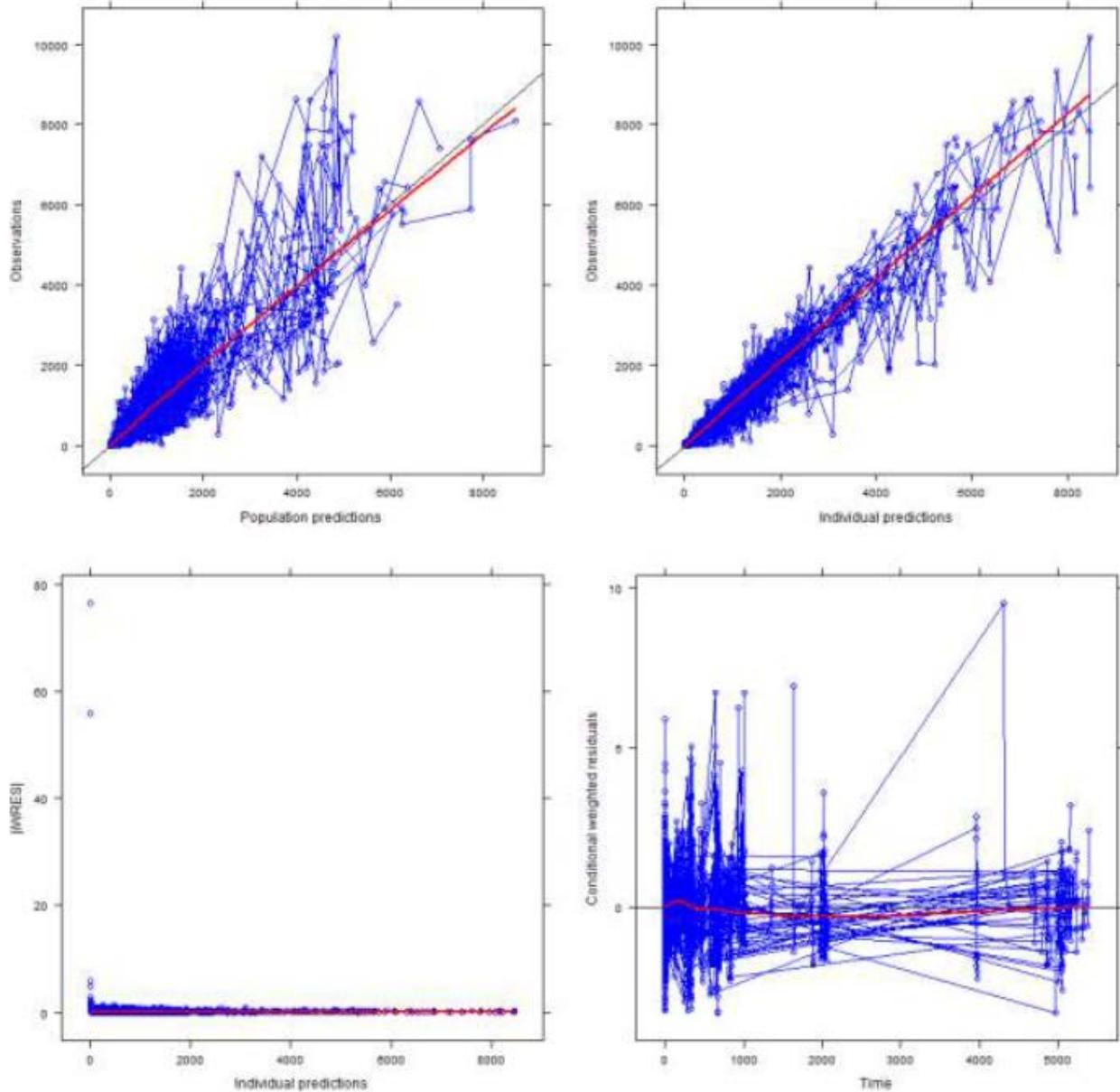
The Applicant's final model parameters are shown in Table 3 and Goodness-of-Fit plots in Figure 1.

**Table 3. Summary of Parameter Estimates from the Final Population PK Model**

Parameter	NONMEM	Bootstrap	
	Estimate (%RSE)	Estimate (%RSE)	95% Confidence Interval
CL <sub>T</sub> /F (L/h)	17.1 (0.153%)	17.0 (2.70%)	11.77-22.36
V <sub>2</sub> /F (L)	63.6 (0.053%)	63.5 (2.87%)	58.00-69.24
Q/F (L/h)	0.95 (0.052%)	0.949 (0.046%)	0.855 to 1.035
V <sub>3</sub> /F (L)	25.7 (0.108%)	25.7 (2.87%)	20.09 to 31.33
K <sub>a</sub> (intercept) (h <sup>-1</sup> )	0.26 (0.091%)	0.256 (0.024%)	0.210 to 0.302
K <sub>a</sub> (slope)	0.27 (0.089%)	0.270 (0.022%)	0.229 to 0.314
Lag time (h)	0.18 (0.047%)	0.177 (0.008%)	0.161 to 0.192
EGFR-related CL <sub>T</sub> /F	0.95 (0.054%)	0.947 (0.050%)	0.849 to 1.043
WT-related CL <sub>T</sub> /F	0.44 (0.216%)	0.448 (0.098%)	0.250 to 0.633
WT-related V <sub>2</sub> /F	0.65 (0.173%)	0.655 (0.112%)	0.432 to 0.870
Fractional Change in V <sub>2</sub> /F related to Fabry disease	-0.28 (0.146%)	-0.274 (0.037%)	-0.347 to -0.204
CL <sub>T</sub> /F for those with EGFR>120 mL/min/1.73 m <sup>2</sup>	18.6 (0.164%)	18.5 (3.19%)	12.31 to 24.81
Fractional Change in CL <sub>T</sub> /F related to Fabry disease	-0.16 (0.243%)	-0.161 (0.036%)	-0.231 to -0.09
IIV on CL <sub>T</sub> /F	54.8%	30.1%	26.9% to 33.6%
IIV on V <sub>2</sub> /F	57.5%	29.2%	25.4% to 33.0%
IIV on K <sub>a</sub> (slope)	92.2%	33.2%	29.1% to 37.1%
IIV on K <sub>a</sub> (intercept)	77.5%	59.9%	45.2% to 72.4%
<b>Residual (unexplained) Variability</b>			
Proportional Component (%)	51.0%	25.6	23.9% to 27.2%
Additive Component (ng/mL)	2.85	8.03	7.53 to 8.54

(Source: Applicant's Final Population PK Report MGM116016, Table 3)

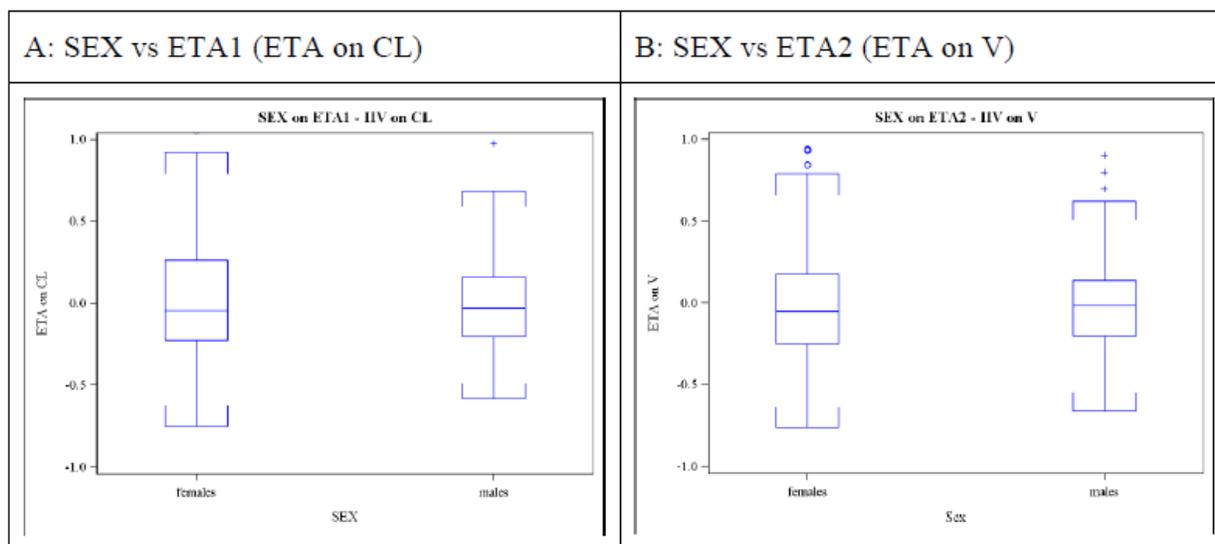
Figure 1. Goodness of Fit Plots for the Final Population PK Model.



(Source: Applicant's Final Population PK Report MGM116016, Figure 1)

Gender did not appear to be a covariate on the clearance or volume of migalastat (Figure 2).

**Figure 2. Plots of Gender vs ETA<sub>CL</sub> (Panel A) and ETA<sub>V</sub> (Panel B)**



Abbreviations: CL=clearance; ETA=vector containing individual subject estimates of the random effect parameters; V=volume of distribution

(Source: Applicant's Summary of Clinical Pharmacology, Figure 4)

#### Final Allometric Population PK Model:

While the (b) (4) model was the foundation for the population PK analysis and followed a more traditional model building approach, the Applicant concluded that it would not be sufficient for predicting pediatric PK.

While the initial population PK model was appropriate for adults, it did not have an allometric component with standard exponents (e.g., 0.75 for CLT/F), which made predictions in pediatrics less feasible. Thus, the adult population PK model required some small adjustments to allow extrapolation of migalastat PK to the pediatric age subjects. In addition, absorption models were re-evaluated and the need for allometric exponents was determined evaluating total CLT/F and non-renal clearance only. The Final Allometric Model was developed as previously described (Report Number AMI-2149-001). Briefly, the Final Allometric Model (run 28.5) was a two-compartment model with linear time-dependent absorption rate and allometric exponents added to the clearance parameters (exponent = 0.75 for CLT/F and Q/F) and volume parameters (exponent = 1 for V2/F and V3/F) for subjects < 70 kg. This model described the plasma concentration-time data well based on goodness of fit, prediction-corrected visual predictive check (pcVPCs) and bootstrap approaches. The PK parameters were presented with respect to the free base migalastat. Model parameters can be found in Appendix 2, Table 1. Model structure/component comparison can be seen in Table 1.

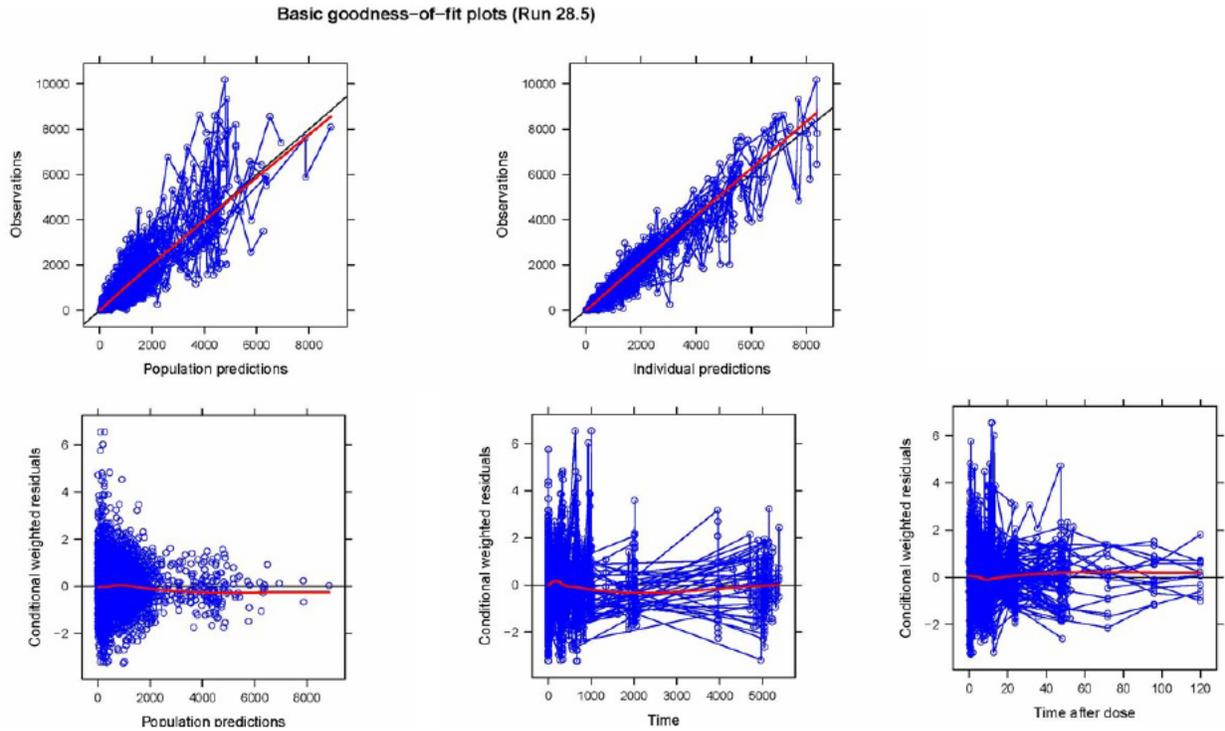
**Table 4. Parameter Estimates for the Final Allometric Model.**

Parameter	NONMEM		Bootstrap	
	Estimate (%RSE); [95% CI]	IIV (%CV)	Estimate (%RSE); [95% CI]	IIV (%CV)
Typical eGFR-related estimate for those with Fabry disease, with eGFR= 90 mL/min/1.73 m <sup>2</sup> , and with body weight >= 70 kg	18.6 (16.3%); [12.6, 24.6]	28.8%	18.5 (16.0%); [14.0, 25.4]	28.5%
Typical eGFR-related estimate for those with Fabry disease, with eGFR>120 mL/min/1.73 m <sup>2</sup> , and with body weight >= 70 kg	20.9 (17.4%); [13.8, 28.0]		20.6 (17.3%); [15.4, 28.8]	
eGFR-related exponential index on CL/F	0.922 (5.64%); [0.820, 1.02]		0.925 (5.25%); [0.832, 1.02]	
Typical total CL/F (L/h) for those with Fabry disease, with eGFR= 90 mL/min/1.73 m <sup>2</sup> , and with body weight >= 70 kg <sup>a</sup>	14.8		14.9	
Typical total CL/F (L/h) for those with Fabry disease, with eGFR>120 mL/min/1.73 m <sup>2</sup> , and with body weight >= 70 kg <sup>b</sup>	16.5		16.4	
Typical V <sub>2</sub> /F (L) for those with Fabry disease, with body weight >=70 kg	70.1 (5.29%); [62.8, 77.4]	34.5%	69.7 (4.8%); [63.8, 76.8]	33.9%
Typical Q/F (L/h) for those with body weight >=70 kg	1.00 (5.17%); [0.899, 1.10]	-	1.01 (4.59%); [0.928, 1.11]	-
Typical V <sub>3</sub> /F (L) for those with body weight >=70 kg	27.5 (11.7%); [21.2, 33.8]	-	27.5 (11.9%); [22.7, 35.2]	-
K <sub>a</sub> (intercept) (h <sup>-1</sup> )	0.256 (9.41%); [0.209, 0.303]	60.4%	0.256 (8.60%); [0.211, 0.298]	59.9%
K <sub>a</sub> (slope)	0.284 (9.12%); [0.233, 0.335]	60.7%	0.282 (7.45%); [0.244, 0.326]	60.6%
Lag time (h)	0.175 (4.65%); [0.159, 0.191]	-	0.176 (4.52%); [0.160, 0.190]	-
WT-related exponential index on CL/F and Q/F for those with body weight <70 kg	Fixed to 0.75	-	Fixed to 0.75	-
WT-related exponential index on V <sub>2</sub> /F and V <sub>3</sub> /F for those with body weight <70 kg	Fixed to 1	-	Fixed to 1	-
Fractional Change in V <sub>2</sub> /F in subjects without Fabry disease (decrease in V <sub>2</sub> /F)	-0.306 (12.8%); [-0.383, -0.229]	-	-0.305 (12.3%); [-0.372, -0.227]	-
Fractional Change in total CL/F in subjects without Fabry disease (decrease in total CL/F)	-0.150 (24.9%); [-0.223, -0.077]	-	-0.151 (23.5%); [-0.223, -0.081]	-
Residual Error (%)	26.2%; [23.2%, 29.0%]	-	26.3%; [24.5%, 27.8%]	-
Residual Error (ng/mL)	2.55; [NA, 3.76]	-	2.47; [1.25, 3.51]	-

- a. Derived total CL/F parameter from typical eGFR-related estimate and eGFR-related exponential index; total CL/F= THETA(1)<sup>THETA(9)</sup>, where THETA(1) is the typical eGFR-related estimate and THETA(9) is the estimate of exponential index for patients with Fabry disease, eGFR= 90 mL/min/1.73 m<sup>2</sup>, and with body weight >= 70 kg.
- b. Derived total CL/F parameter from typical eGFR-related estimate and eGFR-related exponential index; total CL/F= THETA(13)<sup>THETA(9)</sup>, where THETA(13) is the typical eGFR-related estimate and THETA(9) is the estimate of exponential index for patients with Fabry disease, eGFR > 120 mL/min/1.73 m<sup>2</sup>, and with body weight >= 70 kg

(Source: Applicant’s Population PK/PD Report MGM116016-2158-rpt001-pk-pd, Appendix 2, Table 1)

**Figure 3. Goodness of fit plots for the Final Allometric Model.**



(Source: Applicant’s Pediatric Population PK Report ami-2149-001, Figure 1)

**Table 5. Model Comparison**

Model Characteristic	(b) (4) Model	<i>Final Allometric Model</i>
Structure	2-compartment with linear, time-dependent absorption (slope + intercept related to time)	Same as (b) (4) Model except for: upper limit of absorption was fixed to 24 hr)
Random Effects	Inter-individual variability on $CL_T/F$ , $V_2/F$ , $k_a$ (slope) and $k_a$ (intercept)	No Change
Covariate Effects	eGFR (on $CL_T/F$ ) Body weight (on $CL_T/F$ and $V_2/F$ ) Fabry disease (on $CL_T/F$ and $V_2/F$ )	No Change
Allometric Scaling	None	Exponent = 0.75 for $CL_T/F$ and $Q/F$ Exponent = 1 for $V_2/F$ and $V_3/F$ for subjects $\leq 70$ kg

(Source: Applicant’s Population PK/PD Report MGM116016-2158-rpt001-pk-pd, Table 1)

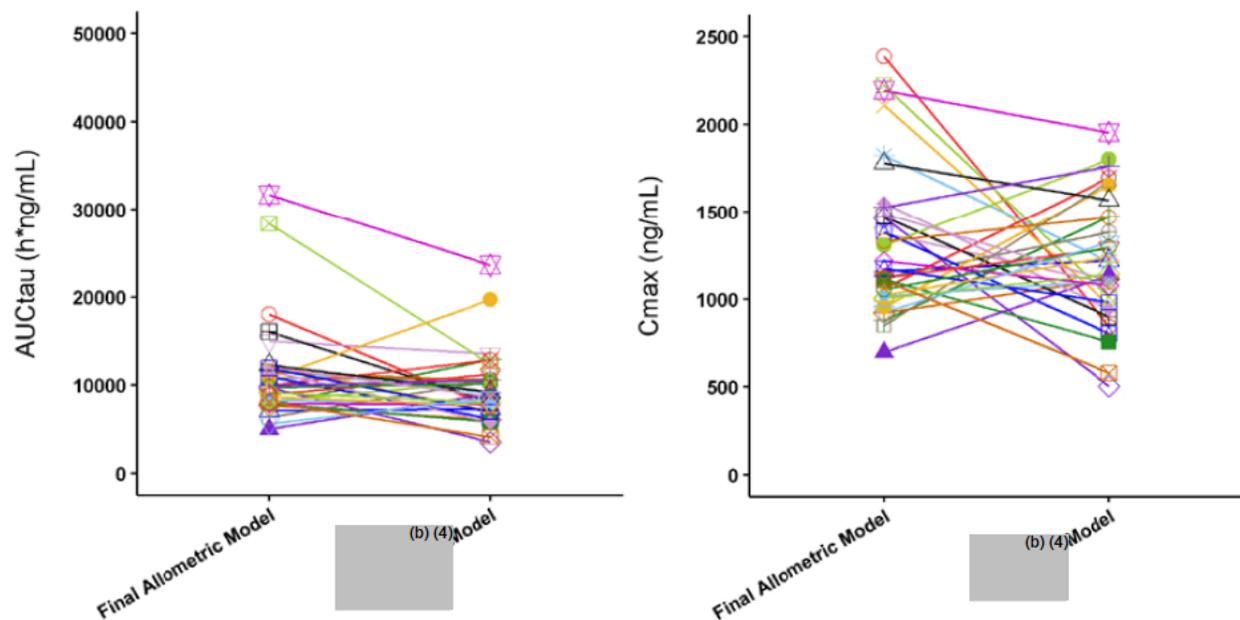
**Justification of the Full Allometric Model for Exposure-Response Assessment:**

Both approaches ((b) (4) and Full Allometric Models) are presented here to illustrate that despite the forced changes to the (b) (4) model (fixing allometric exponents) followed by re-estimation, this did not greatly change the overall parameters and predictions. A combined evaluation of the following figures illustrates that within the range of between subject variation the change between the model

predictions for the exposure metric is not unreasonable. After the first simulation with a single simulation per subject ( $n_{\text{subjects}}=33$ ) the Applicant concluded:

“It was found that the PK parameter values ((AUCtau, Cmax [shown below] and Ctrough [not shown]) changed significantly between the (b) (4) and Final Allometric Models for some patients but not for others (Figure 4).”

**Figure 4. Simulated Individual AUCtau (Left) and Cmax (Right) Values for the Final Allometric (New) Model vs. the (b) (4) (Previous) Model with 1 Simulation per Subject (N=33)**

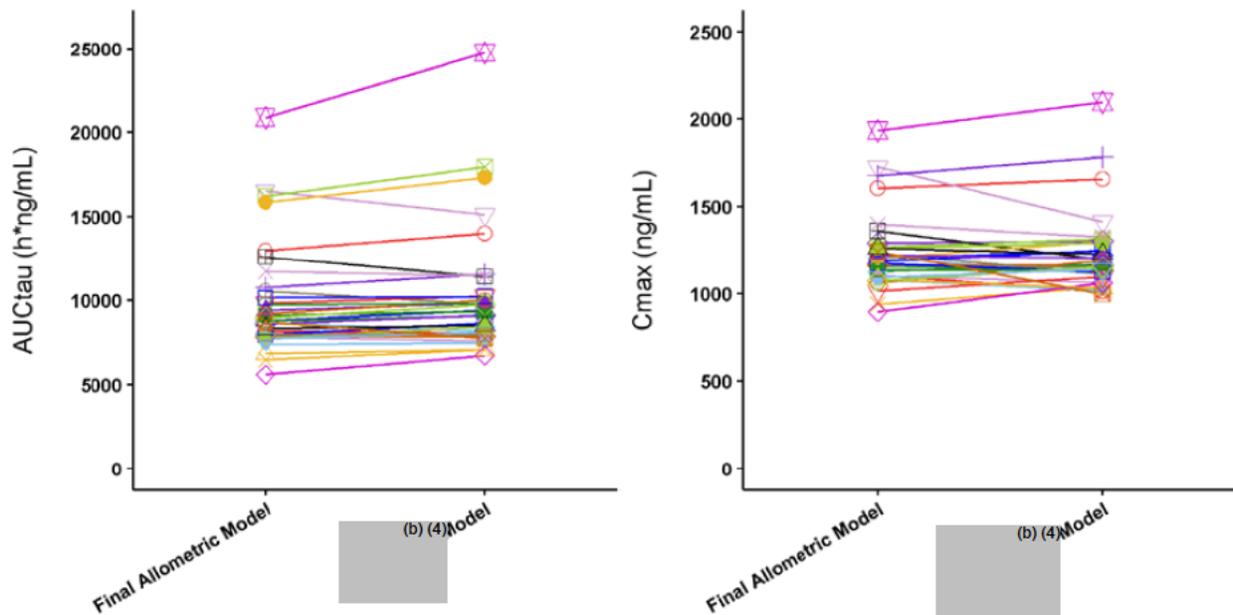


(Source: Applicant’s Population PK/PD Report MGM116016-2158-rpt001-pk-pd, Figure 1)

Based on Figure 4 the Applicant concluded:

A closer look at the results suggested that since the inter-individual and residual variabilities were moderate, the differences observed were likely due to random variability. Therefore, it was decided that 100 simulations should be conducted per subject in Study AT1001-011 (using the SUBPROBLEM=100 function in NONMEM); then noncompartmental analysis would be conducted on the median concentration-time profile for each subject. The concordance in PK parameters between the Final Allometric Model and (b) (4) Model improved significantly (Figure 5) and was maintained.

Figure 5. Simulated Individual AUC<sub>tau</sub> (Left) and C<sub>max</sub> (Right) Values for the Final Allometric (New) Model vs. the (b) (4) (Previous) Model with 100 Simulations per Subject (N=33).



(Source: Applicant's Population PK/PD Report MGM116016-2158-rpt001-pk-pd, Figure 2)

**Reviewer's Comments:**

*Given the minimal change between models when considering the parameter estimates, the degree change in AUC and Cmax values relative to the degree of between subject variation indicated in Figure 5, and the small estimates of shrinkage for ETA<sub>CL</sub>, the choice of final allometric model for the exposure response analysis is acceptable.*

**7.2. Applicant's Exposure-Response for Efficacy:**

The Applicant performed an exposure-response assessment on a number of primary and secondary efficacy endpoints from the phase 3 study AT1001-011.

Sparse blood sampling for plasma migalastat was performed on 2 occasions in the Phase 3 Study AT1001-011. These data were analyzed by population PK (popPK) analysis to predict exposures in the population of subjects with Fabry disease and to provide exposure relationships to renal function and body weight via covariate analysis.

Individual values for C<sub>max</sub>, AUC<sub>tau</sub>, and C<sub>trough</sub> for subjects in Study AT1001-011 were simulated using the Final Allometric Model. Subjects with an amenable mutation status were plotted against individual values of the following change from baseline (CFB) pharmacodynamic markers:

- GL-3 inclusions in ICs, podocytes, endothelial cells, and mesangial cells (referred to as KIC)
- Plasma lyso-GL-3 (also referred to as Lyso)
- Estimated glomerular filtration rate (eGFR, annualized change)
- Improvement in diarrhea subscale of the GSRS

- Left ventricular mass index (LVMI)
- Diarrhea score based on Question 11 (Have you been bothered by diarrhea during the past week?) from the GSRS
- Loose stools score based on Question 11 (Have you been bothered by loose stools during the past week?) from the GSRS
- Urgency score based on Question 14 (Have you been bothered by an urgent need to have a bowel movement during the past week?) from the GSRS
- Average score based on questions 11, 12, and 14 on the GSRS

All GSRS scores were collected using the following scale: 1 = No discomfort at all, 2 = Minor discomfort, 3 = Mild discomfort, 4 = Moderate discomfort, 5 = Moderately severe discomfort, 6 = Severe discomfort, 7 = Very severe discomfort

Data were further parsed by individual sex of the patient.

A subsequent analysis was conducted to evaluate the possible confounding effect of individual patient sensitivity to migalastat (as measured by individual EC50 values) on the PK/PD relationship. PK parameters were normalized to individual EC50 values as follows:

$$(\text{EC50-NormalizedPK})_i = (\text{PKParameter})_i * (\text{arithmetic meanEC50})/\text{EC50}_i$$

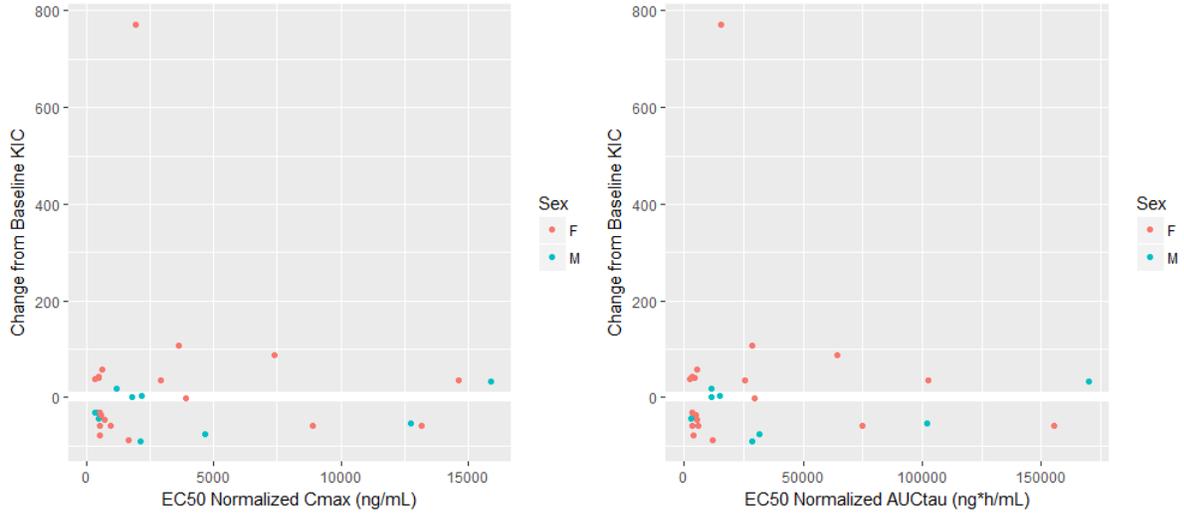
Where (PK Parameter)<sub>i</sub> is the PK parameter (C<sub>max</sub>, AUC<sub>tau</sub> or C<sub>trough</sub>) in the i<sup>th</sup> individual, EC50<sub>i</sub> is the value for the concentration corresponding to half-maximal effect of migalastat in the i<sup>th</sup> individual, and mean EC50 is the population mean EC50 across all genetic variants.

The EC50 for each subject was calculated with individual level HEK assay data from 11 concentrations per subject in triplicate. Molar concentrations evaluated to determine EC50 ranged from 1.69 x10<sup>-8</sup> to 0.001, a 60,000-fold range. The responses varied greatly with a minimum response range of 2-fold to 600-fold over the range of concentrations evaluated. This large variability in HEK response potentially supports an EC50-normalized assessment of the exposure-response as shown in Figure 6 through Figure 13.

As the Applicant's EC50 normalization method aims to reduce variation in the exposure-response between subjects by partially correcting for differences in patient responsiveness to migalastat, the EC50 normalized results are shown below. Figure 6 through Figure 13 generally indicate that there were no clear correlations between C<sub>max</sub> or AUC<sub>tau</sub> of migalastat with potential PD indicators of efficacy. This was also the case for the plots without EC50 normalization.

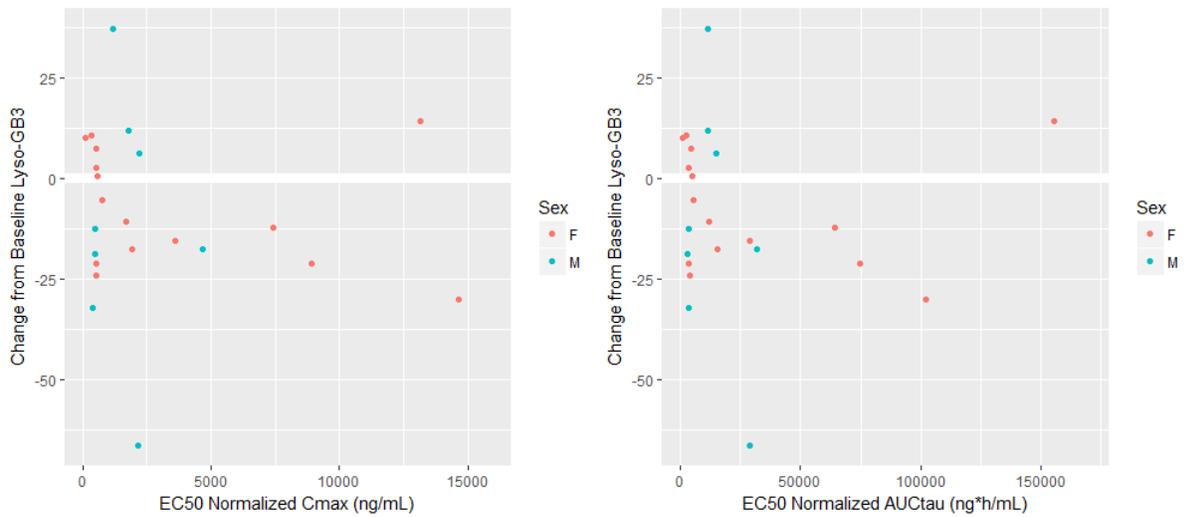
Figure 6 through Figure 9 are generated by the reviewer after removing the spline curves from the applicant's plots.

**Figure 6. Relationship Between Change from Baseline KIC vs. EC50-Normalized Model-Predicted PK Parameters ( $C_{max}$ ,  $AUC_{tau}$ )**



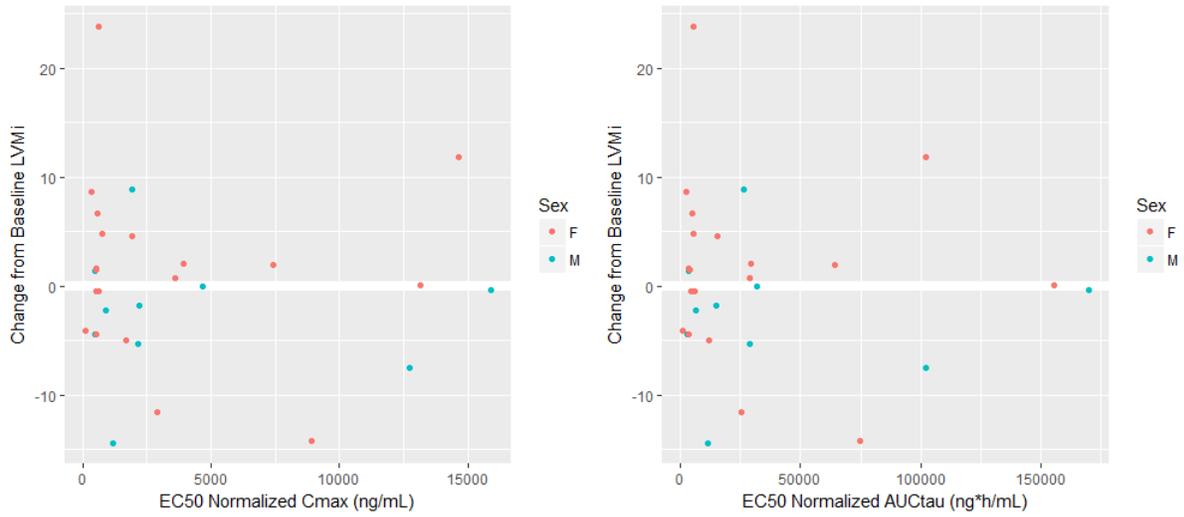
(Source: Reviewer's analysis)

**Figure 7. Relationship Between Change from Baseline Lyso-GB-3 vs. EC50-Normalized Model-Predicted PK Parameters ( $C_{max}$ ,  $AUC_{tau}$ )**



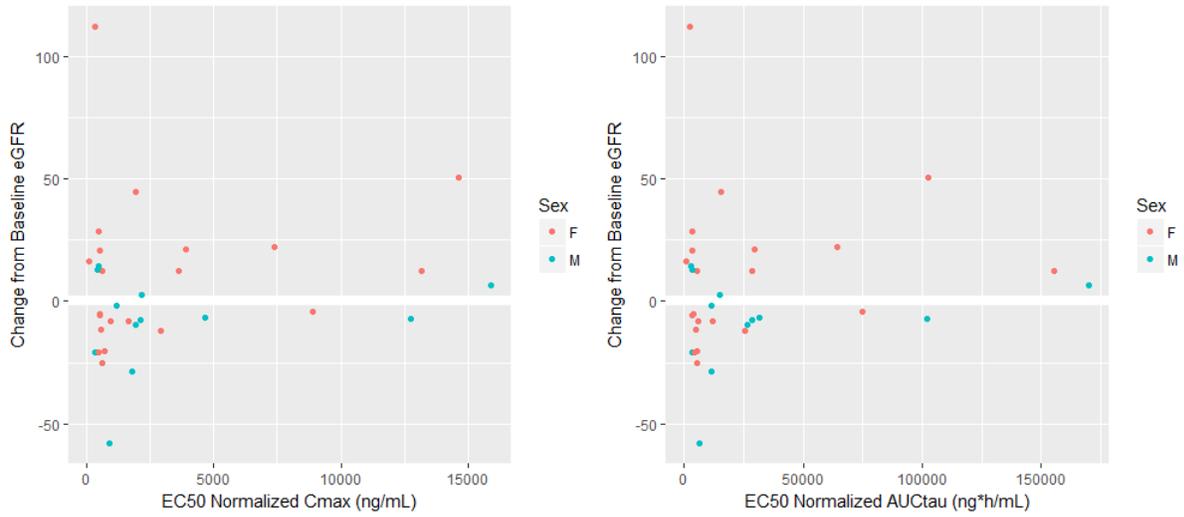
(Source: Reviewer's analysis)

**Figure 8. Relationship Between Change from Baseline LVMI vs. EC50-Normalized Model-Predicted PK Parameters (Cmax, AUCtau)**



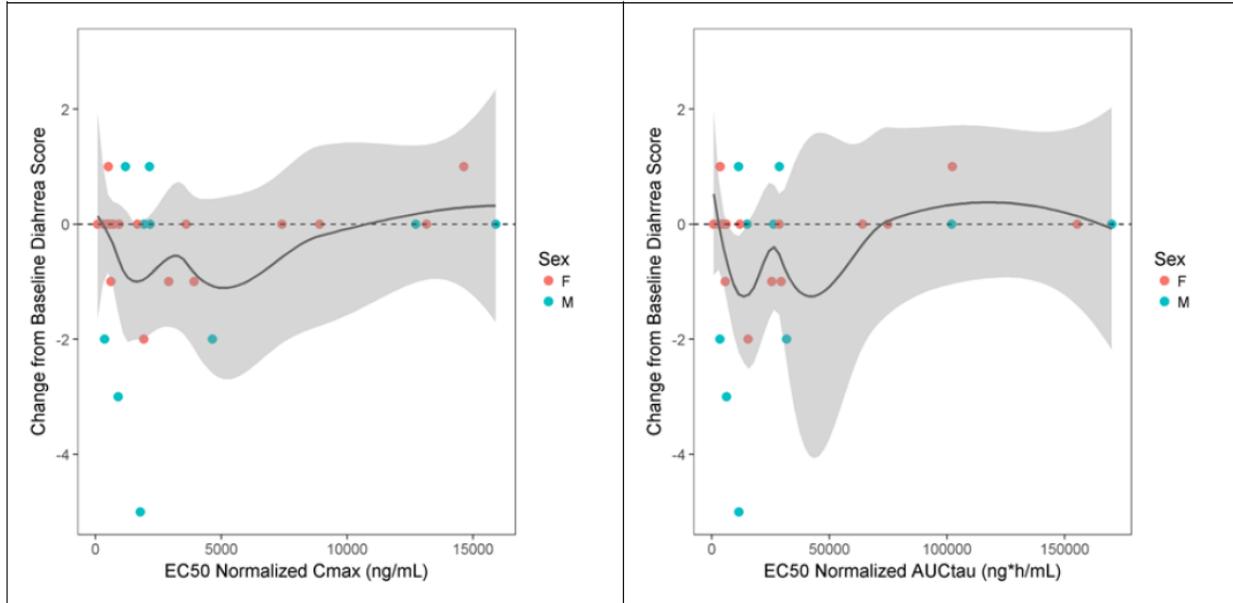
(Source: Reviewer's analysis)

**Figure 9. Relationship Between Change from Baseline eGFR vs. EC50-Normalized Model-Predicted PK Parameters (Cmax, AUCtau)**



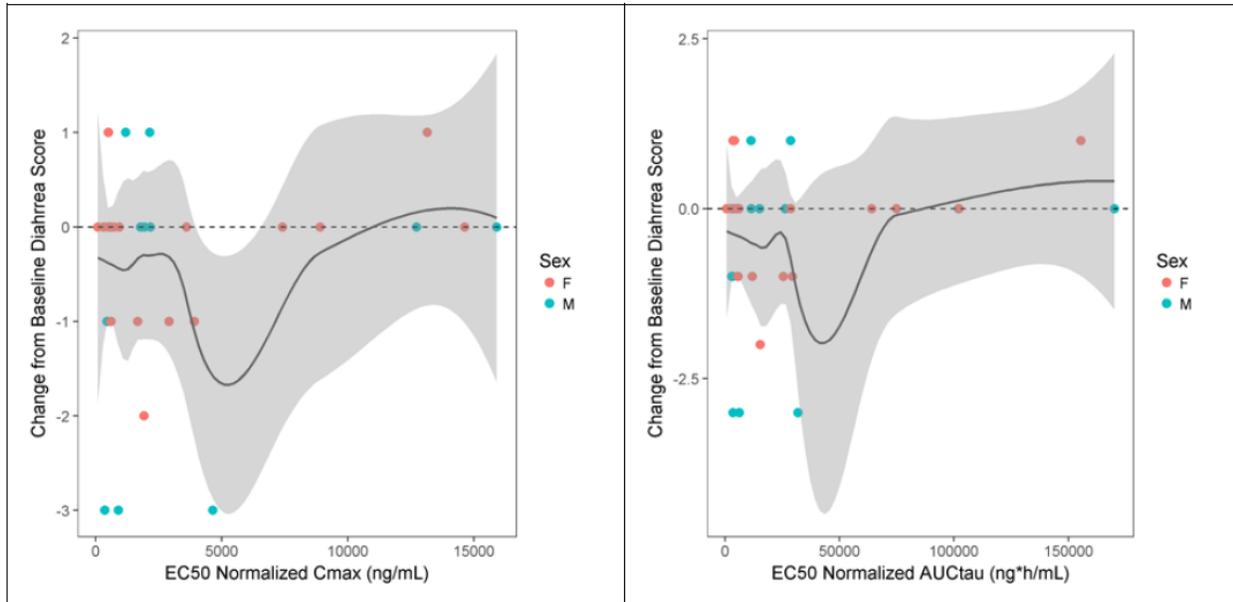
(Source: Reviewer's analysis)

**Figure 10. Relationship Between Improvement in Diarrhea Subscale of the GSRs, CRF Question 11 vs. EC50-Normalized Model-Predicted PK Parameters (Cmax, AUCtau)**



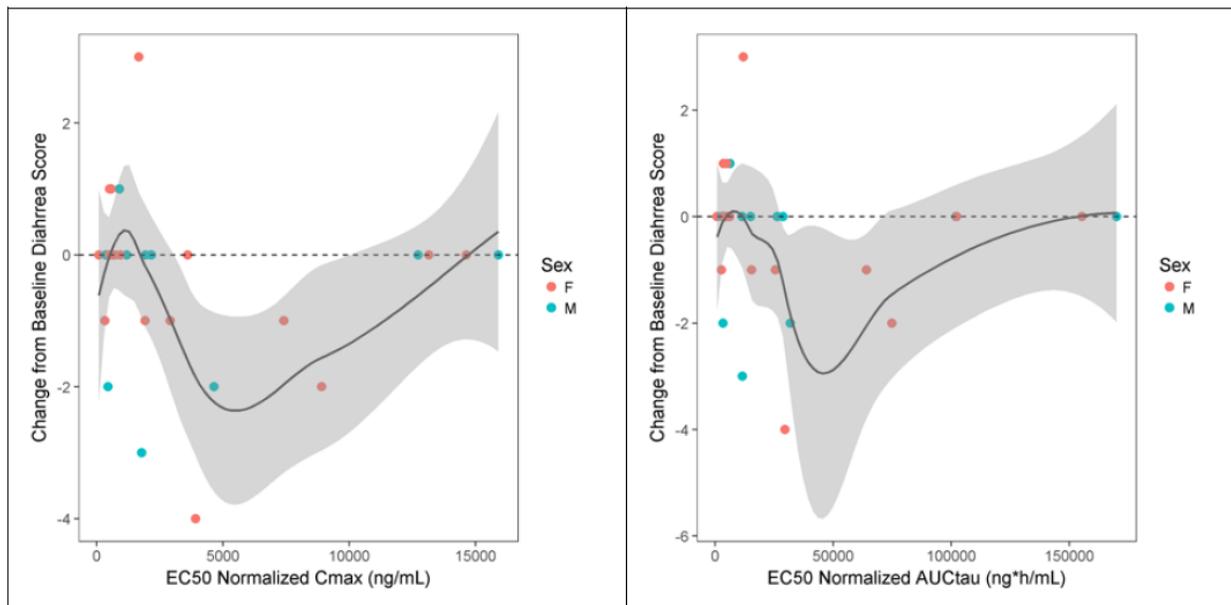
(Source: Applicant's Population PK/PD Report MGM116016-2158-rpt001-pk-pd, Figure 7)

**Figure 11. Relationship Between Improvement in Loose Stools Subscale of the GSRs, CRF Question 12 vs. EC50-Normalized Model-Predicted PK Parameters (Cmax, AUCtau)**



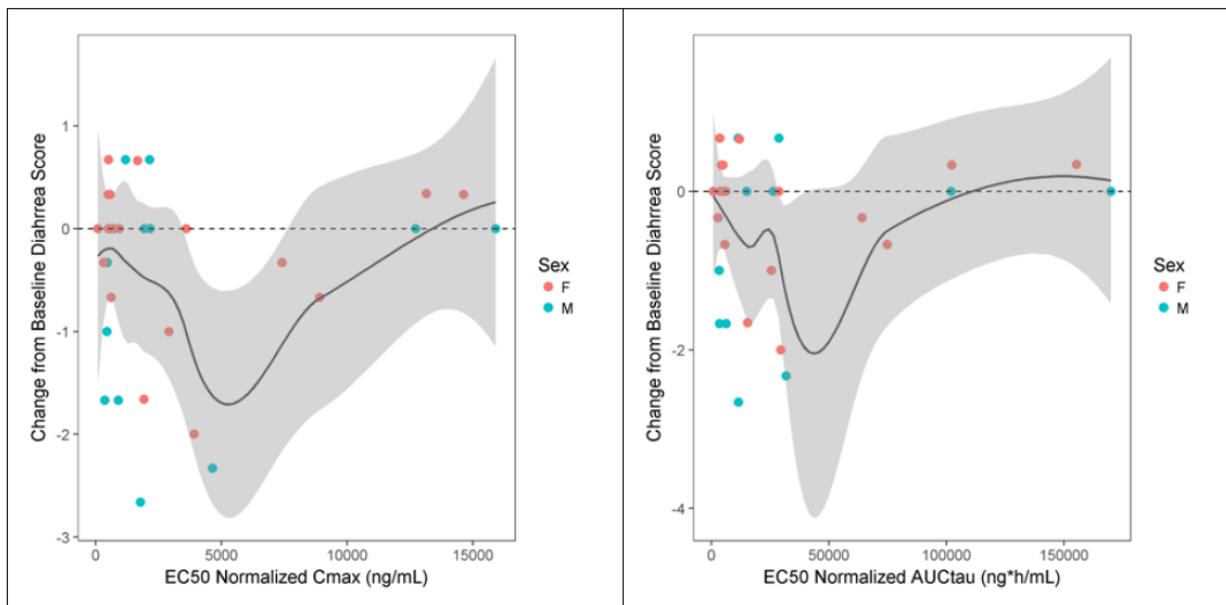
(Source: Applicant's Population PK/PD Report MGM116016-2158-rpt001-pk-pd, Figure 8)

**Figure 12. Relationship Between Improvement in Urgency Subscale of the GSRs, CRF Question 14 vs. EC50-Normalized Model-Predicted PK Parameters (Cmax, AUCtau)**



(Source: Applicant's Population PK/PD Report MGM116016-2158-rpt001-pk-pd, Figure 9)

**Figure 13. Relationship Between Improvement in Average Subscale of the GSRs vs. EC50-Normalized Model-Predicted PK Parameters (Cmax, AUCtau)**



**Reviewer's Comments:**

*The Applicant's exposure-response analyses are acceptable and suggest that exposure changes may not affect patient response to migalastat. However, these results should be interpreted with caution as the analyses were based on a small number of subjects at one dose level.*

## 7.3. Reviewer's Population PK Analysis

### 7.3.1. Introduction

The reviewer performed a sensitivity analysis with each covariate in the final model to determine the degree to which between subject variation, shrinkage on  $ETA_{CL}$ , and trends in  $ETA_{CL}$  versus the covariate changed with removal of the covariate of interest. This analysis also focused on identifying the relevance of body weight as a covariate, and evaluated the change in clearance across the population by body weight.

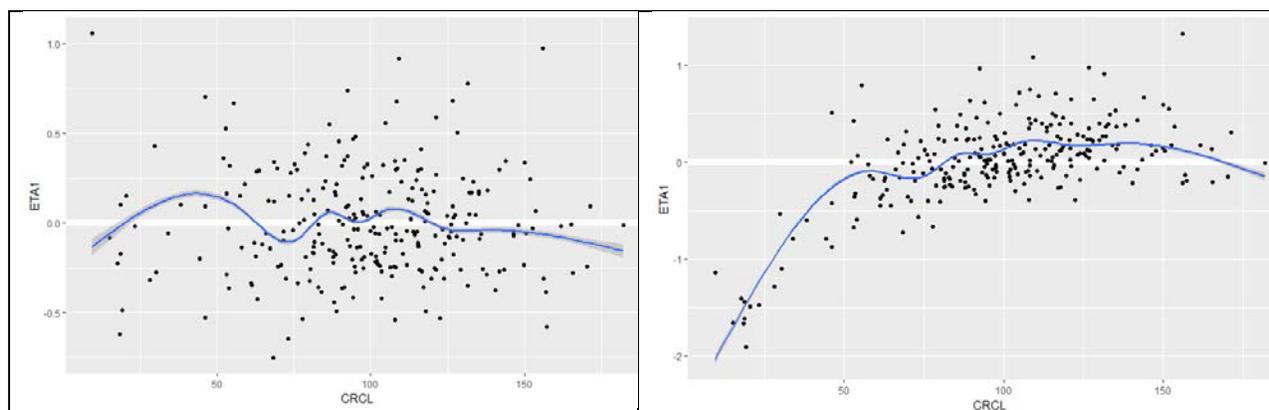
### 7.3.2. Methods

NONMEM (version 7.3) was utilized to evaluate the Applicant's (b) (4) model and variations of that model. The statistical software R was utilized to generate diagnostic plots for each model estimation. Each covariate from the final model was removed and the model was reestimated on the Applicant's final dataset.

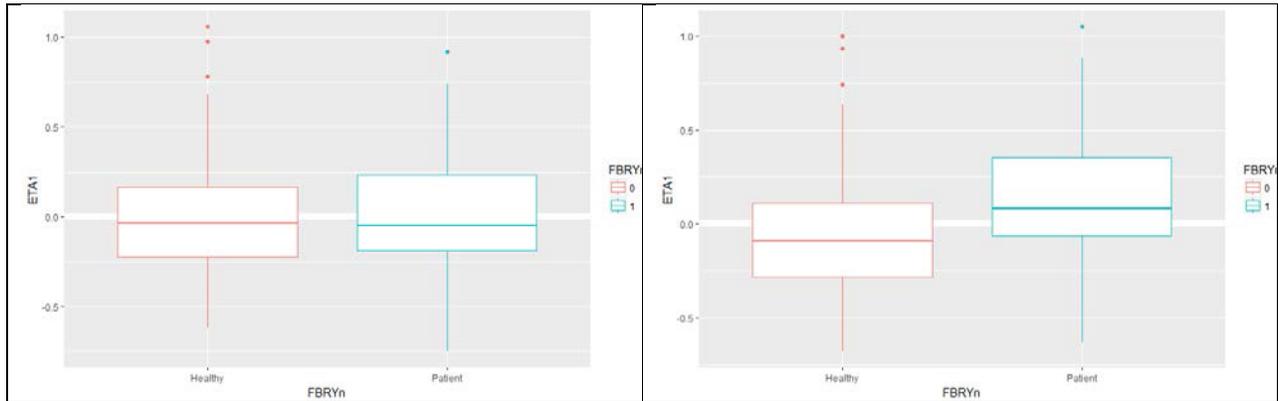
### 7.3.3. Results

The exclusion of either renal function, body weight and patient status were each evaluated to determine the relevance of the parameter on to the pharmacokinetics of migalastat. A sensitivity analysis was performed for each covariate by removing the parameter from the model and refitting the data. Then the  $ETA_{CL}$  for each individual were plotted against the covariate of interest for both before and after the covariate was removed. If the covariate is relevant a trend in the  $ETA_{CL}$  values should be evident when plotted against the tested covariate. Figure 14 through Figure 16 indicate just this. The panel on the right evidences a trend with the covariate compared to the panel on the left (Applicant's final model). The lack of bias in the left panel indicates the mathematical relationship for incorporating the covariate into the model is reasonable. Additionally, the model differences in objective function, between subject variability on CL, and eta shrinkage were evaluated (Table 6).

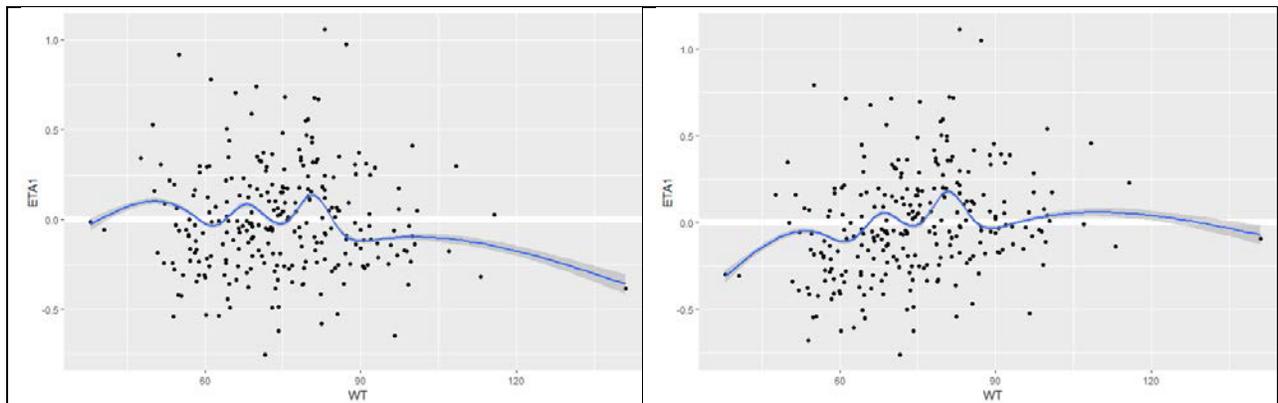
**Figure 14.  $ETA_{CL}$  vs. renal function (RF) before (left panel) and after (right panel) removing renal function as a covariate in the model.**



**Figure 15.  $ETA_{CL}$  vs. Patient Status before (left panel) and after (right panel) removing Patient Status as a covariate in the model.**



**Figure 16.  $ETA_{CL}$  vs. body weight (BW) before (left panel) and after (right panel) removing body weight as a covariate in the model.**



**Table 6. Diagnostics of Covariate Relevance and Model Robustness Before and After Removing Body Weight, Renal Function (RF), and Patient Status**

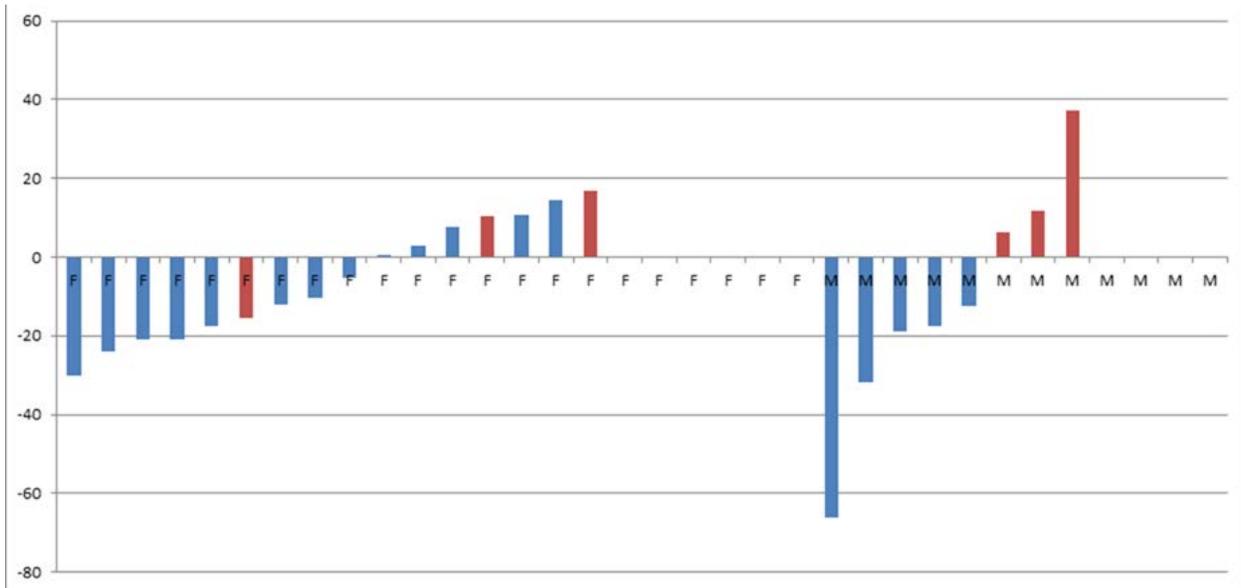
Shrinkage in etaCL		
Parameter Removed	Before (Full Model)	After (Test Model)
Body Weight		3%
RF	4%	1%
Fabry		3%
BSV on CL (%CV)		
Parameter Removed	Before (Full Model)	After (Test Model)
Body Weight		31.5
RF	30.5	45.9
Fabry		31.9
OBJF		
Parameter Removed	Before (Full Model)	After (Test Model)
Body Weight		48833
RF	48806	49188
Fabry		48843

The Applicant’s model appeared to capture the central tendency of the data. Based on the low values of shrinkage, the relevance of the covariates to trends in  $ETA_{CL}$ , and the changes in the objective function and between-subject variability in the sensitivity analyses, the Applicant’s estimates and conclusions regarding covariates were accepted.

#### **7.4. Reviewer’s Response Assessment for Sex and Amenable Status**

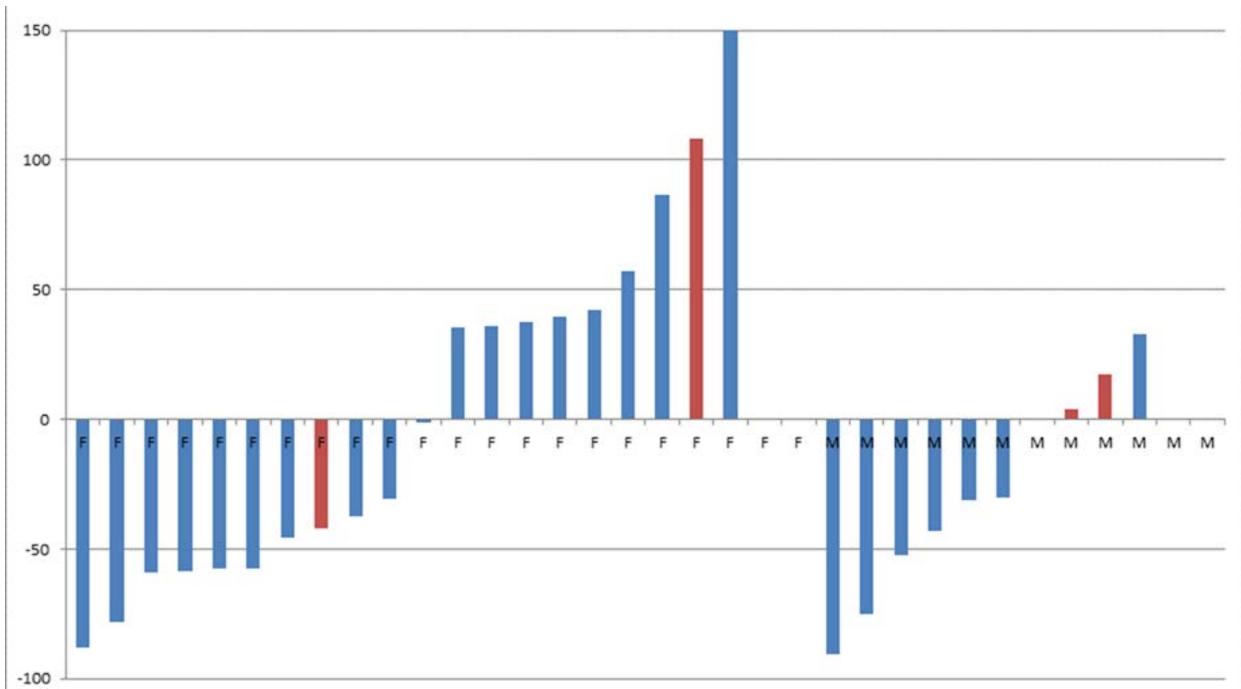
Based on data from the migalastat treated group in Study AT1001-011, two waterfall plots were generated to visualize the range of responses (change from baseline) for lyso-Gb3 (Figure 17) and KIC (Figure 18) by sex and amenable status. Based on these plots two things were apparent. For patients with an amendable mutation status, all but one male patients had a decrease from baseline for both biomarkers, while there is a substantial portion of females whose KIC and lyso-Gb3 levels increased. For the non-amendable patients, all males showed an increase from baseline for both KIC and lyso-Gb3, while female patients had varied response.

**Figure 17. Amenable Status Correlated Greatest With Response in Lyso-Gb3 for Males.**



The y-axis is change from baseline in lyso-Gb3. The left progression from left to right are for females as indicated on the axis. The right set of data are males. The ticks in between represent missing data. Lyso-Gb3 contained more missing data than other endpoints. All missing points were in amenable subjects. The blue bars are for amenable patients, the red bars are not.

**Figure 18. Amenable Status Correlated Greatest With Response in KIC for Males.**



The y-axis is change from baseline in KIC. The left progression from left to right are for females as indicated on the axis. The right set of data are males. The ticks in between represent missing data. All missing points were in amenable subjects. The blue bars are for amenable patients, the red bars are not.

## 8 Appendix 8: Individual Study Review

By: Dilara Jappar & Insook Kim

### 8.1 Renal Impairment Study AT1001-015

**Title:** An Open-Label Study to Determine the Safety and Pharmacokinetics of AT1001 in Subjects with Impaired Renal Function and Healthy Subjects with Normal Renal Function

**Clinical Sites:** Orlando Clinical Research Center, Orlando, Florida;  
Clinical Pharmacology of Miami, Inc, Miami, Florida  
Elite Research Institute, Miami, Florida  
West Coast Clinical Trials, LLC, Costa Mesa, California

**Bioanalytical Site:** (b) (4)

**Study Date:** 31 August 2011 to 23 April 2012

**Test Product:** single oral dose of 150 mg migalastat HCl capsule

**Study Rationale:** Migalastat is primarily eliminated unchanged in the urine. Fabry disease can adversely affect renal function in some patients. Therefore, this study was designed to assess the effect of varying stages of renal impairment on the PK of migalastat.

#### Study Design:

This study was a open-label, non-randomized, multiple-center, sequential group, safety, tolerability, and PK study of a single dose of migalastat HCl administered orally as a 150-mg dose in fasted healthy control male and female subjects with normal renal function compared to mild, moderate, and severe renally impaired subjects (classified by level of creatinine clearance [CL<sub>cr</sub>] as determined by the Cockcroft-Gault formula). The study drugs were administered with 240 mL water following at least 4 hours of fasting. Subjects also fasted from food (but not water) for 2 hours postdose. PK blood samples were collected for up to 120 hours post-dose to determine the plasma concentrations of migalastat.

Cohort	No. of Subjects	Renal Impairment Classification
1	8	normal renal function (CL <sub>cr</sub> ≥90 mL/min)
1	8	mild renal impairment (CL <sub>cr</sub> ≥60 mL/min and <90 mL/min)
1	8	moderate renal impairment (CL <sub>cr</sub> ≥30 mL/min and <60 mL/min)
2	8	severe renal impairment (CL <sub>cr</sub> ≥15 mL/min and <30 mL/min)

#### Study Population:

Thirty-two male and female subjects (18-70 years of age) were enrolled and completed the study. The demographics (age [ $\pm$  5 years], body mass index [BMI;  $\pm$ 15%], and sex) of the healthy control subjects were matched with those of the mild renally impaired subjects as closely as possible.

#### PK Measurements:

PK blood samples were collected predose, and 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 15, 24, 48, 72, 96, and 120 hours postdose to determine the plasma concentration of migalastat.

## Results:

Following oral administration of a single dose of 150 mg migalastat HCl, migalastat exposure (AUC) increased and oral clearance (CL/F) decreased with the severity of renal impairment while C<sub>max</sub> did not vary by renal impairment. Times to maximum concentration (t<sub>max</sub>) increased with severity of renal impairment. Subjects with mild, moderate and severe renal impairment had about 1.2, 1.8 and 4.31-fold higher exposure (AUC) than that of subjects with normal renal function, respectively. Plasma migalastat concentration appears to decrease in biphasic manner. Subjects with mild, moderate and severe renal impairment had about 1.6, 11.3 and 58.6-fold higher mean plasma concentrations at 48 hours postdose (C<sub>48</sub>) than subjects with normal renal function, respectively. Additionally, half-life also increased as function of renal impairment by 1.2, 3.5 and 5-fold in subjects with mild, moderate and severe renal impairment, respectively.

Figure 1: Arithmetic Mean ( $\pm$ SD) Plasma Concentrations-time profiles for Migalastat

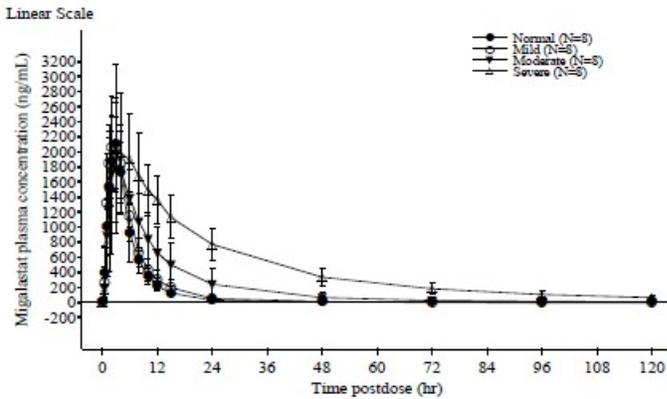


Figure 2: Arithmetic Mean ( $\pm$ SD) AUC<sub>0-t</sub> and Oral Clearance (CL/F) of Migalastat versus Renal Function Group

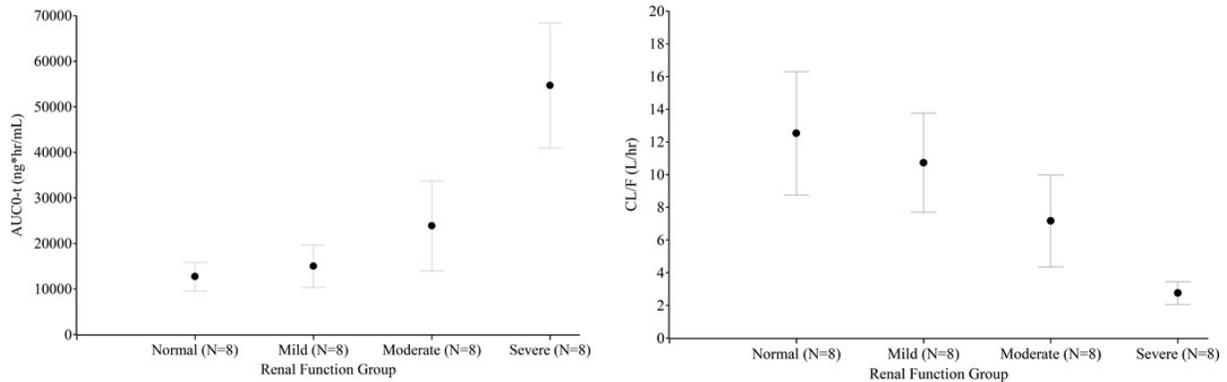


Figure 3: Scatter Plot of Estimated AUC<sub>0-t</sub> of Migalastat Versus CL<sub>cr</sub> (N=32)

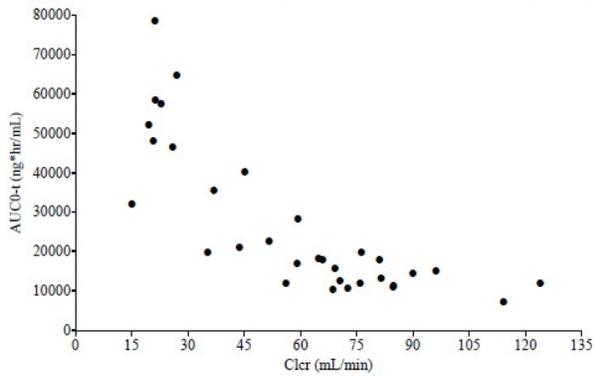


Table 1: Geometric Mean (geo CV%) Migalastat Plasma PK Parameters in Subjects with Various Renal Function

PK Parameter	Units	Renal Function Group			
		Normal (N=8)	Mild (N=8)	Moderate (N=8)	Severe (N=8)
AUC <sub>0-t</sub>	(ng·hr/mL)	12306 (27.9)	14389 (31.1)	22126 (42.8)	53070 (27.0)
AUC <sub>0-∞</sub>	(ng·hr/mL)	12397 (27.7)	14536 (30.7)	22460 (42.2)	56154 (24.9)
C <sub>max</sub>	(ng/mL)	2100 (26.0)	2191 (28.8)	1868 (32.1)	2078 (45.5)
t <sub>max</sub> <sup>a</sup>	(hr)	2.50 (1.50, 3.00)	2.50 (1.50, 4.00)	3.00 (1.50, 4.00)	4.27 (3.00, 8.00)
t <sub>½</sub> <sup>b</sup>	(hr)	6.42 (1.93)	7.66 (3.02)	22.2 (14.2)	32.3 (7.35)
λ <sub>z</sub>	(1/hr)	0.113 (32.9)	0.0965 (40.1)	0.0386 (84.6)	0.0219 (21.2)
CL/F	(L/hr)	12.1 (27.7)	10.3 (30.7)	6.68 (42.2)	2.67 (24.9)
Vd/F	(L)	107 (38.0)	107 (37.7)	173 (102.3)	122 (43.7)
C <sub>48</sub> <sup>b</sup>	(ng/mL)	5.70 (3.63)	9.34 (7.57)	64.5 (68.1)	334 (126)

<sup>a</sup> Median (Min, Max) presented for t<sub>max</sub>.

<sup>b</sup> Arithmetic Mean (SD) is presented

*Table 2. Statistical Analysis of Migalastat Plasma PK Parameters in Subjects with Various Renal Function*

Parameter (unit)	Renal Function Group	N <sup>a</sup>	Geometric Least Squares Means	Ratio of Geometric	90% CI	Between
				Least Squares Means Renally-impaired/Normal <sup>b</sup>	for the Ratio <sup>b</sup> (Lower, Upper)	Subject CV%
AUC <sub>0-∞</sub> (hr·ng/mL)	Mild	8	14536	1.17	(0.90, 1.53)	31.9
	Moderate	8	22460	1.81	(1.39, 2.36)	
	Severe	8	56154	4.53	(3.48, 5.90)	
	Normal	8	12397			
AUC <sub>0-t</sub> (hr·ng/mL)	Mild	8	14389	1.17	(0.89, 1.53)	32.6
	Moderate	8	22126	1.80	(1.37, 2.36)	
	Severe	8	53070	4.31	(3.29, 5.65)	
	Normal	8	12306			
C <sub>max</sub> (ng/mL)	Mild	8	2191	1.04	(0.79, 1.38)	33.7
	Moderate	8	1868	0.89	(0.67, 1.18)	
	Severe	8	2078	0.99	(0.75, 1.31)	
	Normal	8	2100			

<sup>a</sup> N = number of subjects.

<sup>b</sup> The ratio and corresponding CI are back-transformed from the difference and confidence interval calculated on the log scale to the linear scale.

**Safety:** Overall, 14 subjects reported a total of 21 TEAEs. Nine of these TEAEs (reported for 5 subjects) were considered to be related to study drug. The majority of TEAEs (20 of 21) were mild in severity and 1 TEAE was moderate in severity. The incidence of TEAEs, as well as treatment-related TEAEs, was comparable across all renal function.

*Table 3: Summary of Adverse Events*

	Renal Function Group				Overall (N=32)
	Normal (N=8)	Mild (N=8)	Moderate (N=8)	Severe (N=8)	
Subjects with TEAEs	3 (38%) [3]	4 (50%) [5]	4 (50%) [9]	3 (38%) [4]	14 (44%) [21]

**Reviewer’s Comment:**

There was no notable difference in AE profiles across all different renal function subgroups in this single dose PK study. Nonetheless, this safety assessment is very limited due to number of subjects in each group (n = 8).

The Applicant proposed no dose adjustment in patients with mild to moderate renal impairment, and does not propose use of migalastat in patient with severe renal impairment (eGFR <30 mL/min/1.73m<sup>2</sup>). The Applicant’s proposal is acceptable.

The Applicant’s proposal for patients with mild to moderate renal impairment is acceptable for the following reasons:

1. No major accumulation of migalastat is expected from QOD dosing in patients with moderate renal impairment based on multiple dose simulation (approximately 18%) and estimated exposure by population PK approach in patients with moderate renal impairment in phase 3 trial AT1001-011.
2. Migalastat was well tolerated following single doses up to 450 mg (trial AT1001-013), multiple doses up to 250 mg BID dose for up to 2 weeks (FAB-CL-201), and multiple doses at 250 mg QOD dose for up to 48 weeks (study FAB-CL-204) in Fabry patients.

3. From an efficacy standpoint, in phase 3 trial AT1001- 011, Fabry patients with moderate renal impairment ( $30 \leq \text{eGFR} < 60 \text{ mL/min/1.73 m}^2$ ) had similar efficacy responses to 150 mg QOD (e.g., reduction in KIC GL-3 and plasma Lyso-GB3) as patients who had  $\text{eGFR} > 60 \text{ mL/min/1.73 m}^2$  to suggest that an increase in C48h by 11-fold and prolongation in half-life by 4-fold in patients with moderate renal impairment will not affect efficacy.

The Applicant for patients with severe renal impairment is also acceptable as migalastat was not studied in Fabry patients with severe renal impairment ( $\text{eGFR} < 30 \text{ mL/min/1.73m}^2$ ) in this development program. In both phase 3 trials (AT1001-011 and AT101-012), patients with severe renal impairment ( $\text{eGFR} < 30 \text{ mL/min/1.73m}^2$ ) were excluded. Nevertheless, as patients with Fabry disease can develop renal impairment as the disease progresses, OCP recommends that a dosing strategy for patients with severe renal impairment be developed and studied in such patients.

## 8.2 Food Effect Study AT1001-016

**Title:** A Randomized, Open-Label, 5-Period Crossover Study to Evaluate the Effect of Meal Type and Timing on the Pharmacokinetics of Migalastat Hydrochloride in Healthy Volunteers

**Clinical Site:** PPD, Phase I Clinic, Austin, Texas

**Bioanalytical Site:** (b) (4)

**Study Date:** 19 Oct 2011 to 23 Dec 2011

**Test Product:** Migalastat HCl 150 mg capsule

### Study Design:

This was a phase I, randomized, open-label, single-dose, single-center, 5-period crossover study to evaluate the effect of meal type and meal timing on migalastat PK in 20 healthy male and female subjects. In each of the treatment periods, subjects were randomly assigned to receive a single dose of migalastat HCl as follows: while fasting, with the simultaneous consumption of a glucose drink, 1 hour before a high-fat meal, 1 hour before a light meal, or 1 hour after a light meal. There was at least a 7-day washout period between the treatments.

In all treatment regimens (A through E), subjects avoided ingestion of food with the exception of the treatment-designated meal and water 8 hours before and 4 hours after study drug administration. All meals were eaten within a time window of 30 minutes. Water was permitted *ad libitum* except for 1 hour before and 2 hours after dosing. The study drug was administered with 240 mL of water with the exception of treatment regimen B (with simultaneous consumption of a glucose drink). Subjects receiving treatment regimen B were allowed to eat a light snack 3 hours before dosing. The light snack provided approximately 423 calories and consisted of a serving of whole wheat zucchini bread (97 g) and 8 ounces of skim milk. PK blood samples were collected for up to 24 hours postdose to determine the plasma concentrations of migalastat.

Regimen	Description
A	150-mg migalastat HCl in the fasting state (reference treatment)
B	150-mg migalastat HCl with simultaneous consumption of a glucose drink
C	150-mg migalastat HCl 1 hour before consumption of a high-fat meal
D	150-mg migalastat HCl 1 hour before consumption of a light meal
E	150-mg migalastat HCl 1 hour after consumption of a light meal

### Composition of Fed/Fasting Treatment Conditions

Treatment Condition	Composition
Fasting	Not applicable
Glucose drink	Trutol Glucose Tolerance Test Beverage composed of: water – 50%, liquid dextrose 20%, sodium benzoate<0.5%, citric acid <0.5%, and orange flavoring <0.5% <sup>2</sup>
High-fat meal	High-fat breakfast composed of: 850 total calories, 477 calories from fat, 29 g protein, 64 g carbohydrates, 53 g fat, and 457 mg cholesterol
Light meal	Light breakfast composed of: 507 total calories, 150 calories from fat, 13 g protein, 80 g carbohydrates, 17 g fat, and 40 mg cholesterol

The actual contents of high-fat meal or light meal were not provided in the study report. The total calories and calories from fat in the high-fat meal in this study appears to be consistent with the suggested test meal in the FDA guidance “Food Effect Bioavailability and Fed Bioequivalence Studies” where the

recommended standard high-fat breakfast contained approximately 800 to 1000 total calories, with 50% of calories being derived from fat content.

**Study Population:** A total of 20 subjects were enrolled, 19 subjects (95%) completed the study, and 1 subject was discontinued because of a treatment-emergent adverse event (TEAE) of vomiting in period 2.

**PK Measurements:** PK blood samples were collected predose, and 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, and 24 hours postdose.

**Results:**

Reduction in exposure (both AUC and C<sub>max</sub>) and increase in oral clearance (CL/F) were observed with different meal type and meal timing compared to the fasted state.

Figure 1: Mean (±SD) Plasma Concentrations of Migalastat Versus Time by Treatment

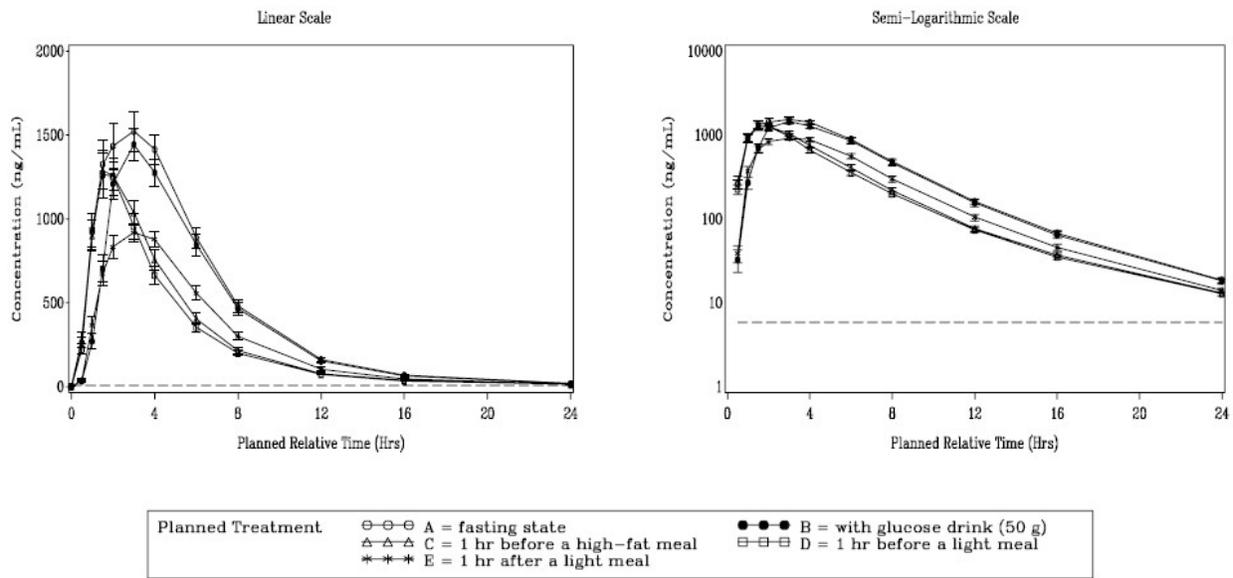


Table 1: Geometric Mean (geometric CV) Plasma PK Parameters of Migalastat

Parameter (unit)	Treatment <sup>1</sup>				
	A n=19	B n=19	C n=19	D n=19	E n=19
AUC(0-t) (h•ng/mL) <sup>2</sup>	9696 (27.1)	8342 (29.2)	6021 (27.6)	5573 (32.0)	5801 (27.0)
AUC(0-inf) (h•ng/mL) <sup>2</sup>	9805 (26.8)	8451 (28.8)	6132 (26.8)	5668 (31.4)	5890 (26.5)
C <sub>max</sub> (ng/mL) <sup>2</sup>	1561 (33.8)	1408 (29.6)	1323 (28.3)	1278 (39.6)	945 (28.3)
t <sub>max</sub> (h) <sup>3</sup>	3.00 (1.50, 6.00)	3.00 (2.00, 4.00)	1.50 (1.00, 3.00)	2.00 (1.50, 3.08)	3.00 (1.52, 6.00)
t <sub>1/2</sub> (h) <sup>2</sup>	3.9 (11.3)	4.0 (11.8)	4.9 (20.5)	4.9 (13.4)	4.3 (10.9)
CL/F (L/h) <sup>2</sup>	12.5 (26.8)	14.5 (28.8)	20.0 (26.8)	21.6 (31.4)	20.8 (26.5)
V <sub>z</sub> /F (L) <sup>2</sup>	70.2 (32.7)	84.3 (39.5)	141 (39.9)	151 (42.5)	128 (34.4)

Data Source: Table 14.2.2

%CV<sub>b</sub>=between-subject coefficient of variation; HCl=hydrochloride; PK=pharmacokinetic

Note: Subject 102 discontinued early and was not included in the PK Population.

- 1 A=150-mg migalastat HCl in the fasting state  
B=150-mg migalastat HCl with simultaneous consumption of glucose drink  
C=150-mg migalastat HCl 1 hour before consumption of a high-fat meal  
D=150-mg migalastat HCl 1 hour before consumption of a light meal  
E=150-mg migalastat HCl 1 hour after consumption of a light meal
- 2 Geometric mean (geometric %CV<sub>b</sub>)
- 3 Median (minimum, maximum)

Table 2: Statistical Analysis of Migalastat Plasma PK Parameters

Parameter (unit)	Treatment <sup>1</sup>	n	Geometric LS Means	Treatment Comparison	Ratio of Geometric LS Means	90% CI of the Ratio
AUC(0-inf) (h•ng/mL)	A	19	9835.95	–	–	–
	B	19	8497.48	B/A	0.864	0.771-0.968
	C	19	6165.57	C/A	0.627	0.559-0.702
	D	19	5703.58	D/A	0.580	0.518-0.650
	E	19	5924.40	E/A	0.602	0.538-0.675
AUC(0-t) (h•ng/mL)	A	19	9727.84	–	–	–
	B	19	8390.66	B/A	0.863	0.769-0.968
	C	19	6056.09	C/A	0.623	0.555-0.699
	D	19	5609.48	D/A	0.577	0.514-0.647
	E	19	5836.75	E/A	0.600	0.535-0.673
Cmax (ng/mL)	A	19	1579.14	–	–	–
	B	19	1426.64	B/A	0.903	0.799-1.021
	C	19	1340.30	C/A	0.849	0.751-0.959
	D	19	1295.79	D/A	0.821	0.726-0.927
	E	19	956.36	E/A	0.606	0.536-0.684

Data Source: Table 14.2.3

CI=confidence interval; HCl=hydrochloride; LS=least squares; PK=pharmacokinetic

Note: Subject 102 discontinued early and was not included in the PK Population. An analysis of variance was performed on the natural log-transformed Cmax, AUC(0-t), and AUC(0-inf) using a mixed-effects model with fixed effects terms for treatment, period, and sequence, and subjects nested within sequence as a random effect.

- 1 A=150-mg migalastat HCl in the fasting state  
 B=150-mg migalastat HCl with simultaneous consumption of glucose drink  
 C=150-mg migalastat HCl 1 hour before consumption of a high-fat meal  
 D=150-mg migalastat HCl 1 hour before consumption of a light meal  
 E=150-mg migalastat HCl 1 hour after consumption of a light meal

**Safety:** According to the sponsor, there was no death or serious adverse event (SAE). Overall, 2 subjects (10%) reported at least 1 TEAE during the study. All TEAEs were mild to moderate in intensity, and all AEs resolved by the end of the study. One subject prematurely discontinued from study because of a TEAE of vomiting that occurred 2.5 hours after 150 mg migalastat HCl was administered in the fasting state.

**Reviewer’s Comment:**

- Administration of 150 mg migalastat HCl 1 hour before a high-fat meal reduced the AUC and Cmax by 37% and 15%, respectively, and shortened the tmax by 1.5 hours compared to the fasted state.
- Food effects were observed regardless of type of meal (high-fat vs. light). Administration of 150 mg migalastat HCl 1 hour before a light meal reduced AUC and Cmax by 42% and 18%, respectively, and shortened the tmax by 1.0 hours compared to the fasted state.
- Food effects were observed regardless of the timing of meal (1 hour before vs. after). Administration of 150 mg migalastat HCl 1 hour after a light meal reduced AUC and Cmax by 40% while tmax remained unchanged compared to the fasted state.
- Administration of 150 mg migalastat HCl with glucose drink (50 g) reduced the AUC and Cmax by only 14% and 10%, respectively, and did not alter tmax compared to the fasted state.
- Sampling time of 24 hours and washout period of 7 days were appropriate as the half-life of migalastat was approximately 4 to 5 hours.

- A similar effect of food on migalastat PK was seen in a supportive food effect study FAB-CL-103 where administration of 100 mg (four 25 mg capsule) migalastat HCl with high fat meal reduced AUC and C<sub>max</sub> by 38% and 40%, respectively, and shortened t<sub>max</sub> from 3.9 hour to 3.1 hour. Please note that as study FAB-CL-103 was conducted with different formulation and different strength compared to TBM formulation (100 mg vs. 150 mg), study FAB-CL-103 was considered as a supportive study and was not reviewed in detail.
- In phase 3 trials (Studies AT1001-011 and AT1001-012), patients were instructed to take 150 mg migalastat HCl QOD and required to fast 2 hours before and 2 hours after taking each migalastat dose. Based on the result of the current food effect study and design of phase 3 studies in regards to food, the applicant proposed not to consume food at least 2 hour before and 2 hour after taking migalastat in the proposed label. This current food effect study does not address the effect of food on migalastat PK when food is administered 2 hour prior or 2 hour after the drug administration, as recommended in the proposed label.

### 8.3 Absolute BA and IV SAD Study: AT1001-018:

**Title:** A Phase 1, Randomized, Double-Blind, Placebo-Controlled, Single Dose Escalation Trial to Evaluate the Safety, Tolerability And Pharmacokinetics Of Migalastat Hydrochloride Given Intravenously To Healthy Volunteers With An Open-Label, Randomized, Two-Way Crossover Arm

**Note:** Migalastat is referred as AT1001 in this study report  
**Study Date:** 11 March 2014 – 12 June 2014  
**Clinical Site:** PRA Health Sciences, Hanzeplein 1, Groningen, the Netherlands  
**Analytical Site:** (b) (4)

**Test Product:** 50 mg/mL migalastat HCl IV solution and 150 mg migalastat HCl oral capsule

#### Study Design:

This was a single-center, phase I, randomized, double-blind, placebo-controlled, single ascending IV dose study to evaluate the safety, tolerability and PK of AT1001 in healthy subjects (Cohort 1-3). Cohort 4 was an open –label, randomized, two-way crossover study to assess the absolute bioavailability of orally administered migalastat HCl relative to IV administered migalastat HCl in healthy subjects. In all cohorts, the duration of the IV infusion with migalastat HCl was 2 hours ( $\pm$  10 min) at a constant rate of 125 mL/h.

Cohort	Study Design	Dose	Subjects
1	Double-blinded	0.3 mg/kg IV	N=5
		Placebo	N=2
2	Double-blinded	1.0 mg/kg IV	N=5
		Placebo	N=2
3	Double-blinded	10.0 mg/kg IV	N=5
		Placebo	N=2
4 Cross-over	Open-label	150 mg IV	N=5
		150 mg Oral Capsule	N=5

Dosing of Cohorts 1, 2, and 3 was staggered to allow for safety and tolerability review before proceeding with the next group. Therefore, Cohorts 1, 2, and 3 were each divided into 2 sub-groups: Group A (2 received migalastat HCl and 1 received placebo) and Group B (3 received migalastat HCl and 1 received placebo). Subjects in Group B were dosed the day following completion of Group A dosing. Additionally, initiation of infusion was staggered by 60 minutes for Group A subjects, and by 30 minutes for Group B subjects. In cohort 4, 2 treatment periods were separated by 7 days of washout period. For all cohorts, including both IV infusion and oral administration, the study drug was administered after at least 4 hours of fasting. For subjects administered oral migalastat HCl, fasting continued for additional 2 hours after drug administration.

**Study Population:** A total of 31 healthy male and female subjects (ages between 19-45) were enrolled into this study and all of them completed the study per protocol.

#### PK Samples:

- Cohort 1, 2 and 3:
  - Plasma PK samples were collected at pre-dose, and at 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 16, 24 and 48 hours post-dose.
  - Urine samples were collected at the following time intervals: pre-dose, 0-6 hours, 6-12 hours, and 12-24 hours after the start of infusion.
- Cohort 4:

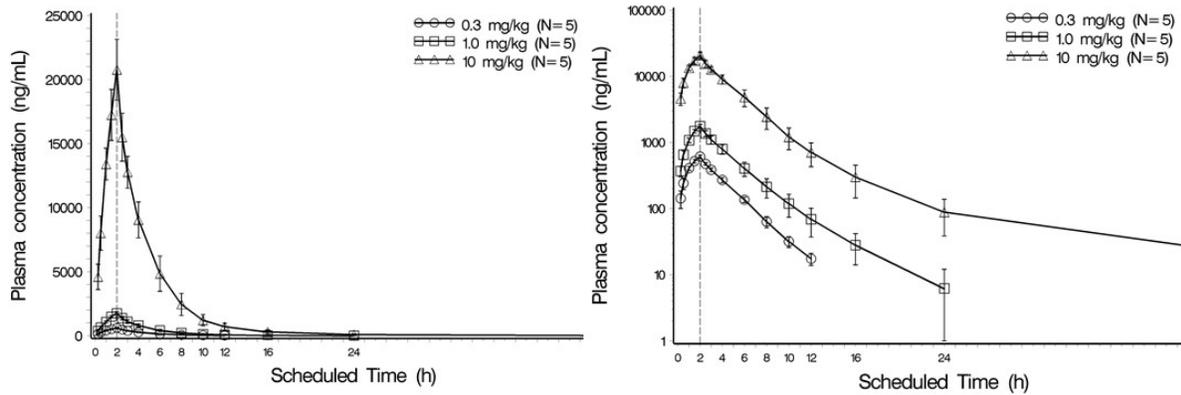
- Plasma PK samples were collected at pre-dose, and at 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 16, 24 and 48 hours post-dose.

## RESULTS:

### Pharmacokinetics:

- Migalastat plasma concentration appears to decline in biphasic manner.
- Half-life of migalastat increased from 2.09 hr to 10.0 hrs over 0.3 – 10 mg/kg IV dose ranges. As plasma migalastat concentration declines in biphasic manner where the second elimination phase appears to start at 24 hours post-dose, this increased in estimated half-life with dose appears to be driven by fewer quantifiable concentration during the terminal elimination phase at lower IV dose levels (12 h post-dose for the 0.3 mg/kg IV group and up to 24 h post-dose for the 1.0 mg/kg IV group). Consequently, the arithmetic mean  $V_z$ , which was derived from the  $CL_T$  and  $t_{1/2}$ , also increased from 19.9 to 107 L with increasing IV dose.

Figure 1: Arithmetic Mean (SD) Plasma Concentration-Time Profiles of Migalastat after a Single IV Dose of Migalastat HCl (Cohort 1-3)



Note: Arithmetic mean migalastat concentrations above LLOQ could be calculated up to 12 h post-dose for the 0.3 mg/kg IV group (Cohort 1), up to 24 h post-dose for the 1.0 mg/kg IV group (Cohort 2), and up to 48 h post-dose for the 10 mg/kg IV group (Cohort 3)

Table 1: Summary Statistics of Pharmacokinetic Parameters for Migalastat in Plasma

PK Parameter	0.3 mg/kg IV (N=5)	1.0 mg/kg IV (N=5)	10 mg/kg IV (N=5)	150 mg oral (N=10)	150 mg IV (N=10)
$C_{max}^a$ (ng/mL)	609 (5.5%)	1760 (5.9%)	20652 (11.4%)	1786 (25.9%)	3378 (12.6%)
$t_{max}^b$ (h)	1.93 (1.93 - 1.95)	1.93 (1.93 - 1.97)	1.93 (1.93 - 1.93)	2.75 (1.50 - 4.00)	1.93 (1.93 - 2.00)
$AUC_{0-t}^a$ (h·ng/mL)	2282 (7.7%)	6861 (14.7%)	81106 (14.5%)	9777 (25.7%)	13167 (14.9%)
$AUC_{0-\infty}^a$ (h·ng/mL)	2317 (7.4%)	6917 (14.6%)	81447 (14.6%)	9881 (25.6%)	13253 (15.0%)
$t_{1/2}^c$ (h)	2.09 (8.9%)	3.21 (19.5%)	10.0 (3.1%)	7.28 (59.2%)	4.54 (44.8%)
$CL_T^{c,d}$ (L/h)	6.59 (4.3%)	7.68 (14.8%)	7.40 (9.7%)	12.8 (26.1%)	9.34 (14.6%)
$V_z^{c,d}$ (L)	19.9 (7.5%)	35.0 (15.4%)	107 (10.6%)	123 (46.0%)	59.4 (33.7%)
$V_{ss}^{c,e}$ (L)	17.9 (6.3%)	24.2 (12.8%)	27.2 (10.8%)		30.2 (15.0%)

CV% = coefficient of variation; IV = intravenous; N = number; PK = pharmacokinetic.

<sup>a</sup> For  $C_{max}$ ,  $AUC_{0-t}$ , and  $AUC_{0-\infty}$  geometric mean (CV%) are presented.

<sup>b</sup> For  $t_{max}$  median and (minimum - maximum) are presented.

<sup>c</sup> For  $t_{1/2}$ ,  $CL_T$ ,  $V_z$ , and  $V_{ss}$  arithmetic mean (CV%) are presented.

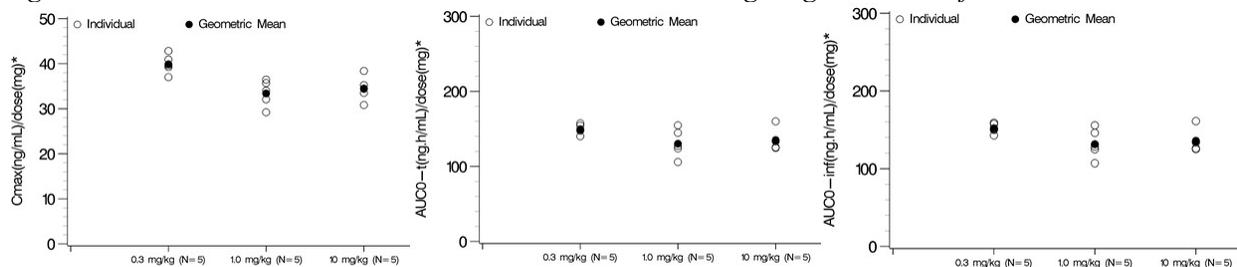
<sup>d</sup>  $CL_T$  and  $V_z$  for IV treatment and  $CL_T/F$  and  $V_z/F$  for oral treatment.

<sup>e</sup>  $V_{ss}$  was calculated for IV treatment only.

### Dose Proportionality:

Following IV infusion over 2 hours, migalastat exposure (both  $C_{max}$  and AUC) increased in approximately dose –proportional manner between 0.3 mg/kg to 10 mg/kg. Dose normalized  $C_{max}$  and AUC vs. dose were almost flat.

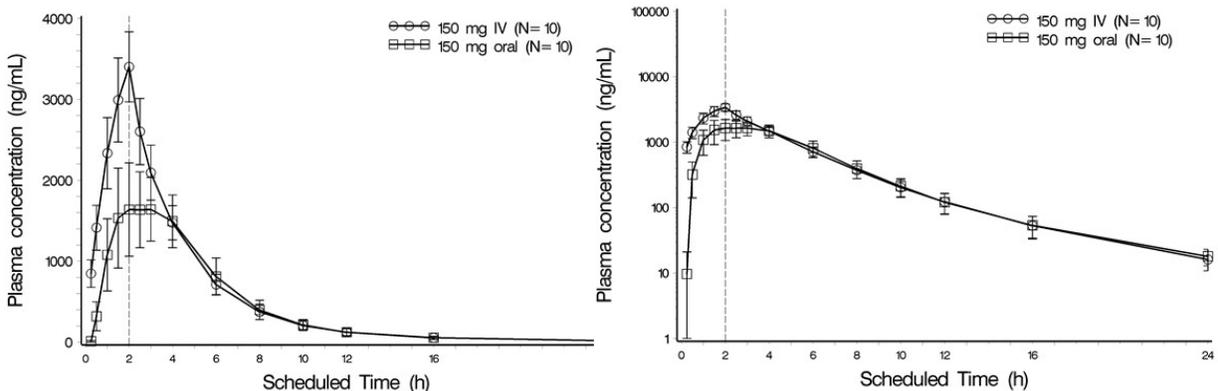
Figure 2: Dose-Normalized AUC and  $C_{max}$  vs. Dose Following Single IV Dose of AT-1001



### Absolute BA:

150 mg Migalastat IV infusion (over 2 hours) has 1.89-fold higher  $C_{max}$  and 1.35-fold higher AUC compared to oral 150 mg migalastat HCl capsule. The absolute bioavailability of oral migalastat is approximately 75% compared to IV administration.

Figure 3: Arithmetic Mean (SD) Plasma Concentration-Time Profiles of Migalastat after a Single Oral or IV Dose of 150 mg Migalastat HCl (Cohort 4)



Note: Arithmetic mean migalastat concentrations above LLOQ could be calculated up to 24 h post-dose after both oral and IV administration of 150 mg migalastat HCl.

Table 2: Summary of Statistical Analysis of Bioavailability (Cohort 4)

PK Parameter	Geometric Least Square means			Percentage Test/Reference	
	Test		Reference	Estimate	(90% CI)
AUC <sub>0-4</sub>	Oral 150 mg	9777	IV 150 mg	13167	74.3 (67.0, 82.3)
AUC <sub>0-∞</sub>	Oral 150 mg	9881	IV 150 mg	13253	74.6 (67.2, 82.7)

CI = confidence interval; IV = intravenous; PK = pharmacokinetic.

PK parameters were compared using a model with treatment (oral or IV), period and sequence as a fixed factors, and subject within sequence as a random factor. The data were natural log transformed and back transformed after the analysis to obtain ratios.

#### Urinary Excretion:

Urine was collected over a 24-hour period following a single IV dose administration and the percentage of migalastat recovered in the urine as unchanged drug was 69% to 85% suggesting that renal excretion is a major elimination pathway for migalastat after IV administration. The mean cumulative amount of migalastat excreted ( $Ae_{0-24}$ ) in urine increased in approximately dose proportional manner from 0.3 mg/kg to 10 mg/kg.

Table 3: Arithmetic Mean (CV%) of PK parameters for migalastat in urine (cohort 1-3)

PK Parameter	0.3 mg/kg IV (N=5)	1.0 mg/kg IV (N=5)	10 mg/kg IV (N=5)
$Ae_{0-24}$ (mg)	10.7 (50.0%) <sup>a</sup>	42.2 (11.2%)	512 (6.5%)
$Fe$ (%)	69.2 (49.7%)	79.7 (5.0%)	85.1 (2.3%)
$CL_r$ (L/h)	4.51 (49.0%)	6.16 (16.3%)	6.41 (8.3%)

**Safety:** Overall, single IV doses of migalastat HCl in the range of 0.3 to 10.0 mg/kg as well as single IV and oral doses of 150 mg migalastat HCl were well-tolerated by healthy male and female subjects. There was no death or serious adverse event (SAE) or discontinuation due to AEs in this study. No subject was withdrawn due to AEs. In total, 20 treatment-emergent AEs (TEAEs) were reported by 9 of the 31 (29%) subjects. All TEAEs were of mild intensity; they were all transient and had resolved without sequelae by follow-up. The number of TEAE did not increase with increasing dose levels. There were no clinically significant findings for clinical laboratory, 12-lead ECG, vital signs, or physical examination during the study.

Table 4: Summary of TEAEs by Treatment, Relationship and Intensity

Treatment	Unrelated		Mild Relation to test drug Related		All TEAEs	
	e	n %	e	n %	e	n %
Placebo (N=6)	4	2 33%	2	1 17%	6	2 33%
0.3 mg/kg IV (N=5)	2	1 20%			2	1 20%
1.0 mg/kg IV (N=5)	5	2 40%			5	2 40%
10 mg/kg IV (N=5)	2	1 20%	1	1 20%	3	2 40%
150 mg oral (N=10)	1	1 10%	1	1 10%	2	2 20%
150 mg IV (N=10)	2	1 10%			2	1 10%
Total Active (N=25)	12	5 20%	2	2 8%	14	7 28%
Total (N=31)	16	7 23%	4	3 10%	20	9 29%

e = the number of adverse events per treatment; IV = intravenous; N = number of subjects exposed per treatment; n = the number of subjects that experienced the adverse event per treatment; TEAE = treatment-emergent adverse event; % = the percentage of subjects that experienced the adverse event per treatment: (n/N)\*100%.

**Reviewer's Conclusion:**

- Following IV infusion over 2 hours, migalastat exposure (both Cmax and AUC increased in approximately dose -proportional manner between 0.3 mg/kg to 10 mg/kg doses.
- The absolute bioavailability of migalastat following oral administration of 150 mg migalastat HCl is approximately 75%.
- Following a single dose IV administration, 69% to 85% of administered migalastat was recovered in urine over 24 hours as unchanged drug suggesting that renal excretion is a major elimination pathway for migalastat after IV administration.

## 8.4 SAD Study: FAB-CL-101:

**Title:** A Phase I, Randomized, Double Blind, Placebo Controlled, Ascending Single Oral Dose Study to Evaluate the Safety, Tolerability and Pharmacokinetics of AT1001 in Healthy Volunteers

**Note:** Migalastat is referred as AT1001 in this study report

**Study Date:** 25 July 2004 to 27 November 2004

**Clinical Site:** MDS Pharma Services in Lincoln, Nebraska, USA

**Analytical Site:** (b) (4)

**Test Product:** AT1001 oral solution

### Study Design:

This was the first-in man, single-center, phase I, randomized, double-blind, single-dose, placebo-controlled, ascending dose study to evaluate the safety, tolerability and pharmacokinetics of AT1001. The study consisted of 4 dose levels (25, 75, 225 and 675 mg) of a single oral dose of AT1001 with 6 subjects allocated to the active treatment and 2 subjects to the placebo, in a dose escalation regimen, with a 7-day safety evaluation period between successive cohorts. The study drug was administered following an overnight fasting of at least 10 hours and subjects fasted 4 additional hours post-dose. Blood and urine samples were collected over 24- hour post-dose to assess the PK of AT1001.

Group	Subjects	Dose Level	Dose
1	6	1	25 mg
	2		placebo
2	6	2	75 mg
	2		placebo
3	6	3	225 mg
	2		placebo
4	6	4	675 mg
	2		placebo

**Study Population:** A total of 32 healthy male subjects (ages between 19-50) were enrolled into this study and all of them completed the study per protocol.

**PK Blood Samples:** PK Blood samples (10 mL) were collected at pre-dose, and at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 16 and 24 hours post-dose.

**Urine Samples:** Urine samples were pooled for the entire collection interval at pre-dose (-4-0), 0-4, 4-8, 8-12 and 12-24 hours post-dose.

## RESULTS:

### Pharmacokinetics:

- C<sub>max</sub> increased in a dose-proportional manner between 25 mg to 675 mg.
- AUC increased in more than dose proportional manner between 25 mg and 75 mg and increased in approximately dose-proportional manner between 75 mg to 675 mg.

Figure 1: Mean plasma concentration vs. time profile of AT-1001 Following Single Dose Administration

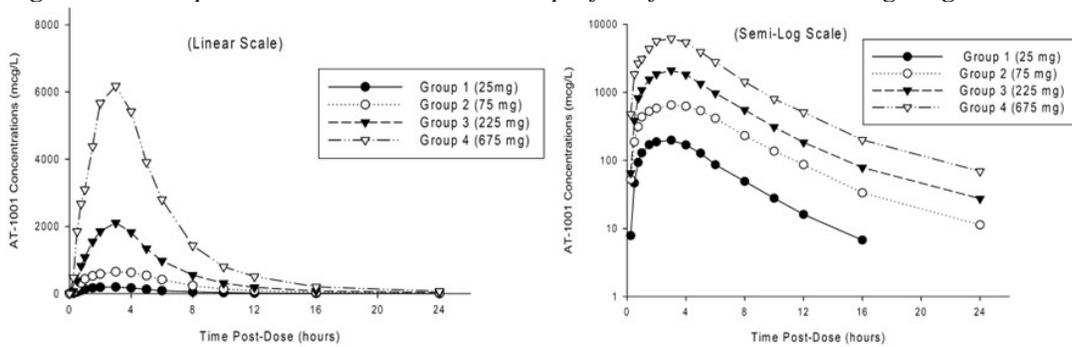
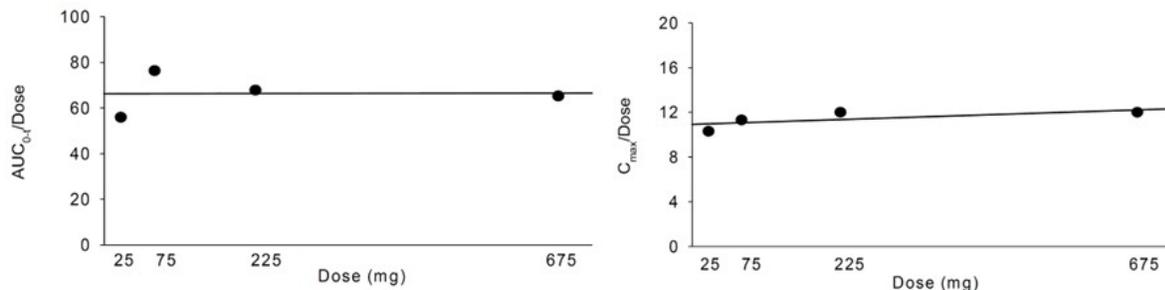


Table 1: Arithmetic Mean (CV%) of Pharmacokinetic Parameters for AT1001 Following Ascending Single Oral Doses

Dose Level (mg)	AUC <sub>0-4</sub> <sup>*</sup> (mcg*h/L) (n=6)	AUC <sub>inf</sub> <sup>*</sup> (mcg*h/L) (n=6)	C <sub>max</sub> <sup>*</sup> (mcg/L) (n=6)	t <sub>max</sub> (h) (n=6)	t <sub>1/2</sub> (h) (n=6)	CL/F (L/h) (n=6)	Varea/F (L) (n=6)
25	1092 (34.2)	1129 (33.6)	201 (35.5)	3.00 (21.1)	3.04 (15.7)	18.95 (34.5)	81.16 (27.2)
75	4661 (9.0)	4730 (8.8)	685 (16.7)	2.92 (41.2)	4.05 (16.4)	13.0 (8.3)	76.47 (22.7)
225	11177 (59.8)	11353 (59.2)	1997 (56.1)	3.17 (23.8)	4.62 (15.4)	18.63 (65.1)	133.09 (83.6)
675	35275 (22.3)	35675 (22.4)	6492 (24.4)	2.67 (30.6)	4.19 (7.3)	15.78 (21.9)	94.27 (15.8)

\*Geometric mean and geometric CV% were used to present the AUC(0-t), AUCinf and Cmax parameters.

Figure 2: AUC/Dose & Cmax/Dose (SD) vs. Dose Following Single oral Dose Administration of AT-1001



Urine Data:

Urine was collected over a 24-hour period following a single oral dose administration and the percentage of dose recovered in the urine as unchanged drug was between 32% to 49%. The mean cumulative amount of AT1001 excreted (Ae0-24) in urine increased from 75 mg to 675 mg.

*Table 2: Arithmetic Mean (CV%) of Cumulative Amounts Excreted and % Excreted for AT1001 in urine*

Dose Level (mg)	Ae <sub>0-24</sub> (mcg)	Cumulative % of Dose Excreted	CL <sub>R</sub> (L/h)
25*	6584 (58.7) n = 2	32.22 (58.7) n = 2	5.90 (1.5) n = 2
75	26378 (31.7) n = 4	43.0 (31.7) n = 4	5.73 (29.9) n = 4
225	90699 (37.2) n = 6	49.32 (37.2) n = 6	7.54 (17.4) n = 6
675	267468 (14.9) n = 6	48.48 (14.9) n = 6	7.66 (20.9) n = 6

\*Subjects on active treatment with all urine collections resulting in BLQ were not included in descriptive statistics due to the limit of quantitation for the urine assay.

**Safety:** According to the sponsor, there was no death or serious adverse event (SAE) in this study. No subject was withdrawn due to AEs. Five subjects (16%) reported 10 treatment-emergent AEs during this study. Two subjects had AEs in Cohort B, and 3 subjects had AEs in Cohort D. No subjects presented with AEs in Cohorts A or C. Nine AEs were mild in severity, 1 was moderate, and none were severe. No trends were observed with respect to increasing dose levels. No clinically relevant abnormality occurred in any vital sign, ECG and physical examination parameter.

**Reviewer's Conclusion:**

- AT1001 appeared to be safe and well tolerated up to 675 mg single dose administration in healthy male subjects.
- C<sub>max</sub> increased in dose-proportional manner between 25 mg to 675 mg. AUC increased in more than dose proportional manner between 25 mg and 75 mg and increased in approximately dose-proportional manner between 75 mg to 675 mg.
- Following a single dose administration, % of dose recovered in the urine as unchanged drug over 24 hour was between 32% to 49%.

## 8.5 MAD Study: FAB-CL-102

**Title:** A Phase I, Randomized, Double Blind, Placebo Controlled, Ascending Multiple Oral Dose Study to Evaluate the Safety, Tolerability and Pharmacokinetics of AT-1001 in Healthy Volunteers

**Note:** Migalastat HCl is referred as AT-1001 in this study report

**Study Date:** 3 December 2004 to 14 Jan 2004

**Clinical Site:** MDS Pharma Services, Neptune, New Jersey

**Analytical Site:** (b) (4)

**Test Product:** AT-1001, Oral solution

### Study Design:

This was a single-center, phase 1, randomized, double-blind, multiple-dose, placebo-controlled, orally administered, ascending dose study to evaluate the safety, tolerability and PK of AT-1001 in healthy subjects. The study evaluated 2 dose levels (50 and 150 mg) of multiple oral dose AT-1001 given twice daily for 7 days. Six subjects were allocated to the active treatment group and 2 subjects to the placebo, in a dose-escalation regimen with a 2-week safety evaluation period (from the first dose) between successive cohorts.

Group	Subjects	Dose Level	Dose	Daily Dose	Total Study Dose
1	6	1	50 mg BID	100 mg	650 mg
	2		Placebo	Placebo	Placebo
2	6	2	150 mg BID	300 mg	1950 mg
	2		placebo	Placebo	Placebo

**Study Population:** 16 healthy male subjects (ages between 18-55) were enrolled and all of them completed the study as designed.

**PK Blood Samples:** Blood samples (10 mL each) were collected before the initial drug administration on Day 1, before the 9th, 11th and 13th doses (AM doses on Days 5, 6 and 7) (for  $C_{min}$  determination), and at the following times after the 1st (Day 1) and 13th (Day 7) doses: 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 9, 10 and 12 hours post-dose.

**Urine Samples:** Urine samples were collected at the following intervals on Days 1 and 7: (-4-0), 0-4, 4-8 and 8-12 hours post-dose.

## RESULTS:

### Pharmacokinetics:

Figure 1: Mean Plasma AT-1001 Concentrations vs. Time - Day 1 in Healthy Subjects

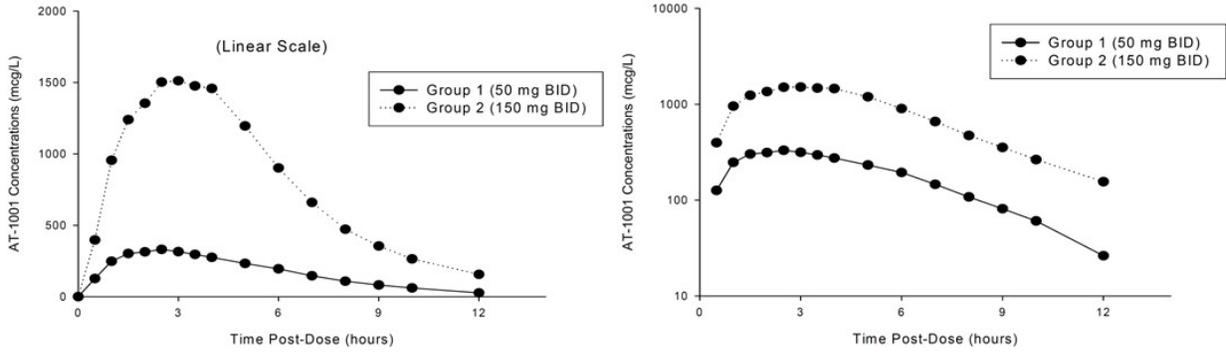


Figure 2: Mean Plasma AT-1001 Concentrations vs. Time - Day 7

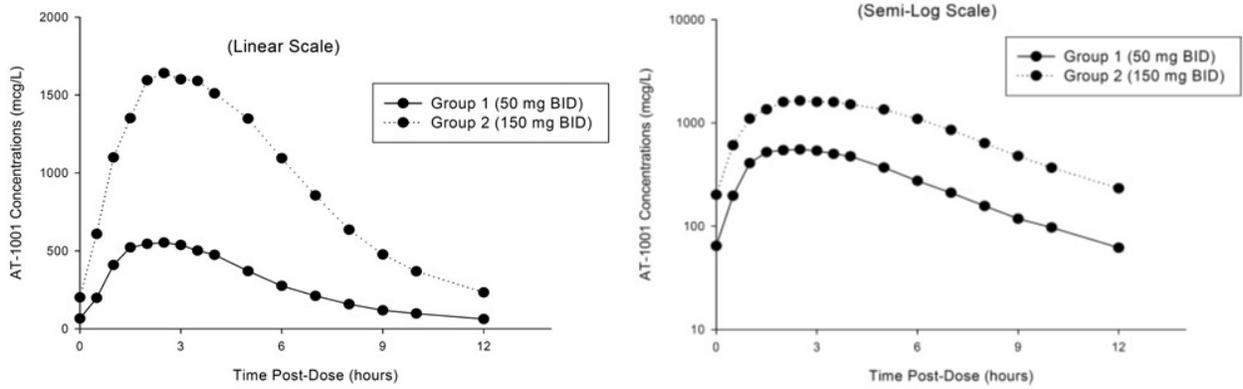


Table 1: Day 1 Mean (CV%) of AT-1001 Plasma PK Parameters

Dose Level	AUC <sub>0-t</sub> (mcg*h/L) (n=6)	AUC <sub>inf</sub> (mcg*h/L) (n=6)	C <sub>max</sub> (mcg/L) (n=6)	t <sub>max</sub> (h) (n=6)	t <sub>1/2</sub> (h) (n=6)	CL/F (L/h) (n=6)	V <sub>area</sub> /F (L) (n=6)
50 mg BID	1897 (48.0)	2017 (47.8)	344 (40.9)	2.92 (36.6)	2.56 (14.6)	22.1 (47.6)	84.6 (57.4)
150 mg BID	8955 (40.5)	9482 (40.2)	1723 (46.6)	3.08 (36.1)	2.44 (5.5)	13.9 (49.9)	48.4 (46.2)

Geometric mean and Geometric CV% was used to present the AUC<sub>0-t</sub>, AUC<sub>inf</sub> and C<sub>max</sub> pharmacokinetic parameters, whereas arithmetic means were used to estimate the other parameters.

Table 2: Day 7 Mean (CV%) of AT-1001 Plasma PK Parameters

Dose Level	AUC <sub>0-t</sub> (mcg*h/L) (n=6)	C <sub>max</sub> (mcg/L) (n=6)	C <sub>min</sub> (mcg/L) (n=6)	t <sub>max</sub> (h) (n=6)
50 mg BID	3259 (25.6)	617 (35.1)	60.5 (22.7)	2.50 (35.8)
150 mg BID	10680 (33.6)	1659 (40.5)	224 (32.1)	2.92 (29.4)

Geometric mean and geometric CV% were used to present the AUC<sub>0-t</sub>, C<sub>max</sub> and C<sub>min</sub> parameters, whereas arithmetic means were used to estimate the other parameters.

*Table 3: Arithmetic Mean (CV%) of Cumulative Amounts Excreted and % Excreted of AT-1001 in Urine*

Day	Dose Level (mg)	Ae <sub>0-12</sub>	Cumulative % Excreted of AT-1001	CL <sub>R</sub> (L/h)
1	50 mg BID*	11697 (46.8) n = 4	28.6 (49.48) n = 4	4.52 (26.6) n = 4
	150 mg BID	72564 (34.2) n = 6	59.19 (34.2) n = 6	7.57 (18.0) n = 6
7	50 mg BID	19241 (33.6) n = 6	47.1 (33.6) n = 6	5.73 (20.1) n = 6
	150 mg BID	82265 (24.6) n = 6	67.1 (24.6) n = 6	7.61 (19.4) n = 6

\*Subjects on active treatment who had no AT-1001 recovered were not included in summary statistics due to the limitations of the assay.

**Accumulation:**

Following 50 mg BID dosing for 7 days, migalastat appears to accumulate in systemic circulation as both systemic exposure and urinary excretion of migalastat on Day 7 were higher than that of Day 1. AUC, C<sub>max</sub> and amount excreted in urine on Day 7 following 50 mg BID dosing were about 1.72-, 1.79- and 1.65-fold higher than that of Day 1, respectively .

However, following 150 mg BID dosing, migalastat does not appear to accumulate much as the systemic exposure (AUC and C<sub>max</sub>) and urinary excretion of migalastat on Day 7 following 150 mg BID dosing were similar to that of Day 1.

**Dose Linearity:**

Following a single dose administration on Day 1, both AUC and C<sub>max</sub> increase in more than dose-proportional manner between 50 mg and 150 mg. Nonetheless, following multiple dose administration (BID) for 7 days, AUC and C<sub>max</sub> increased in a dose-proportional manner between 50 mg and 150 mg doses on Day 7.

*Figure 3: AUC/Dose and C<sub>max</sub>/Dose vs. Dose following a single dose administration of AT-1001(Day 1)*

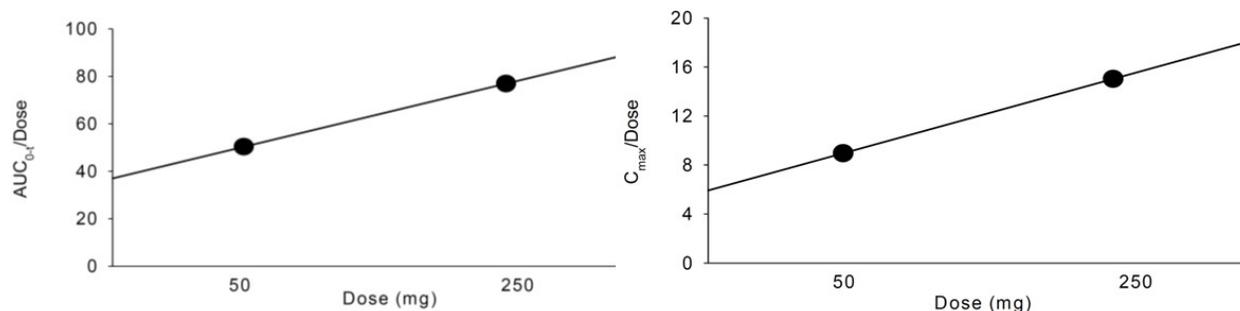
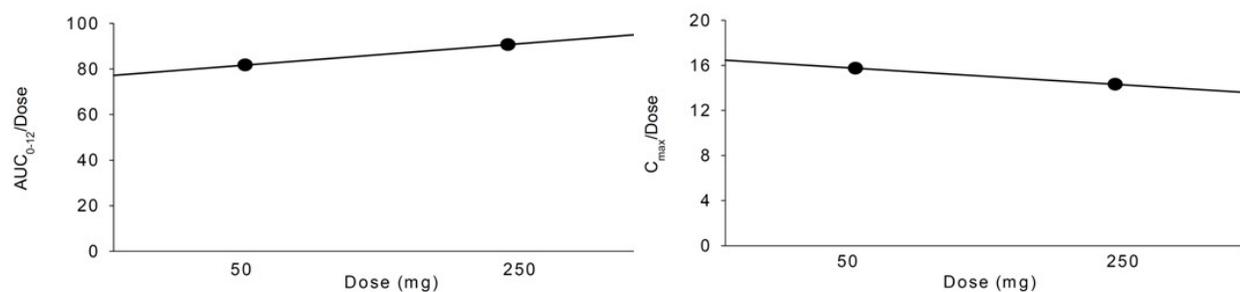


Figure 4: AUC/Dose and C<sub>max</sub>/Dose vs. Dose following multiple dose administration of AT-1001 (Day 7)



**Assessment of Steady State:**

Based on the pre-dose levels on Day 5, 6 and 7, the steady state appears to have reached by Day 5 following BID dosing as there is no specific trend of increasing trough concentration after Day 5 for both dose levels.

Table 4: Geometric Mean Trough Concentration (C<sub>min</sub>) of AT-1001 on Days 5, 6, and 7 following multiple BID dosing

Dose Level	C <sub>min</sub> Pre-Dose (ug/L)		
	Day 5	Day 6	Day 7
50 mg BID	56.568	51.333	63.561
150 mg BID	177.567	158.063	194.905

**SAFETY:**

According to the sponsor, there was no death or serious adverse event (SAE) or discontinuation due to AEs during the study. AT-1001 appeared to be well tolerated in healthy male subjects at doses of 50 mg and 150 mg.

**Reviewer’s Conclusion:**

- Following a single dose administration, the systemic exposure (C<sub>max</sub> and AUC) increased in more than dose-proportional manner between 50 and 150 mg doses on Day 1. However, following multiple BID dosing for 7 days, the systemic exposure (C<sub>max</sub> and AUC) increased in dose proportional manner.
- Steady state for AT1001 following BID dosing seems to have reached by Day 5.
- Following 50 mg BID dosing for 7 days, migalastat appears to accumulate in systemic circulation as both systemic exposure and urinary excretion of migalastat on Day 7 are higher than that of Day 1. However, following 150 mg BID dosing, migalastat does not appear to accumulate significantly as the systemic exposure (AUC and C<sub>max</sub>) and urinary excretion of migalastat on Day 7 following 150 mg BID dosing were similar to that of Day 1.
- The accumulation potential of proposed dosing regimen of 150 mg QOD was not evaluated in this study. Nonetheless, as there was no significant accumulation following 150 mg BID dosing, no major accumulation is anticipated with less frequent dosing of 150 mg QOD.

## 8.6 Oral Solution SAD Study: Study FAB-CL-104

**Title:** A Double-Blind, Placebo-Controlled, Single-Dose Study of the Safety and Tolerability of Oral AT1001 in Healthy Male and Female Subjects

**Note:** Migalastat is referred as AT1001 in this study report

**Study Date:** 21 Dec 2008 – 22 Jan 2009

**Clinical Site:** MDS Pharma Services, Neptune, New Jersey

**Analytical Site:** (b) (4)

**Test Product:** AT1001 Powder for Oral solution

### Study Design:

This was a single-center, randomized, double-blind, placebo-controlled, single-dose, ascending-dose study to evaluate the safety, tolerability, PK and QTcF changes following a single dose of AT1001 in healthy subjects. Oral solution was administered following a 10-hours of overnight fasting.

Dose Level	Dose	Subjects
1	500 mg	N=6
	Placebo	N=2
2	1250 mg	N=6
	Placebo	N=2
3	2000 mg	N=6
	Placebo	N=2

**Study Population:** A total of 24 healthy male and female subjects (ages between 18-50) were enrolled into this study, and all of them completed the study per protocol.

**PK Samples:** PK samples were collected at pre-dose, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10, 12, 16, and 24 hours post-dose to determine concentrations of AT1001 in plasma.

### RESULTS:

#### Pharmacokinetics:

The exposure (both AUC and C<sub>max</sub>) increased in dose proportional manner between 500 mg and 1250 mg doses. However, the exposure appears to plateau after 1250 mg dose as the PK profiles and exposure of 2000 mg dose is very similar to that of 1250 mg dose.

Figure 1: Mean (SD) Plasma Concentration-Time Profiles of Migalastat after a Single Escalating Doses of AT1001 Oral solution

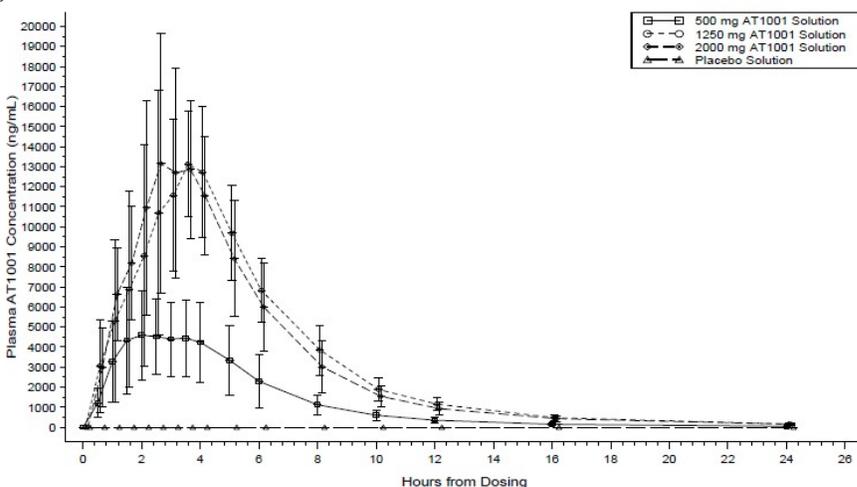


Table 1: Summary Statistics of Pharmacokinetic Parameters Following Single Ascending Doses of AT1001 Oral Solution

Pharmacokinetic Parameters	500 mg (N=6)	1250 mg (N=6)	2000 mg (N=6)
$C_{max}$ (ng/mL)			
Geometric Mean (CV%)	5028 (39%)	14342 (32%)	13844 (42%)
$t_{max}$ (hr)			
Median (min, max)	3.01 (1.50, 5.00)	3.50 (2.50, 4.02)	2.51 (2.50, 3.50)
$AUC_{0-t}$ (ng*hr/mL)			
Geometric Mean (CV%)	27144 (40%)	75108 (29%)	72794 (27%)
$AUC_{0-inf}$ (ng*hr/mL)			
Geometric Mean (CV%)	27428 (40%)	76091 (29%)	73838 (27%)
$k_{el}$ (1/hr)			
Mean ( $\pm$ SD)	0.16 $\pm$ 0.01	0.16 $\pm$ 0.01	0.15 $\pm$ 0.02
$t_{1/2}$ (hr)			
Mean ( $\pm$ SD)	4.29 $\pm$ 0.28	4.28 $\pm$ 0.32	4.79 $\pm$ 0.52
CL/F (L/hr)			
Mean ( $\pm$ SD)	15.8 $\pm$ 5.4	13.9 $\pm$ 4.4	22.9 $\pm$ 6.8

**Safety:** Overall, single oral solution of migalastat HCl in the range of 500 mg to 2000 mg doses were well-tolerated by healthy male and female subjects. There was no death or serious adverse event (SAE) or discontinuation due to AEs in this study. A total of five AEs occurred in four subjects. Of the five AEs reported, one (20%) was moderate in severity while the remaining AEs were mild. All AEs were either unrelated or unlikely related to the study treatments. The number of TEAE did not increase with increasing dose levels. There were no clinically significant findings for clinical laboratory, vital signs, or physical examination during the study.

All mean ECG results were within normal limits, and changes from baseline were minor. The cardiodynamic ECG analysis of this study showed no statistically significant or clinically meaningful QTc at the studied doses of AT1001 (500 mg to 2000 mg).

## 8.7 Mass Balance Study AT1001 – 014

**Title:** A Phase 1 Study To Investigate The Absorption, Metabolism And Excretion of [<sup>14</sup>C] AT1001 (Migalastat Hydrochloride) Following a Single Oral Administration in Healthy Volunteers

**Clinical Site:** PRA Clinical Research Unit, Groningen, The Netherlands

**Bioanalytical site:** (b) (4): migalastat concentration in plasma & urine  
(b) (4): total [<sup>14</sup>C]-radioactivity in plasma and blood using an AMS method  
(b) (4): total [<sup>14</sup>C]-radioactivity in urine and feces using LSC  
(b) (4): [<sup>14</sup>C]-radioactivity in expired air samples by AMS counting

**Study Date:** 08 August 2011 – 30 September 2011

**Test product:** Single 150 mg oral dose of [<sup>14</sup>C]-labeled migalastat HCl as aqueous solution containing 37 kBq (1 μCi) <sup>14</sup>C

**Study Design:** This study was phase 1, an open-label, single-center, single-dose, mass balance study in 6 healthy male subjects to evaluate the absorption, distribution, metabolism and excretion of a single oral dose of 150 mg oral dose of 1 μCi [<sup>14</sup>C] migalastat HCl. The drug was administered with 240 mL of water following an overnight fasting of approximately 10 hours and no food was allowed for additional 5.5 hours post-dose.

**Study Population:** This study had 6 healthy male volunteers (ages between 30-55 with good health with a BMI between 18-30 kg/m<sup>2</sup>) enrolled and all of them completed the study as planned and were included in PK and safety analysis.

### Pharmacokinetic Measurements:

- **Blood Samples:**

*Blood samples for plasma migalastat PK and plasma radioactivity assessments:*

Blood samples (10 mL) for the analysis of migalastat and total radioactivity were collected at predose and at 30 minutes, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 24, 36, 48, 72, 96, 120, 144, 168, 192, 216, and 240 hours post-dose. Additional samples (5 mL) were collected at screening and Day -1 for the measurement of total radioactivity.

*Blood samples for blood radioactivity assessment and plasma metabolite profiling:*

Blood samples (30 mL) for measurement of radioactivity in blood and for derivation of plasma for migalastat metabolite elucidation and identification were collected at predose, and at 2, 4, 6, 24, and 48 hours post-dose.

- **Urine samples:** Urine samples were collected to determine the concentration of migalastat, for analysis of total radioactivity and for metabolic profiling at the following time points: predose (a blank sample was collected within 12 hours prior to dosing), between 0 to 12 and 12 to 24 hours on Day 1, and thereafter at 24-hour intervals on Days 2-10; the last sample was collected before release from the clinical unit on Day 11.
- **Fecal samples:** Feces samples for radioactivity counting and possible metabolite elucidation and identification were collected at predose (-24 to 0 hours) and from all bowel movements post dose on Days 1 to 10; the last sample was collected before release from the clinical unit on Day 11.

- Expired Air Sampling: Expired air samples were collected predose, and at 2, 4, 6, and 24 hours after dosing.
- Bile Sampling: Duodenal bile samples for total radioactivity and analysis of metabolites were collected via an Entero-Test capsule. A predose sample was collected following ingestion of the capsule at least 2 hours after the final meal on Day -1 and removed prior to dosing on Day 1. A post dose sample was collected using an Entero-Test capsule ingested approximately 2 hours post dose and removed at approximately 6.5 hours post dose on Day 1.  
A food stimulus was given at 5.5 hours post dose (i.e., one hour prior to string withdrawal) on Day 1 to encourage the release of bile onto the string.

#### PK Parameters Analysis:

Plasma concentrations of migalastat were analyzed by non-compartmental analysis.

PK parameters determined from plasma concentrations:

$AUC_{0-last}$ ,  $AUC_{0-inf}$ ,  $C_{max}$ ,  $t_{max}$ ,  $t_{last}$ ,  $t_{1/2}$ ,  $K_{el}$ ,  $CL/F$ ,  $Vz/F$ , ratio of migalastat  $^{14}C$  total radioactivity in plasma, calculated as  $AUC_{0-inf, migalastat} / AUC_{0-inf, total radioactivity}$

The ratio of plasma to blood radioactivity was calculated at 2, 4, 6, 24, and 48 hours after administration of migalastat HCl

PK parameters determined from total radioactivity in urine:

$A_{eu_t}$	Amount of radioactivity excreted in urine expressed as percent of dose, calculated from the sum of the products of the analyte concentrations in urine and the urine volumes for all collection intervals
$CL_R$	Renal clearance, defined as $CL_R = A_{eu} / AUC_{0-inf}$
%Excreted	Percent of the migalastat dose excreted unchanged and percent of radioactivity excreted in urine, calculated as: $\%Excreted = 100 * A_{eu} / Dose$
Excretion rate	Amount excreted in a urine sample divided by the duration of the collection of the urine collection interval
$\%Excreted_{last-inf}$	Percent of the migalastat dose remaining to be excreted, estimated by extrapolation of the area under the excretion rate-time curve to infinity

PK parameters determined from total radioactivity in feces:

$A_{ef}$	Amount of radioactivity excreted in feces expressed as percent of dose, calculated from the sum of the products of the radioactive concentrations in feces and the feces weight for all collection intervals
%Excreted	Percentage of the radioactive dose excreted in feces, calculated as $\%Excreted = 100 * A_{ef} / Dose$
Excretion rate	Amount excreted in a feces sample divided by the duration of the collection of the feces collection interval
$\%Excreted_{last-inf}$	Percent of the migalastat dose remaining to be excreted, estimated by extrapolation of the area under the excretion rate-time curve to infinity

Total Radioactivity Recovery:

The lower limit of quantitation (LLOQ) for  $^{14}C$ -radioactivity was 4.44 ng eq/mL in plasma and 11.6 ng eq/mL in whole blood.

$A_{et}$	Amount of radioactivity excreted in feces, urine, and expired air (if applicable) for all collection intervals
%Excreted	Percentage of the radioactive dose excreted in feces, urine, and expired air (if applicable) calculated as $\%Excreted = 100 * A_{et} / Dose$

### Metabolic Profiling:

Plasma, urine, bile and feces samples from six healthy male subjects who were administered 150 mg AT1001 were used for metabolite identification and profiling.

- *Plasma*: A plasma pool was prepared for each subject from samples taken at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 16 and 24 hours post-dose by combining the time point samples in proportion to the time interval between individual samples. The six pools from individual subjects were then used to produce a single (0 to 24 hour) human plasma pool for metabolite identification.
- *Urine*: A pooled urine sample was prepared from the 0-12 hour samples across all six subjects (which contained > 85% of the total drug related material excreted by that route). A proportion (0.025% by total volume excreted) of each 0-12 hour sample was combined to make one sample.
- *Feces*: Aliquots of fecal homogenates from each subject (containing  $\geq 3.7\%$  of the administered dose) were extracted. For subjects 1 to 4 these samples were from the 0 to 24 hour collection and for subjects 5 and 6 were from the 24 to 48 hour collection

Radio-metabolite profiles were determined by analysis of appropriate aliquots of urine, extracts of plasma, and fecal homogenates using HPLC. Representative plasma and fecal extracts and urine were analyzed by UPLC-MS<sup>n</sup>.

### Results:

- Plasma migalastat was detectable in all 6 subjects up to 24 hours (Day 2) after dosing, was detectable in 5 out of 6 subject at 36 hours post-dose and was detectable in only 1 out of 6 subjects at 48 hours post-dose (LLOQ = 5.88 ng/mL).
- Plasma [<sup>14</sup>C]-radioactivity was detectable in all subjects up to 24 hours (Day 2) after dosing, was detectable in 5 out of 6 subject at 36 hours post dose and was detectable in only 1 subject after 72 hours post dose (LLOQ = 4.4 ng eq/mL).
- At all-time points, plasma [<sup>14</sup>C]-concentrations were higher than plasma migalastat concentrations. Additionally, plasma migalastat AUC only accounts for approximately 58% of plasma total [<sup>14</sup>C]-radioactivity AUC suggesting the presence of a migalastat metabolite(s) in the plasma accounting for the rest of the [<sup>14</sup>C]-radioactivity.

Figure 1: Arithmetic Mean Concentration-Time Profiles ( $\pm$  SD) for Migalastat in Plasma and Radioactivity in Plasma and Whole Blood

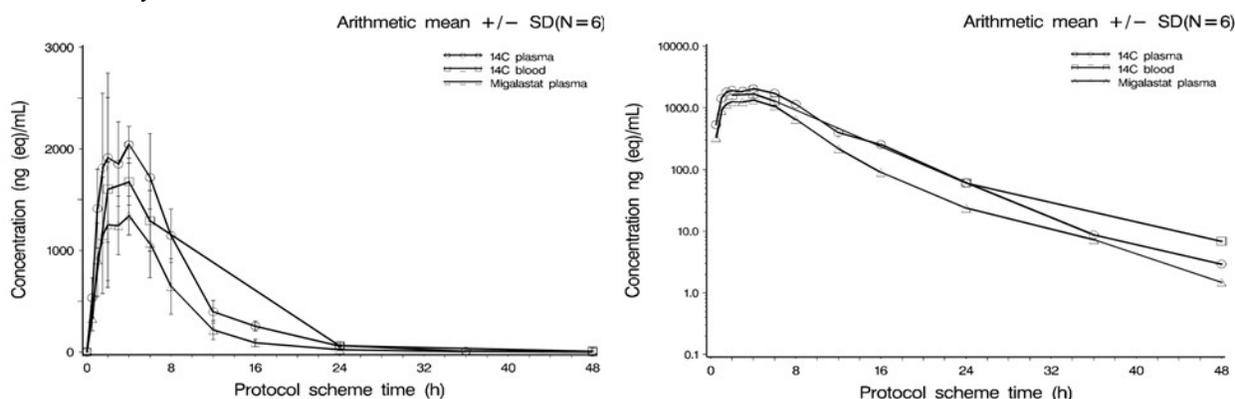


Table 1: Summary Statistics of PK Parameters of Radioactivity and Migalastat in Plasma (N=6)

Analyte	C <sub>max</sub> <sup>a</sup> (ng (eq)/mL)*	t <sub>max</sub> <sup>b</sup> (h)	AUC <sub>0-last</sub> <sup>a</sup> (ng (eq).h/mL)*	AUC <sub>0-inf</sub> <sup>a</sup> (ng (eq).h/mL)*	t <sub>1/2</sub> <sup>c</sup> (h)
Radioactivity [ <sup>14</sup> C]	2246 (27)	4.00 (2.00-6.00)	18718 (14)	18849 (14)	7.68 (6.90)
Plasma Migalastat	1516 (27)	4.00 (2.00-6.00)	10957 (22)	11029 (22)	6.34 (2.50)

<sup>a</sup> Geometric mean (CV%)

<sup>b</sup> Median (range)

<sup>c</sup> Arithmetic mean (SD)

Geometric mean [<sup>14</sup>C]-blood/[<sup>14</sup>C]-plasma ratios were relatively constant between 2 and 6 hours post-dose (ranging between 0.76 and 0.82) and further increased to 1.12 at 24 hours post-dose. The data suggest that [<sup>14</sup>C]-radioactivity equilibrated slowly between plasma and red blood cells and may have reached equilibrium by 24 hours post-dose with some preferential association of [<sup>14</sup>C]-radioactivity with red blood cells. [<sup>14</sup>C]-blood was below LLOQ in 5 out of 6 subject at 48 hours post-dose (LLOQ = 11.6 ng eq/mL).

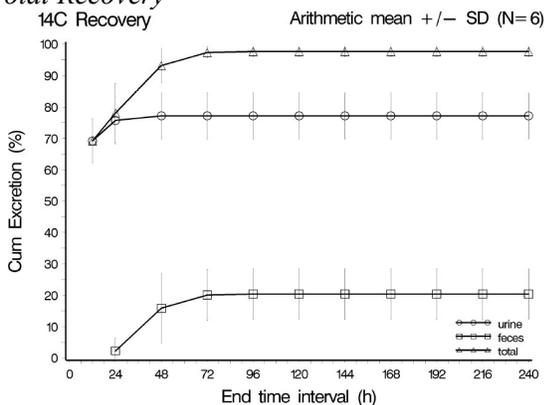
Following an oral administration of 150 mg [<sup>14</sup>C] migalastat, 77% of the [<sup>14</sup>C]-radioactivity was recovered in urine and 20.4% was recovered in feces with overall total recovery of 97.6% within 96 hours post-dose. No [<sup>14</sup>C]-radioactivity was detected in expired air samples. Of 77.2% of [<sup>14</sup>C]-radioactivity recovered in urine, 64.5% is unchanged migalastat suggesting that 12.7% difference is probably accounted by migalastat metabolite(s).

Table 2: Summary Statistics of [<sup>14</sup>C]-Radioactivity and Migalastat Excretion Parameters (N=6)

Radioactivity [ <sup>14</sup> C]	Ae <sub>urine</sub> (%)	Ae <sub>feces</sub> (%)	Ae <sub>total</sub> (%)
Arithmetic mean (range)	77.2 (67.1 - 85.8)	20.4 (11.9 - 33.5)	97.6 (95.7 - 100.6)
Urine Migalastat	Ae <sub>urine</sub> (%)	Ae <sub>urine</sub> (mg)	CL <sub>R</sub> (L/hr)
Arithmetic mean (range)	64.5 (57.0 - 70.4)	79.1 (69.9 - 86.3)	7.32 (4.49 - 9.09)

N = number; Ae<sub>urine</sub> = amount of radioactivity or migalastat excreted in urine, Ae<sub>feces</sub> = amount of radioactivity excreted in feces, Ae<sub>total</sub> = total amount of radioactivity excreted in urine and feces, CL<sub>R</sub> = renal clearance

Figure 2: Arithmetic Mean Profile (± SD) of Cumulative Radioactivity Excretion in Urine and Feces and Total Recovery

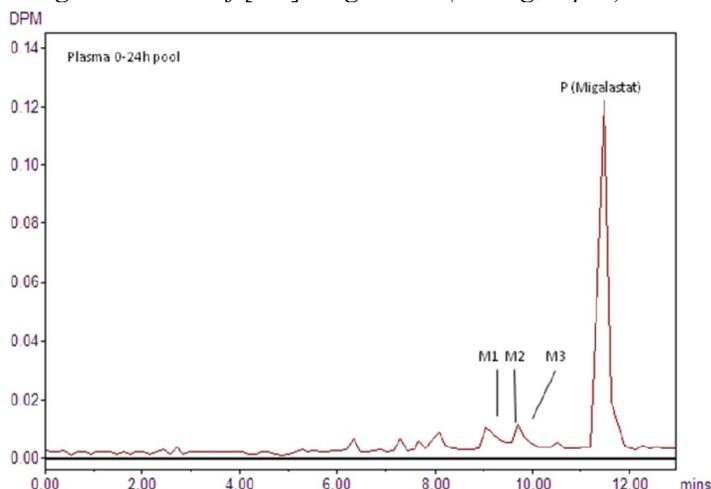


### Metabolic Profiling:

*In-vitro:* AT1001 was stable in human cryopreserved hepatocytes after 4 hours of incubation. However, hepatocytes that were employed in that in-vitro study were not adequately characterized in respect to all possible phase I and phase II enzymes prior to the study. In addition, this study did not have adequate positive control. The sponsor only had included naloxone as a metabolic positive control. However, it is not clear what metabolic capacity or specific enzyme that naloxone was used as positive control for. According to the Naloxone label, Naloxone is metabolized primarily by glucuronide conjugation. Therefore, human cryopreserved hepatocytes were not adequately validated prior to the use with respect to phase I and II drug metabolizing enzymes and did not have proper positive controls during the study making it difficult to interpret the result of that in-vitro metabolism study in hepatocyte.

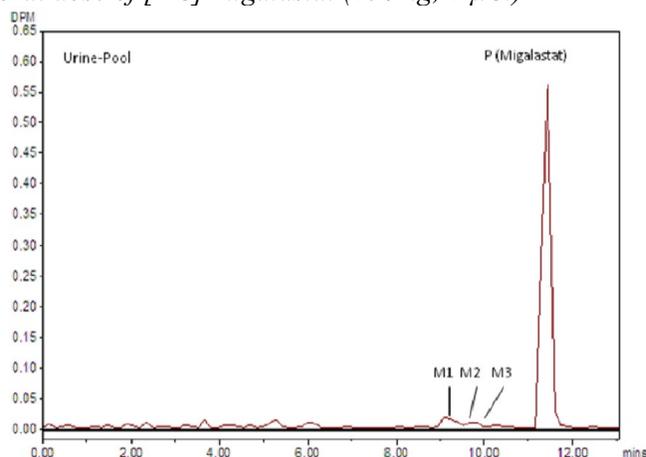
*Plasma:* Chromatographic analysis of plasma extracts indicated the major circulating component in human plasma was migalastat accounting for approximately 77% of the plasma radioactivity. Dehydrogenated O-glucuronide conjugated metabolites (M1, M2 and M3) accounted for 5%, 2% and 6% of the plasma radioactivity. Approximately 9% of the total sample radioactivity was unassigned.

*Figure 3: Reconstructed AMS radiochromatogram of pooled human plasma extract (0 to 24h), following a single oral dose of [<sup>14</sup>C] Migalastat (150mg; 1 μCi)*



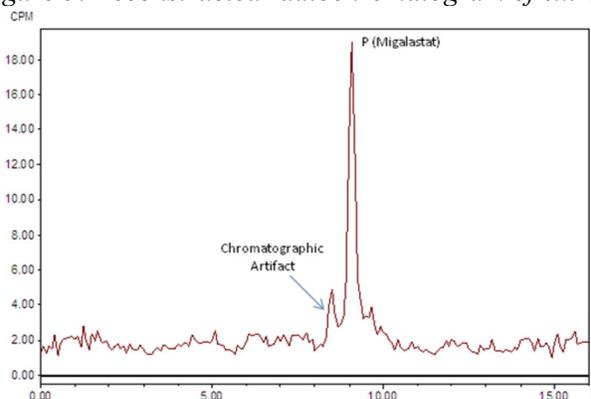
*Urine:* Approximately 77% of the total administered dose was excreted in the urine. In a pooled sample extract, migalastat and M1, M2 and M3 were observed to account for approximately 80%, 3%, 1% and 2% of the sample radioactivity which equated to approximately 62% and 4% (combined metabolites) of the administered dose, respectively. Approximately 14 % of the total sample radioactivity urine was unassigned components.

Figure 4: Reconstructed AMS radiochromatogram of pooled human urine (0 to 12h) following a single oral dose of [<sup>14</sup>C] migalastat (150mg; 1 μCi)



**Feces:** Approximately 20% of the administered dose was excreted via the feces. Unchanged migalastat was the only drug related component observed in feces extracts.

Figure 5: Reconstructed radiochromatogram of extracted human feces, from subject 6



Note 1: Retention time of parent peak in the above chromatogram is earlier due to the chromatogram being obtained from a different HPLC system which has less dead volume.

**Bile:** Duodenal bile contained insufficient levels of radioactivity to warrant further investigation,

### Safety:

A total of 6 TEAEs were reported by 4 of the 6 subjects in the study. All 6 TEAEs were of mild intensity. Three of the TEAEs (headache and diarrhea reported by Subject 1, and headache reported by Subject 4) were reported as possibly related to the study medication. The relationship for the other TEAEs was recorded as unrelated or unlikely related. There were no deaths or other SAEs during the study. None of the subjects discontinued due to AEs. All TEAEs were transient and had recovered at the end of the study. There were no clinical significant findings with respect to clinical laboratory, ECGs, vital signs, physical examination, or body weight measurements.

**Reviewer's Comment:**

- Following oral administration of 150 mg [<sup>14</sup>C] migalastat HCl, 77.2% of the [<sup>14</sup>C]-radioactivity was recovered in urine and 20.4% was recovered in feces with overall total recovery of 97.6% within 96 hours post-dose. No [<sup>14</sup>C]-radioactivity was detected in expired air samples.
- Total [<sup>14</sup>C] radioactivity and unchanged migalastat in plasma and urine suggest the evidence of migalastat metabolism.
  - At all-time points, plasma [<sup>14</sup>C]-concentrations were higher than plasma migalastat concentrations. Plasma migalastat AUC only accounts approximately 58% of total [<sup>14</sup>C] radioactivity AUC suggesting the presence of migalastat metabolite(s) in the plasma accounting for rest of the total [<sup>14</sup>C] radioactivity AUC.
  - Furthermore, of 77.2% of [<sup>14</sup>C] radioactivity recovered in urine, 64.5% is the unchanged migalastat suggesting that 12.7% difference in [<sup>14</sup>C] radioactivity is probably accounted by migalastat metabolites.
- AT1001 was stable in human cryopreserved hepatocytes after 4 hours of incubation. However, human cryopreserved hepatocytes were not adequately validated prior to the use with respect to phase I and II drug metabolizing enzymes and did not have proper positive controls during the study making it difficult to interpret the result of that in-vitro metabolism study in hepatocyte.

## 8.8 SAD Study in Japanese Subjects: MGM115806:

**Title:** A Phase I, Randomized, Single-Blind, Four-Period Cross-Over, Placebo-Controlled, Dose-Escalation Study to Evaluate the Safety and Pharmacokinetics of Single Oral Doses of GR181413A/AT1001 in Healthy Japanese Subjects

**Note:** Migalastat is referred as GR181413A/ AT1001 in this study report

**Study Date:** 30 Sept 2011 to 19 Dec 2011

**Clinical Site:** GlaxoSmithKline Medicines Research Unit, Australia

**Analytical Site:** (b) (4)

**Test Product:** 50 mg as an oral solution in water (10 mg/mL)  
150 mg and 450 mg doses as 1 and 3 hard gelatin capsules

### Study Design:

This study was a single-center, phase I, randomized, single-blind, single-dose, cross-over, placebo-controlled, ascending dose study to evaluate the safety, tolerability and PK of GR181413A/AT1001 in healthy Japanese subjects. Each subject received 4 dosing on separate periods, three of four treatments of GR181413A/AT1001 50 (D1 or X1), 150 (D2 or X2), and 450 mg (D3) and the matching placebo (P1, P2 or P3) under fasting condition. Each treatment period was separated by at least 7 days.

Sequence	n	Period 1	Period 2	Period 3	Period 4
A	3	D1	D2	D3	P3
B	3	D1	D2	P3	D3
C	3	D1	P2	X2	D3
D	3	P1	X1	X2	D3

**Study Population:** A total of 14 healthy male and female Japanese subjects (ages between 20-55) were enrolled into this study, and 13 of them completed the study per protocol. One subject withdrew consent at Period 2.

**PK Blood Samples:** PK Blood samples were collected at pre-dose, and at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10, 12, 16 and 24 hours post-dose.

**Urine Samples:** Urine samples were pooled for the entire collection interval at pre-dose (-4-0), 0-, 4-8, 8-12 and 12-24 hours post-dose.

## RESULTS:

### Pharmacokinetics:

C<sub>max</sub> and AUC increased in a dose-proportional manner between 50 mg to 450 mg in healthy Japanese subjects.

Figure 1: Mean plasma concentration vs. time profile of AT-1001 Following Single Dose Administration

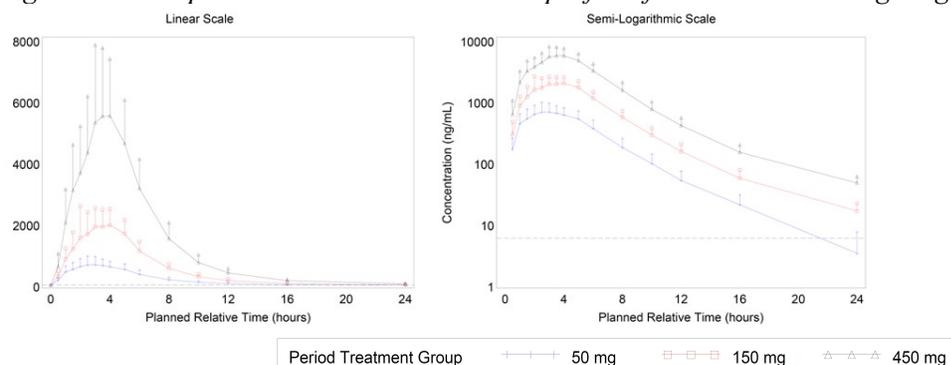


Table 1: Summary of Plasma AT1001 PK Parameters Following a Single Dose Administration <sup>a</sup>

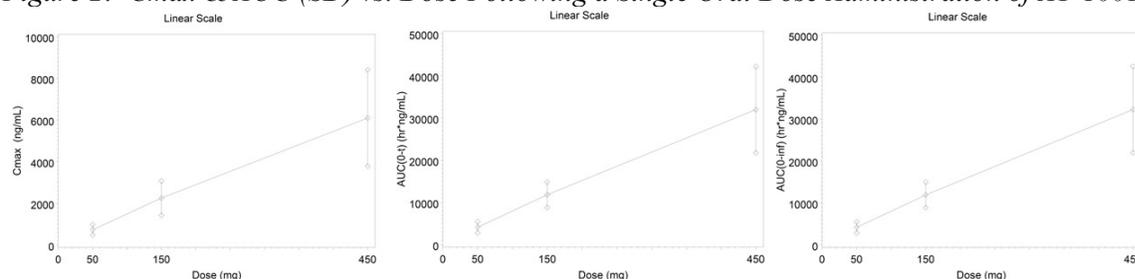
Dose	N	C <sub>max</sub> (ng/mL)	T <sub>max</sub> <sup>b</sup> (hr)	AUC(0-t) (hr*ng/mL)	AUC(0-∞) (hr*ng/mL)	t <sub>1/2</sub> (hr)
50 mg	14	695.1 (36.0)	3.00 (1.5-5.0)	3905.4 (35.0)	3961.5 (34.6)	3.176 (22.5)
150 mg	13	2124.3 (36.3)	3.50 (2.0-5.0)	11430.7 (27.4)	11519.4 (27.4)	3.816 (6.6)
450 mg	13	5694.9 (40.4)	3.50 (2.5-5.0)	30453.9 (34.2)	30721.7 (34.1)	4.009 (5.6)

Source: Table 3.02, Table 3.03

a. Geometric mean (%CV<sub>b</sub>)

b. Median (range)

Figure 2: C<sub>max</sub> & AUC (SD) vs. Dose Following a Single Oral Dose Administration of AT-1001



### Urine Data:

Urine was collected over a 24-hour period following a single oral dose administration, and the percentage of dose recovered in the urine as unchanged drug was between 45% to 50%. The mean cumulative amount of AT1001 excreted (Ae<sub>0-24</sub>) in urine increased in approximately dose proportional manner from 50 mg to 450 mg.

Table 2: Geometric Mean (%CV<sub>b</sub>) of Urine AT1001 PK Parameters Following a Single Dose Administration

Dose	N	%F <sub>x</sub> (0-24)(%)	Ae(0-24) (mg)	CL <sub>r</sub> (L/hr)
50 mg	14	49.67 (24.6)	24.84 (24.6)	6.303 (20.6)
150 mg	13	46.65 (22.9)	69.97 (22.9)	6.121 (17.8)
450 mg	13	44.54 (20.1)	200.43 (20.1)	6.008 (16.0)

**Safety:** According to the sponsor, there was no death or serious adverse event (SAE) in this study. No subject was withdrawn due to AEs. Nine subjects (64%) reported a total of 25 AEs from period 1 to period 4 and the most frequent AE, irrespective of causality, was headache. All AEs reported were considered mild in severity. No trends were observed with respect to increasing dose levels. No clinically relevant abnormality occurred in any vital sign, ECG, clinical laboratories and physical examination parameter.

## 8.9 DDI Study with Agalsidase: AT1001-013:

**Title:** An open-label Phase 2A study to investigate drug-drug interactions between AT1001 (migalastat hydrochloride) and agalsidase in subjects with Fabry disease

**Note:** Migalastat may also referred as AT1001 in this study report

**Study Date:** 02 Feb 2011 – 09 Oct 2012

**Test Products:** Migalastat HCl capsule at 150 mg and 450 mg  
Agalsidase alfa, Replagal® IV infusion at 0.2 mg/kg  
Agalsidase beta (Fabrazyme®) IV infusion at 0.5 mg/kg and 1.0 mg/kg

### Study Design:

This was an open-label, single dose, non-randomized study consisted of 2 stages to assess the drug-drug interaction between migalastat and agalsidase. Two dose levels of migalastat HCl (150 mg and 450 mg) were selected to evaluate interaction with each of the 3 doses of recombinant agalsidase: 0.2 mg/kg agalsidase alfa (Replagal®, Shire Human Genetic Therapies), 0.5 mg/kg agalsidase beta (Fabrazyme®, Genzyme Corporation), or 1.0 mg/kg agalsidase beta (Fabrazyme).

- Stage 1 of the study consisted of a 3 treatment periods to evaluate the effect of 150 mg migalastat HCl on the PK and safety of agalsidase (0.2 mg/kg agalsidase alfa, 0.5 mg/kg agalsidase beta, or 1.0 mg/kg agalsidase beta), and the effect of agalsidase on the PK and safety of 150 mg migalastat.
- Stage 2 consisted of 2 treatment periods to evaluate the effect of 450 mg migalastat HCl on the PK and safety of agalsidase (0.2 mg/kg agalsidase alfa, 0.5 mg/kg agalsidase beta, or 1.0 mg/kg agalsidase beta). In Stage 2, the effect of agalsidase on the PK and safety of 450 mg migalastat was not evaluated.

	Stage 1 (n=12)	Stage 2 (n=13)
Period 1	IV infusion of 0.2 mg/kg agalsidase alfa (n=4), or 0.5 mg/kg agalsidase beta (n=5) or 1.0 mg/kg agalsidase beta (n=3)	IV infusion of 0.2 mg/kg agalsidase alfa (n=4), or 0.5 mg/kg agalsidase beta (n=2) or 1.0 mg/kg agalsidase beta (n=7)
Period 2	A single 150 mg oral dose (1 capsule) of migalastat HCl 2 hours before initiation of an intravenous (IV) infusion of 0.2 mg/kg agalsidase alfa or 0.5 mg/kg or 1.0 mg/kg agalsidase beta	A single 450 mg oral dose (3 × 150-mg capsules) of migalastat HCl 2 hours before initiation of an IV infusion of 0.2 mg/kg agalsidase alfa or 0.5 mg/kg or 1.0 mg/kg agalsidase beta
Period 3	A single 150 mg oral dose (1 capsule) of migalastat HCl	---

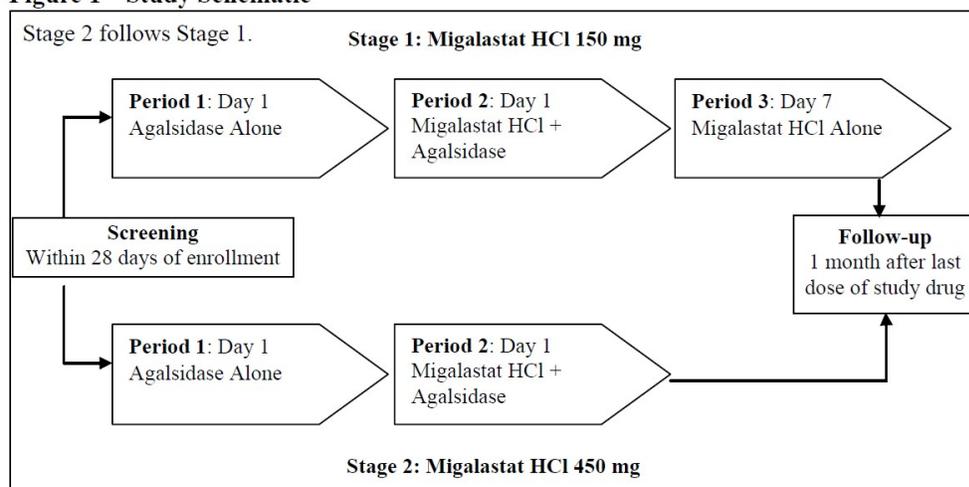
Agalsidase alfa was administered as a 40-minute infusion, and agalsidase beta was administered as a 2-hour infusion. Subjects fasted for at least 2 hours before and 2 hours after migalastat HCl administration.

### Reviewer's Comments:

*While Fabrazyme (agalsidase beta) is approved in USA for use in patients with Fabry disease at recommended dose of 1 mg/kg given every two week as an IV infusion, Replagal (agalsidase alfa) is not approved in USA, but approved in EU for Fabry patients at recommended dose of 0.2 mg/kg administered every 2 week as IV infusion over 40 minutes. Therefore, the doses used in this study to assess the drug-drug interaction were appropriate.*

*This study was not designed to show the PD effect as it was a single dose study and one sequence treatment (migalastat with second dose of Fabrazyme).*

**Figure 1 Study Schematic**



**Table 1: Schedule of Assessments (Stages 1 and 2)**

Activity	Screening <sup>a</sup>	Period 1 (Stages 1 and 2)					Period 2 (Stages 1 and 2)					Period 3 (Stage 1)				Follow-up <sup>b</sup>	
		Day					Day					Day					
		-1	1	2	7	14	-1 <sup>c</sup>	1	2	7	14 <sup>c</sup>	1	6	7	8		
eGFR	X																X
Urine GL-3/ Lyso-Gb <sub>3</sub> <sup>e</sup>		X	X			X	X	X			X						X
Plasma Lyso-Gb <sub>3</sub>			X		X	X	X		X	X	X						
Check-in to the Clinic		X					X						X				
Agalsidase Dose			X <sup>f</sup>				X <sup>f</sup>					X <sup>f</sup>					
Migalastat HCl Dose							X						X				
PK/PD Blood Sampling <sup>g</sup>			X	X	X	X	X	X	X	X			X	X			
Skin Biopsy <sup>h</sup>		X		X	X			X	X								
WBC Collection <sup>i</sup>			X	X	X	X	X	X	X	X							X
Antibody Titer <sup>g</sup>			X				X										
Adverse Events			←-----X-----→				←-----X-----→				←-----X-----→						X

<sup>c</sup> For subjects on a bi-weekly infusion schedule, Day -1/Period 2 may have overlapped with Day 14/Period 1.

Day 14/Period 2 may have overlapped with Day 1/Period 3. All procedures listed were performed once and transcribed on the applicable CRF page.

<sup>d</sup> Vital signs included temperature, blood pressure, heart rate, and respiration.

<sup>e</sup> First morning void was collected for urine GL-3 and lyso-Gb<sub>3</sub> determination.

<sup>f</sup> Outpatient administration of agalsidase alfa or agalsidase beta. In Period 2, each subject received the identical dose and infusion rate of agalsidase as administered in Period 1.

<sup>g</sup> Sample collection times and analytes are summarized in the protocol (see Appendix 16.1.1).

<sup>h</sup> If sufficient sample was available, skin GL-3 was also measured.

<sup>i</sup> This assessment took place at 2, 4, and 24 hours after infusion.

**Study Population:** Male Fabry patients (ages between 18-65) who had been receiving a stable dose of agalsidase beta (0.3-1.0 mg/kg) or of agalsidase alfa ( $\geq 0.2$  mg/kg) for at least 1 month before study entry and had received at least 2 infusions before the screening visit were enrolled in the study. 23 patients were enrolled into this study and all of the enrolled 23 patients completed the study per protocol. Of 23 subjects, 3 subjects were enrolled into both stage 1 and stage 2.

**PD samples:**

Urine GL-3, urine lyso-Gb3, plasma lyso-Gb3, skin  $\alpha$ -Gal A activity, white blood cell (WBC)  $\alpha$ -Gal A activity

Agalsidase plasma PK parameter values were measured by active  $\alpha$ -Gal A levels ( $\alpha$ -Gal A enzyme activity) and total  $\alpha$ -Gal A protein levels after agalsidase infusion alone and in combination with oral migalastat HCl

**PK Samples Collection Time:**

Blood for plasma  $\alpha$ -Gal-A Activity and Western Blot: Period 1 and period 2, Stage 1 and 2:  
FABRAZYME® (agalsidase beta): Pre-dose, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 12, 24 hr, 7 day, and 14 days following IV infusion

REPLAGAL™ (agalsidase alfa): re-infusion, 0.33 (20 min), 0.66 (40 min), 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 12, 24 hr, 7 day, and 14 days following IV infusion

**Plasma AT1001:**

Period 2, Stage 1 and 2: Pre-dose, -1, 1, 2, 3, 4, 5, 6, 8, 10, 24 hr after oral dose administration

Period 3, Stage 1: Pre-dose, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 24 hr after oral dose administration

**WBC  $\alpha$ -Gal-A Activity:**

Period 1 and 2 and Stage 1 and 2: Pre-dose, 2, 4, 24 hr 7day, 14 days following IV infusion.

**Plasma Lyso-GB3:**

Period 1 and 2, Stage 1 and 2: Pre-dose, 7 and 14 days post-dose.

**Bioanalytical:**

*$\alpha$ -Gal A Activity and Total Protein in Plasma:* The  $\alpha$ -Gal A activity level in plasma was determined by a qualified assay that measured the rate of enzyme activity using an artificial, fluorescent substrate, 4-methylumbelliferone glucuronide (4-MUG). The total  $\alpha$ -Gal A protein level was measured by Western blot using anti-human Gal A antibody.

*Migalastat Plasma Pharmacokinetics:* Concentrations of migalastat were measured in plasma using a validated liquid chromatography - tandem mass spectrometry (LC-MS/MS) assay.

*Skin  $\alpha$ -Gal A Enzyme Activity:* Skin  $\alpha$ -Gal A enzyme activity was examined in skin biopsy samples. The  $\alpha$ -Gal A activity in skin was determined by a qualified assay that measured the rate of enzyme activity using an artificial, fluorescent substrate, 4-MUG. The  $\alpha$ -Gal A protein level was measured by Western blotting using anti-human Gal A antibody

*Urinary GL-3:* Urinary GL-3 was measured using a validated assay and was expressed as a function of urinary creatinine concentration (ng/mg creatinine).

*WBC  $\alpha$ -Gal A Activity:* The WBC  $\alpha$ -Gal A activity level was determined by a qualified assay measuring enzyme activity using 4-MUG. The WBC  $\alpha$ -Gal A protein level was measured by Western blotting using anti-human Gal A antibody.

*Plasma and Urinary Lyso-Gb3*: Plasma and urinary concentrations of lyso-Gb3 were measured with qualified liquid chromatography – mass spectrometry (LC-MS) assay.

*Antibody Titer*: Total antibody titers (IgG) were measured using a qualified research-grade enzyme-linked immunosorbent assay (ELISA).

## RESULTS:

### PK:

#### Plasma $\alpha$ -Gal A Enzyme Activity

- Co-administration of migalastat with agalsidase increased the levels of active  $\alpha$ -Gal A AUC<sub>infinity</sub> by 2.0 - to 4.15-fold relative to agalsidase (alfa or beta) administered alone.
- For the US, approved product agalsidase beta (Fabrazyme), the relative increase in active  $\alpha$ -Gal A AUC<sub>infinity</sub> was less than that of agalsidase alfa where the co-administration of migalastat with agalsidase beta increased active  $\alpha$ -Gal A AUC<sub>infinity</sub> by 2-to 2.8- folds.
  - The increase in active  $\alpha$ -Gal A level in presence of migalastat was observed in 22 out of 23 subjects (95.7%) in the study. Only one subject (2017-005) showed no change in active  $\alpha$ -Gal A AUC<sub>infinity</sub> following co-administration with 450 mg migalastat HCl relative to when 1.0 mg/kg agalsidase beta administered alone. Please note that this subject had very high active  $\alpha$ -Gal A AUC<sub>infinity</sub> following administration of 1.0 mg/kg agalsidase beta alone compared to any other subjects.
- The magnitude of the increase in  $\alpha$ -Gal A activity was not correlated with migalastat HCl dose.

*Table 2: Summary of Plasma Active  $\alpha$ -Gal A PK Parameters by Treatment*

Treatment Group	C <sub>max</sub> <sup>a</sup> (nmol/hr/mL)	t <sub>max</sub> <sup>b</sup> (hr)	AUC <sub>infinity</sub> <sup>a</sup> (hr <sup>2</sup> nmol/hr/mL)	t <sub>1/2</sub> <sup>c</sup> (hr)	AUC Ratio <sup>a</sup>
0.2 mg/kg agalsidase alfa alone (N = 4)	299.52 (29.08)	0.66 (0.7;1.0)	381.48 (21.56)	4.48 (3.132)	
0.2 mg/kg agalsidase alfa + 150 mg migalastat HCl (N = 4)	511.09 (14.88)	0.66 (0.7;1.0)	1583.90 (28.01)	4.27 (1.536)	4.15 (20.15)
0.2 mg/kg agalsidase alfa alone (N = 4)	358.10 (31.37)	0.66 (0.7;1.0)	672.01 (79.59)	5.15 (3.645)	
0.2 mg/kg agalsidase alfa + 450 mg migalastat HCl (N = 4)	605.20 (25.81)	0.66 (0.7;1.0)	2108.57 (46.69)	5.31 (2.462)	3.14 (38.24)
0.5 mg/kg agalsidase beta alone (N = 5)	508.55 (16.52)	2.00 (2.0;2.3)	1128.90 (19.56)	3.91 (2.054)	
0.5 mg/kg agalsidase beta + 150 mg migalastat HCl (N = 5)	877.31 (25.17)	2.00 (2.0;3.0)	3191.54 (27.48)	3.51 (1.304)	2.83 (30.94)
1.0 mg/kg agalsidase beta alone (N = 3)	1645.96 (26.63)	2.00 (1.5;3.0)	4765.43 (26.26)	5.33 (4.082)	
1.0 mg/kg agalsidase beta + 150 mg migalastat HCl (N = 3)	2291.63 (37.92)	2.22 (2.0;3.0)	9464.36 (30.60)	4.30 (1.712)	1.99 (16.85)
0.5 mg/kg agalsidase beta alone (N = 1)	684.36	3.00	2523.87	6.50	
0.5 mg/kg agalsidase beta + 450 mg migalastat HCl (N = 1)	1351.18	3.00	6197.71	3.49	2.46
1.0 mg/kg agalsidase beta alone (N = 6)	1655.35 (44.44)	2.25 (2.0;4.0)	4931.07 (65.14)	3.11 (1.875)	
1.0 mg/kg agalsidase beta + 450 mg migalastat HCl (N = 6)	2315.55 (31.39)	2.25 (2.0;4.0)	9676.48 (42.94)	4.96 (1.532)	1.96 (53.36)

Abbreviations:  $\alpha$ -Gal A =  $\alpha$ -galactosidase A; AUC = area under the plasma concentration versus time curve; AUC<sub>infinity</sub> = area under the plasma concentration versus time curve extrapolated from time 0 to infinity; C<sub>max</sub> = maximum observed plasma concentration; HCl = hydrochloride; PK = pharmacokinetic; t<sub>max</sub> = time to maximum observed plasma concentration; t<sub>1/2</sub> = terminal elimination half-life

<sup>a</sup> Geometric mean (% Coefficient of variation of geometric mean)

<sup>b</sup> Median (Range)

<sup>c</sup> Arithmetic Mean (Standard deviation)

Figure 1: Individual Plasma Active  $\alpha$ -Gal A AUCs when 0.2 mg/kg Agalsidase Alfa was administered with and without 150 mg or 450 mg Migalastat HCl

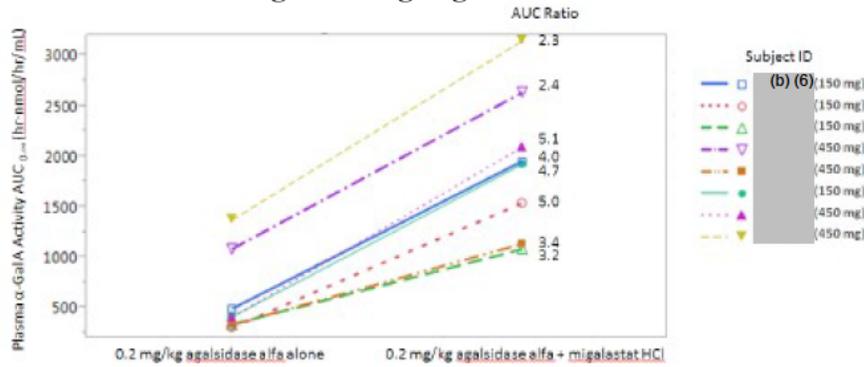


Figure 2: Arithmetic Mean (SD) Plasma-  $\alpha$ -Gal A Enzyme Activity Time Profiles of when 0.2 mg/kg Agalsidase Alfa (Replagal) was administered with and without 150 mg or 450 mg Migalastat HCl

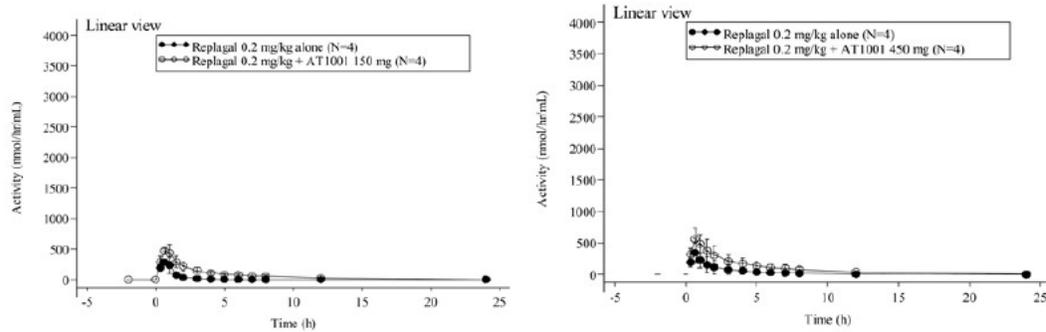


Figure 3: Individual Plasma Active  $\alpha$ -Gal A AUCs when 0.5 mg/kg Agalsidase Beta (Fabrazyme) was administered with and without 150 mg or 450 mg Migalastat HCl

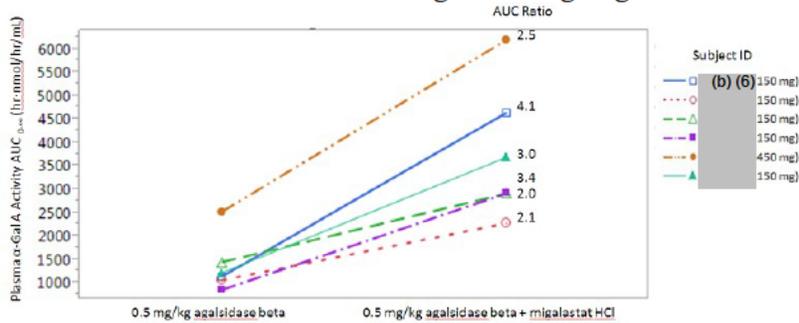


Figure 4: Arithmetic Mean (SD) Plasma  $\alpha$ -Gal A Enzyme Activity Time Profiles when 0.5 mg/kg Agalsidase Beta (Fabrazyme) was administered with and without 150 mg or 450 mg Migalastat HCl

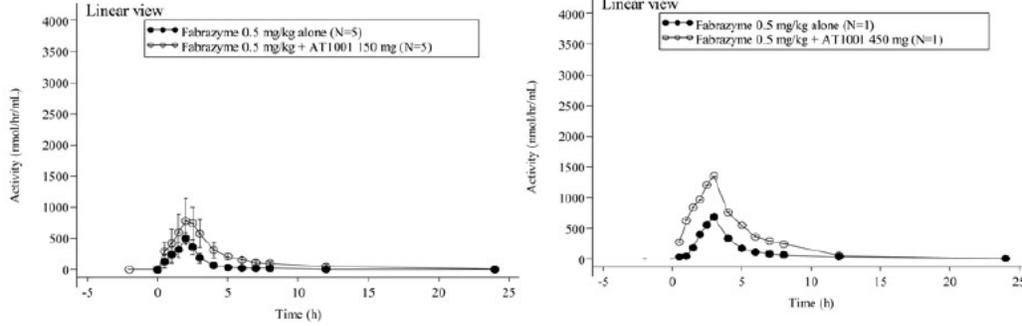


Figure 5: Individual Plasma Active  $\alpha$ -Gal A AUCs when 1.0 mg/kg Agalsidase Beta (Fabrazyme) was administered with and without 150 mg or 450 mg Migalastat HCl

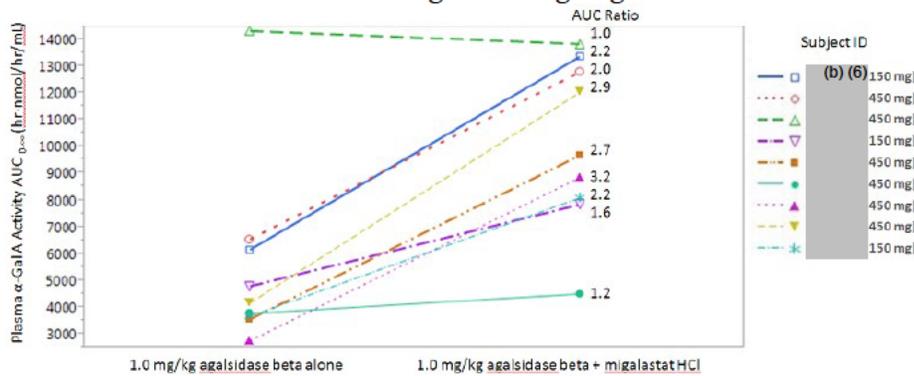
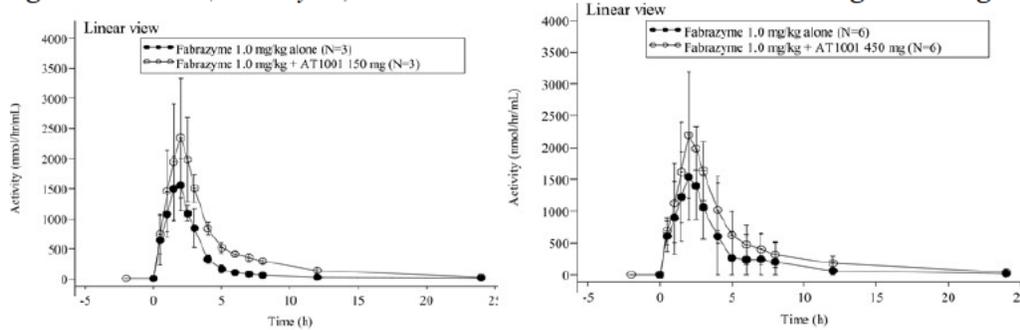


Figure 6: Arithmetic Mean (SD) Plasma  $\alpha$ -Gal A Enzyme Activity -Time Profiles when 1.0 mg/kg Agalsidase Beta (Fabrazyme) was administered with and without 150 mg or 450 mg Migalastat HCl



**Plasma Total  $\alpha$ -Gal A Protein Level**

As expected, unlike the increase in plasma  $\alpha$ -Gal A enzyme activity, the plasma total  $\alpha$ -Gal A protein level did not change significantly when agalsidase (alfa or beta) were co-administered with migalastat compared to when agalsidase (alfa or beta) administered alone.

Table 3: Statistical Analysis of PK Parameters of Plasma Total  $\alpha$ -Gal A Protein, by Agalsidase mg/kg Dose Level with and without 150 mg Migalastat HCl- Stage 1

Treatment Category	PK Parameter: Arithmetic Mean (SD)	PK Parameter: Arithmetic Mean (SD)			
		AUC <sub>0-t</sub> (hr*ng/mL)	C <sub>max</sub> (ng/mL)	t <sub>max</sub> (hr)	t <sub>1/2</sub> (hr)
Period Ratio (Period 2/ Period 1)					
Agalsidase beta (0.5 mg/kg) (N = 5)					
Period 1 (agalsidase alone)	57187.48 (47381.96)	7159.13 (4599.113)	2.07 (0.149)	57.78 (40.019)	
Period 2 (agalsidase with migalastat HCl)	56895.40 (45753.77)	7060.26 (5016.147)	2.10 (0.548)	23.10 (22.338)	
Ratio	0.95 (0.216)	0.97 (0.286)			
Agalsidase beta (1.0 mg/kg) (N = 3)					
Period 1 (agalsidase alone)	29905.87 (20470.28)	5156.74 (2411.601)	2.16 (0.778)	17.77 (8.837)	
Period 2 (agalsidase with migalastat HCl)	34298.23 (17022.13)	5572.63 (1915.073)	2.24 (0.750)	9.15 (5.621)	
Ratio	1.25 (0.246)	1.14 (0.198)			
Agalsidase beta (Overall) (N = 8)					
Period 1 (agalsidase alone)	46956.88 (40024.64)	6408.23 (3849.989)	2.10 (0.434)	42.78 (36.965)	
Period 2 (agalsidase with migalastat HCl)	48421.46 (37627.07)	6502.40 (4002.345)	2.15 (0.581)	17.87 (18.607)	
Ratio	1.06 (0.260)	1.03 (0.256)			
Agalsidase alfa (0.2 mg/kg) (N = 4)					
Period 1 (agalsidase alone)	42256.74 (13868.00)	3813.49 (1036.627)	0.75 (0.170)	86.19 (92.048)	
Period 2 (agalsidase with migalastat HCl)	42528.55 (14652.93)	4196.18 (1264.763)	0.75 (0.166)	32.95 (20.282)	
Ratio	1.00 (0.029)	1.10 (0.150)			

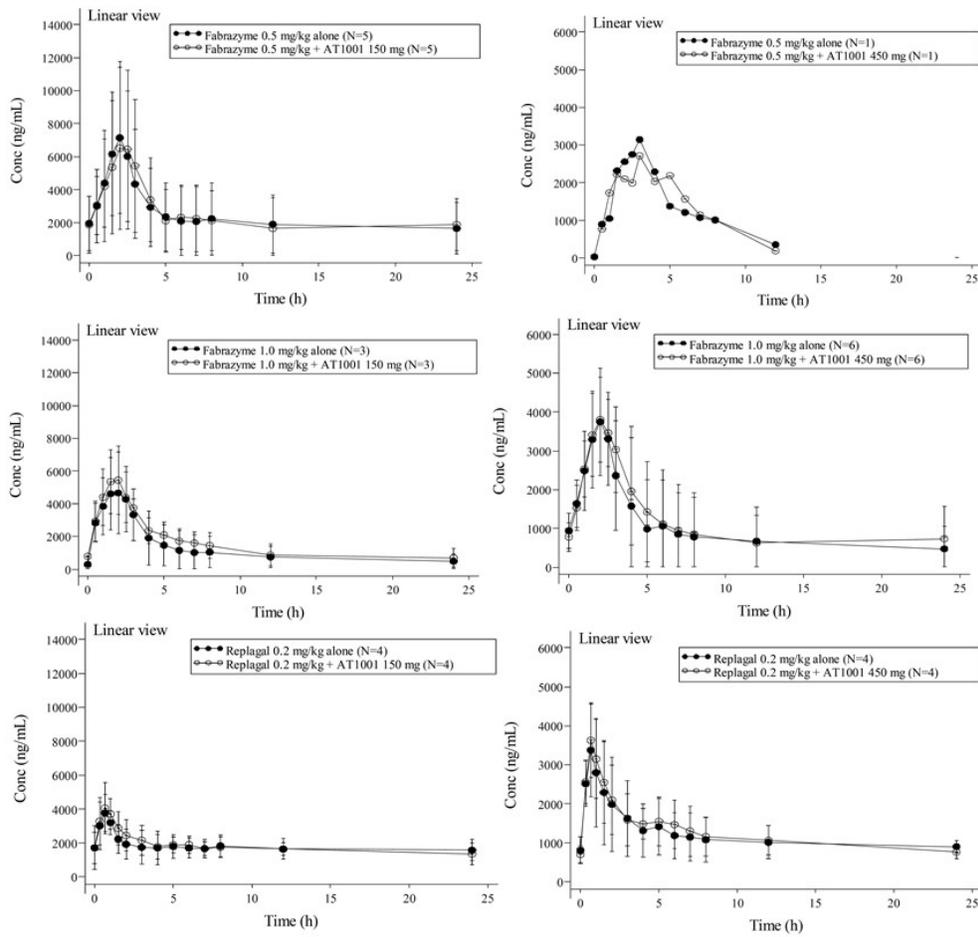
Abbreviations:  $\alpha$ -Gal A =  $\alpha$ -galactosidase A; AUC<sub>0-t</sub> = area under the plasma concentration versus time curve to t<sub>last</sub> (last time point at which concentration is quantified); CI = confidence interval; C<sub>max</sub> = maximum observed plasma concentration; HCl = hydrochloride; PK = pharmacokinetic; SD = standard deviation; t<sub>max</sub> = time to maximum observed plasma concentration; t<sub>1/2</sub> = terminal elimination half-life

Table 4: Statistical Analysis of Primary PK Parameters of Plasma Total  $\alpha$ -Gal A Protein, by Agalsidase mg/kg Dose Level with and without 450 mg Migalastat – Stage 2

Treatment Category	PK Parameter: Arithmetic Mean (SD)	PK Parameter: Arithmetic Mean (SD)			
		AUC <sub>0-t</sub> (hr*ng/mL)	C <sub>max</sub> (ng/mL)	t <sub>max</sub> (hr)	t <sub>1/2</sub> (hr)
Period Ratio (Period 2/ Period 1)					
Agalsidase beta (0.5 mg/kg) (N = 1)					
Period 1 (agalsidase alone)	16229.13 (NA)	3141.84 (NA)	3.00 (NA)	2.96 (NA)	
Period 2 (agalsidase with migalastat HCl)	16272.03 (NA)	2715.38 (NA)	3.00 (NA)	2.00 (NA)	
Ratio	1.00 (NA)	0.86 (NA)			
Agalsidase beta (1.0 mg/kg) (N = 6)					
Period 1 (agalsidase alone)	17200.63 (16906.97)	3983.05 (1422.734)	2.50 (0.775)	6.71 (7.702)	
Period 2 (agalsidase with migalastat HCl)	22031.50 (16754.04)	4170.15 (935.512)	2.50 (0.548)	5.55 (5.553)	
Ratio	1.59 (0.708)	1.12 (0.235)			
Agalsidase beta (Overall) (N = 7)					
Period 1 (agalsidase alone)	17061.84 (15438.25)	3862.87 (1337.124)	2.57 (0.732)	6.09 (7.057)	
Period 2 (agalsidase with migalastat HCl)	21208.72 (15448.42)	3962.33 (1015.705)	2.57 (0.535)	5.04 (5.245)	
Ratio	1.51 (0.683)	1.08 (0.234)			
Agalsidase alfa (0.2 mg/kg) (N = 4)					
Period 1 (agalsidase alone)	28770.37 (10941.44)	3587.46 (1181.299)	0.75 (0.170)	30.32 (5.010)	
Period 2 (agalsidase with migalastat HCl)	29816.41 (8956.789)	3773.21 (950.710)	0.75 (0.170)	28.05 (18.582)	
Ratio	1.06 (0.102)	1.07 (0.091)			

Abbreviations:  $\alpha$ -Gal A =  $\alpha$ -galactosidase A; AUC<sub>0-t</sub> = area under the plasma concentration versus time curve to t<sub>last</sub> (last time point at which concentration is quantified); C<sub>max</sub> = maximum observed plasma concentration; CI = confidence interval; HCl = hydrochloride; NA = not applicable; PK = pharmacokinetic; SD = standard deviation; t<sub>max</sub> = time to maximum observed plasma concentration; t<sub>1/2</sub> = terminal elimination half-life

Figure 7: Arithmetic Mean (SD) Plasma Total  $\alpha$ -Gal A Protein -Time Profiles by Agalsidase mg/kg Dose Level with and without 150 mg or 450 mg Migalastat HCl



### Migalastat PK

Co-administration of agalsidase did not significantly affect the plasma exposure of migalastat in Fabry patients. PK of migalastat observed in these Fabry subjects following a single dose administration were similar to those observed in healthy volunteers.

Table 5: Summary of Plasma Migalastat PK Parameters by Treatment

Treatment Group	C <sub>max</sub> <sup>a</sup> (ng/mL)	t <sub>max</sub> <sup>b</sup> (hr)	AUC <sub>0-t</sub> <sup>a</sup> (hr*ng/mL)	AUC <sub>infinity</sub> <sup>a</sup> (hr*ng/mL)	AUC F <sub>rel</sub>	t <sub>1/2</sub> <sup>c</sup> (hr)
150 mg migalastat HCl + Agalsidase (N = 12)	1625.99 (35.78)	3.04 (2.0;4.4)	13104.50 (40.00)	13520.99 (41.23)	1.06 (31.70) <sup>d</sup>	5.11 (0.845)
150 mg migalastat HCl Alone (N = 12)	1630.11 (32.25)	3.00 (2.0;4.0)	12371.21 (36.19)	12858.27 (37.15)	-	5.08 (0.874)
450 mg migalastat HCl + Agalsidase (N = 11)	3935.34 (30.82)	4.00 (2.0;6.0)	31846.98 (34.50)	33101.26 (35.07)	2.57 <sup>e</sup>	4.85 (1.244)

<sup>a</sup> Geometric mean (% Coefficient of variation of geometric mean)

<sup>b</sup> Median (Range)

<sup>c</sup> Arithmetic Mean (Standard deviation)

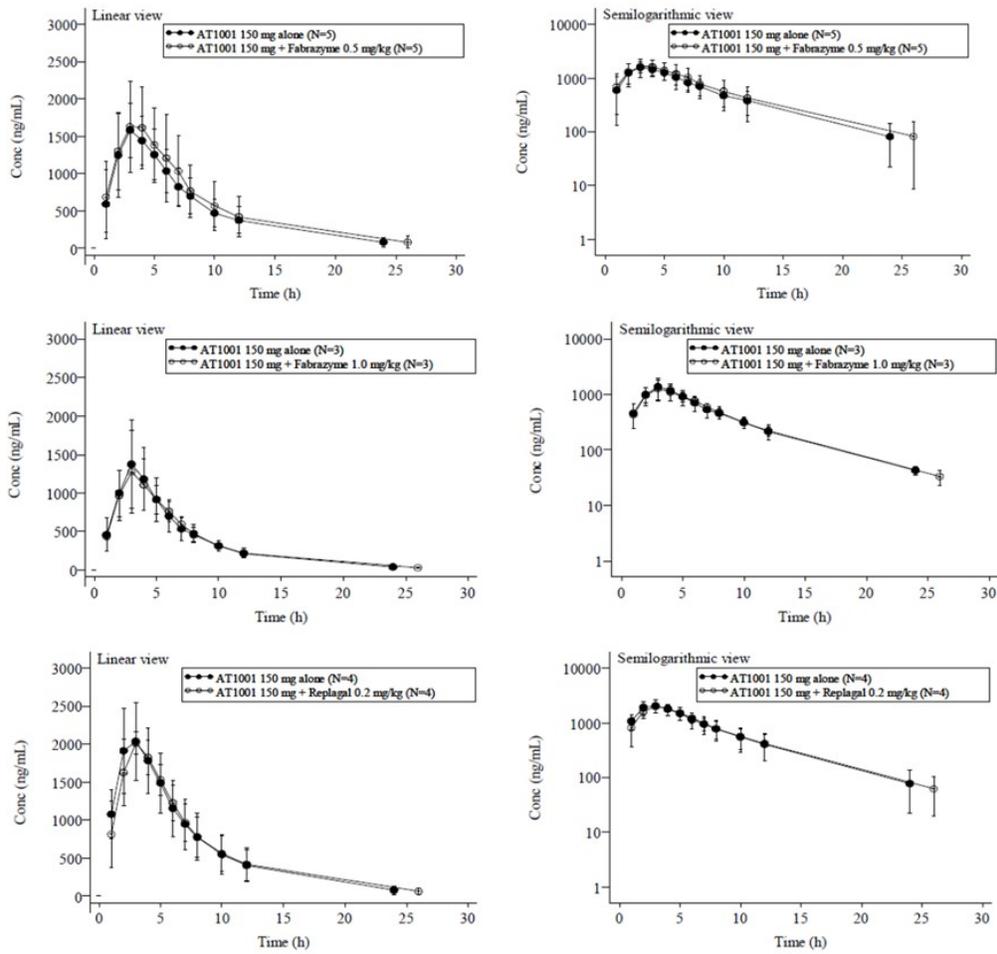
<sup>d</sup> Geometric mean AUC<sub>0-t</sub> for 150 mg migalastat HCl + agalsidase/ geometric mean AUC<sub>0-t</sub> for 150 mg migalastat HCl alone

<sup>e</sup> Geometric mean AUC<sub>0-t</sub> for 450 mg migalastat HCl + agalsidase/ geometric mean AUC<sub>0-t</sub> for 150 mg migalastat HCl alone

Table 6: Statistical Analysis of Plasma Migalastat Primary PK Parameters when 150 mg Migalastat HCl was administered with and without Agalsidase (Stage 1)

Treatment Category Period	PK Parameter: Geometric Mean (90% CI) Value			
	Ratio (Period 2/ Period 3)	AUC <sub>infinity</sub> (hr*ng/mL)	AUC <sub>0-t</sub> (hr*ng/mL)	C <sub>max</sub> (ng/mL)
Agalsidase beta (0.5 mg/kg) (N = 5)				
Period 2 (agalsidase + migalastat HCl)		14493 (10313, 20369)	13942 (10076, 19292)	1606 (1241, 2078)
Period 3 (migalastat HCl alone)		13152 (9358, 18484)	12547 (9068, 17361)	1594 (1231, 2063)
Ratio	1.102 (0.681, 1.783)		1.111 (0.702, 1.759)	1.007 (0.700, 1.451)
Agalsidase beta (1.0 mg/kg) (N = 3)				
Period 2 (agalsidase + migalastat HCl)		9470 (6975, 12859)	9232 (6744, 12637)	1227 (735, 2046)
Period 3 (migalastat HCl alone)		9406 (6928, 12771)	9085 (6637, 12437)	1297 (777, 2162)
Ratio	1.007 (0.653, 1.552)		1.016 (0.652, 1.584)	0.946 (0.459, 1.950)
Agalsidase alfa (0.2 mg/kg) (N = 4)				
Period 2 (agalsidase + migalastat HCl)		16191 (12025, 21801)	15771 (11905, 20893)	2041 (1713, 2432)
Period 3 (migalastat HCl alone)		15804 (11737, 21279)	15322 (11566, 20297)	1991 (1671, 2372)
Ratio	1.025 (0.673, 1.560)		1.029 (0.692, 1.532)	1.025 (0.800, 1.313)
All Agalsidase (N = 12)				
Period 2 (agalsidase + migalastat HCl)		13521 (11209, 16310)	13105 (10919, 15727)	1626 (1379, 1917)
Period 3 (migalastat HCl alone)		12858 (10659, 15511)	12371 (10308, 14847)	1630 (1383, 1921)
Ratio	1.052 (0.807, 1.371)		1.059 (0.818, 1.371)	0.997 (0.791, 1.259)

Figure 8: Arithmetic Mean (SD) of Migalastat Plasma Concentration–Time Profiles when 150 mg Migalastat HCl was administered with and without Agalsidase (Stage 1)



### Skin $\alpha$ -Gal A Enzyme Activity

Co-administration of migalastat with agalsidase consistently increased the change in skin  $\alpha$ -Gal A enzyme activity from baseline on both Day 2 and Day 7 post-dose regardless of the dose and type of agalsidase. However, the change on Day 7 was less pronounced compared to Day 2.

Table 7: Mean Change from Baseline in Active  $\alpha$ -Gal A in Skin following Treatment with Agalsidase Alone and Co-administration with 150 mg and 450 mg Migalastat HCl by Agalsidase mg/kg Dose Level on Days 2 and 7

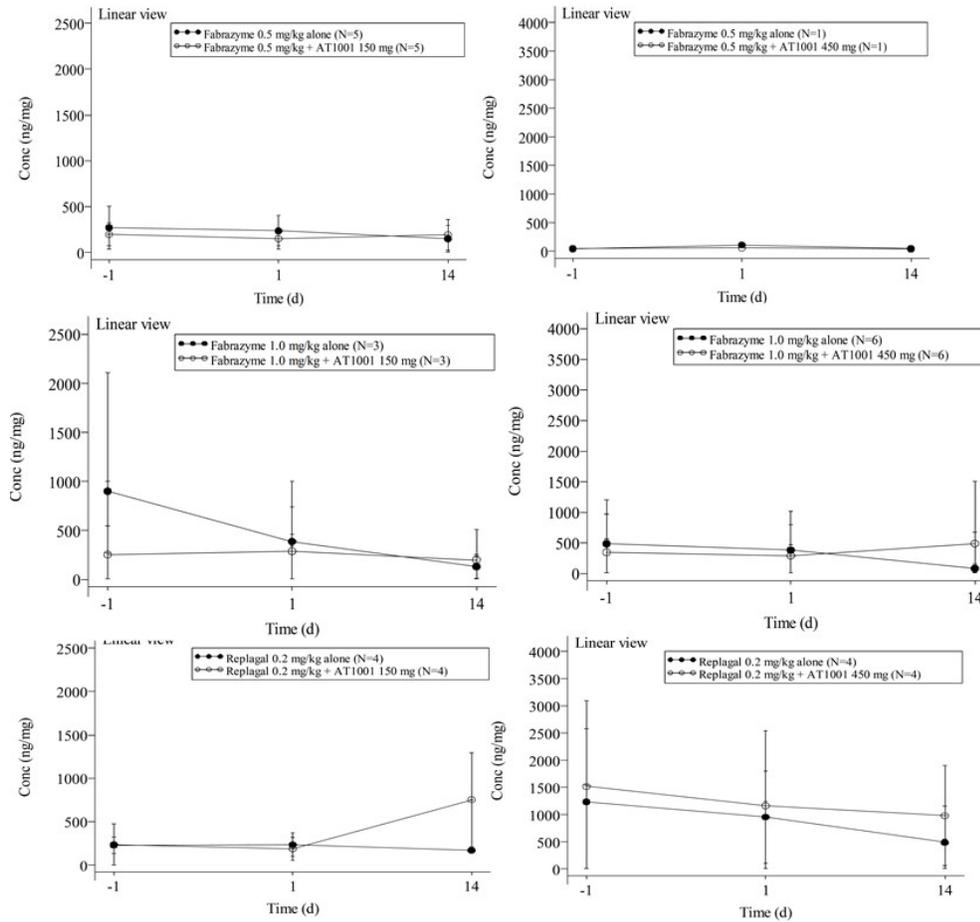
Treatment Category	Change from Baseline: Mean (Min – Max)			
	Day 2		Day 7	
	Period 1 (agalsidase alone)	Period 2 (agalsidase with migalastat HCl)	Period 1 (agalsidase alone)	Period 2 (agalsidase with migalastat HCl)
Agalsidase alfa (0.2 mg/kg) $\pm$ 150 mg (N = 4)	121 (-52 – 371) n = 3	437 (35 – 1146) n = 3	139 (50 – 229) n = 2	228 (11 – 606) n = 3
Agalsidase beta (0.5 mg/kg) $\pm$ 150 mg (N = 5)	334 (-141 – 998) n = 5	1508 (221 – 4103) n = 4	137 (45 – 234) n = 5	574 (-200 – 2588) n = 5
Agalsidase beta (1.0 mg/kg) $\pm$ 150 mg (N = 3)	857 (795 – 919) n = 2	2977 n = 1	553 (315 – 791) n = 2	855 (460 – 1250) n = 2
Agalsidase alfa (0.2 mg/kg) $\pm$ 450 mg (N = 4)	18 n = 1	160 n = 1	NA n = 0	-100 n = 1
Agalsidase beta (0.5 mg/kg) $\pm$ 450 mg (N = 1)	89 n = 1	647 n = 1	72 n = 1	235 n = 1
Agalsidase beta (1.0 mg/kg) $\pm$ 450 mg (N = 6)	1936 (884 – 3162) n = 6	4019 (1777 – 6168) n = 6	613 (252 – 2001) n = 6	789 (-174 – 1935) n = 6

### **Exploratory Endpoints:**

#### Urinary GL-3:

There was no significant change in urinary GL-3 concentration across Days -1, 1, and 14 of Periods 1 and 2, regardless of the dose and type of agalsidase. Urinary GL-3 did not change significantly when agalsidase (alfa or beta) were co-administered with migalastat compared to agalsidase (alfa or beta) administered alone regardless of migalastat dose. Nevertheless, GL-3 had high variability to have a meaningful interpretation.

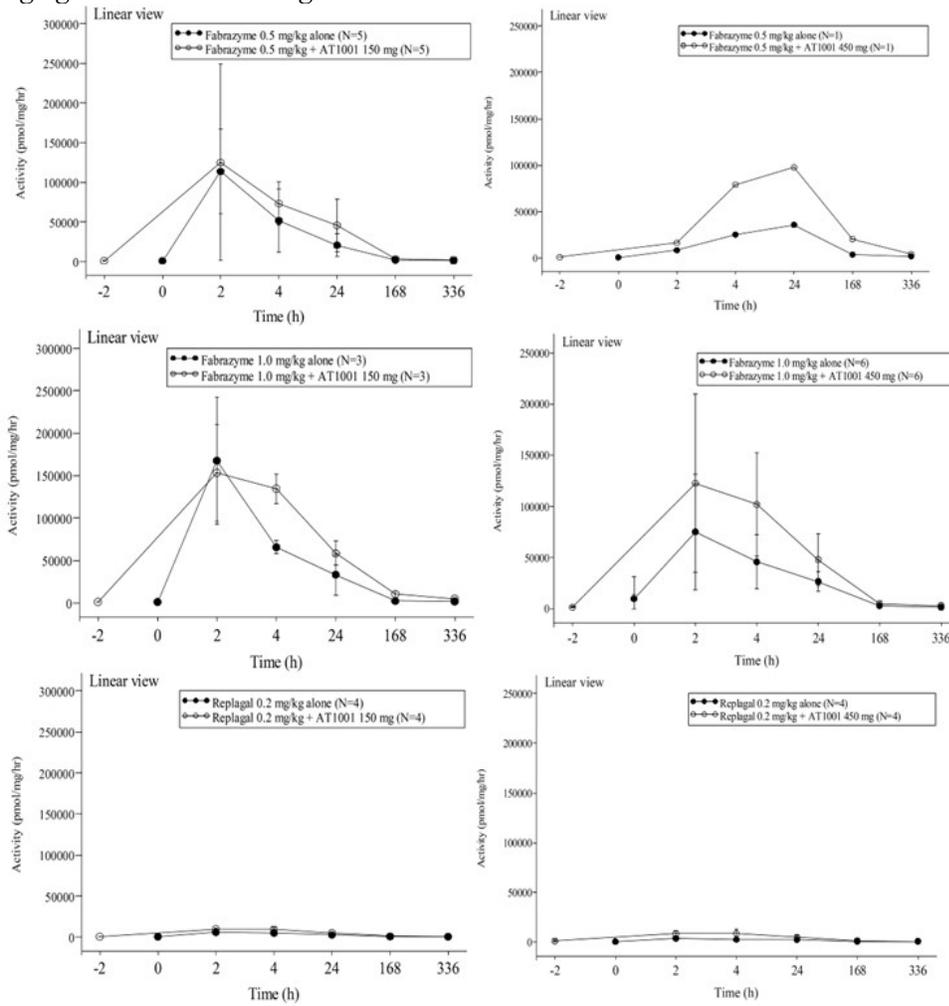
Figure 9: Arithmetic Mean (SD) Urinary GL-3 Concentrations Time Profiles by Agalsidase mg/kg Dose Level and Migalastat Dose Level



*WBC  $\alpha$ -Gal A Activity:*

Co-administration of migalastat with all agalsidase dose groups increased WBC  $\alpha$ -Gal A Activity compared to when agalsidase was administered alone at both migalastat doses. The difference was more pronounced at 450 mg migalastat dose.

Figure 10: Arithmetic Mean (SD) WBC  $\alpha$ -Gal A Activity (pmol/mg/hr) Time Profiles of by Agalsidase mg/kg Dose Level and Migalastat Dose Level



*Plasma Lyso-Gb3:*

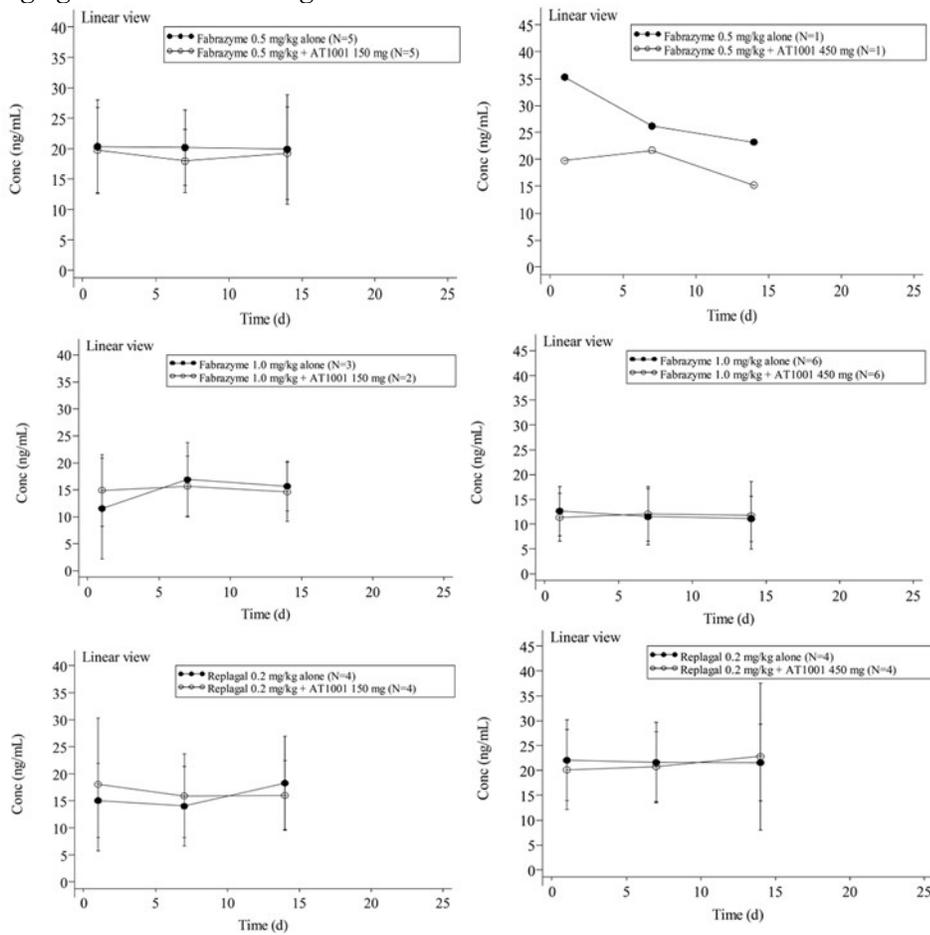
In all treatment groups, the mean plasma Lyso-Gb3 levels across Days 1, 7, and 14 of Periods 1 and 2 were relatively stable, and co-administration of migalastat (150 mg or 450 mg) with agalsidase (Period 2) did not significantly change the plasma Lyso-Gb3 level compared to when agalsidase was administered alone (period 1).

Table 8: Plasma Lyso-Gb3 (ng/mL) Levels by Study Stage, Period, and Agalsidase mg/kg Dose Level

Treatment Category	n, Mean (SD) Plasma Lyso-Gb <sub>3</sub> (ng/mL)			
	Stage 1		Stage 2	
	Migalastat HCl 150 mg	Migalastat HCl 150 mg	Migalastat HCl 450 mg	Migalastat HCl 450 mg
Day	Period 1	Period 2	Period 1	Period 2
0.5 mg/kg Agalsidase Beta				
Day 1	5, 20.34 (7.698)	5, 19.74 (7.008)	1, 35.30	1, 19.80
Day 7	5, 20.18 (6.230)	4, 17.98 (5.175)	1, 26.20	1, 21.70
Day 14	3, 19.90 (9.009)	5, 19.24 (7.567)	1, 23.20	1, 15.20
1.0 mg/kg Agalsidase Beta				
Day 1	3, 11.50 (9.302)	2, 14.90 (6.647)	5, 12.63 (5.005)	6, 11.36 (4.826)
Day 7	2, 16.85 (6.859)	2, 15.65 (5.586)	6, 11.49 (5.635)	6, 12.04 (5.516)
Day 14	2, 15.65 (4.596)	2, 14.60 (5.515)	6, 11.06 (4.626)	6, 11.72 (6.810)
0.2 mg/kg Agalsidase Alfa				
Day 1	4, 15.04 (6.852)	3, 18.06 (12.270)	4, 22.05 (8.105)	4, 20.15 (8.053)
Day 7	4, 14.02 (7.347)	4, 15.92 (7.788)	4, 21.60 (8.053)	4, 20.80 (7.002)
Day 14	2, 18.30 (8.627)	4, 16.00 (6.464)	4, 21.58 (7.768)	3, 22.80 (14.763)

Abbreviations: HCl = hydrochloride; lyso-Gb<sub>3</sub> = globotriaosylsphingosine; PK = pharmacokinetic; SD = standard deviation

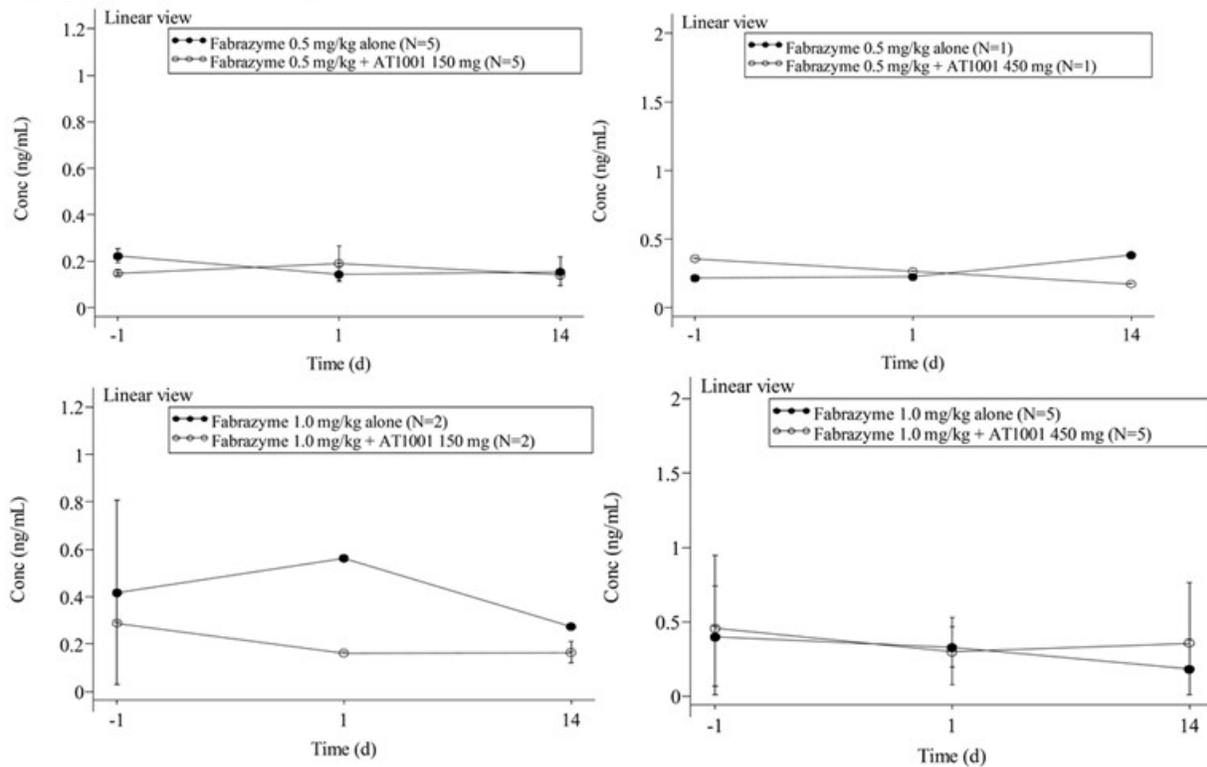
Figure 11: Arithmetic Mean (SD) of Plasma Lyso-GB3 Concentration Time Profiles of by Agalsidase mg/kg Dose Level and Migalastat Dose Level



### Urinary Lyso-Gb3:

Co-administration of migalastat (150 mg or 450 mg) with agalsidase (Period 2) did not significantly change the urinary Lyso-Gb3 level compared to when agalsidase was administered alone (period 1).

Figure 12: Arithmetic Mean (SD) Urinary Lyso-GB3 Concentrations Time Profiles of by Agalsidase mg/kg Dose Level and Migalastat Dose Level



**Safety:** Co-administration of migalastat HCl at doses of 150 mg and 450 mg with agalsidase were generally well-tolerated. There was no death or discontinuation due to AEs in this study. One serious TEAE was reported during Stage 2, Period 1 in the 0.5 mg/kg agalsidase beta treatment category. Most TEAEs reported during the study were mild in intensity. A total of 6 subjects reported moderate TEAEs. Overall, during Stage 1 of the study, out of the 12 subjects, 8, 2, and 5 subjects experienced at least 1 TEAE during Periods 1, 2, and 3, respectively; and during Stage 2 of the study, out of the 11 subjects, a total of 4 and 5 subjects experienced at least 1 TEAE during Periods 1 and 2, respectively. The most commonly reported TEAEs overall were headache (n = 5), diarrhea (n = 3), cardiac murmur (n = 2), nausea (n = 2), peripheral edema (n = 2), paresthesia (n = 2), and dyspnea (n = 2). No treatment-related TEAEs were reported during Stage 1 of the study. During Stage 2 of the study, 2 subjects receiving 0.2 mg/kg agalsidase alfa reported mild, treatment-related TEAEs. All treatment-related events resolved within 3 days with no actions taken. There were no clinically significant findings for clinical laboratory, 12-lead ECG, vital signs, or physical examination during the study.

## 8.10 Phase 2 Dose Ranging Study: FAB-CL-204

**Title:** 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

**Note:** Migalastat may also referred as AT1001 in this study report

**Study Date:** (b) (6)

### Study Design:

This was a phase 2, multicenter, open-label trial of AT1001 in female subjects with Fabry disease. The trial included a 12-week treatment period followed by an optional 36-week treatment extension. Subjects were stratified by  $\alpha$ -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 (two, six, or ten 25-mg capsules, respectively) once every other day (QOD). Subjects fasted for at least 2 hours before and 2 hours after each dose.

Before randomization, subjects were stratified into two groups (high or low) by their baseline  $\alpha$ -Gal A activity as tested in lymphocytes cultured from a blood sample collected on Day -28. High was defined as activity greater than 40% of normal; low was defined as activity less than or equal to 40% of normal.

**Study Population:** 9 Female Fabry patients (ages between 18-65) who had a confirmed diagnosis of Fabry disease with a documented missense gene mutation were enrolled. All nine subjects completed both the 12-week treatment and the 36-week extension periods of the study.

**PD samples:** The PD measures were  $\alpha$ -Gal A activity in leukocytes, kidney, and skin; globotriaosylceramide (GL-3) in urine, kidney, plasma, and skin;

*Table 1: Schedule of Assessments – 12-Week Treatment Phase*

Period	Assessment		Open-Label Treatment					Follow-up
	Visit	0 <sup>a</sup>	Screening	Baseline	1	2	3	
Study Day	-28	-2 to -1	1	14	28	56	83-84	98
Study Week	-4	-1	0	2	4	8	12	14
<b>Procedures and Assessments</b>								
<b>Pharmacodynamic Measures</b>								
Kidney Biopsy <sup>e</sup>		X					X	
Skin Biopsy <sup>e, f</sup>		X					X	
$\alpha$ -Gal A (leukocytes/WBC) <sup>g</sup>	X	X	X	X	X	X	X	X
GL-3 (plasma and urine)	X	X	X	X	X	X	X	X

*Table 2: Schedule of Assessments – 36-Week Extension Phase*

Clinic Visit	6	7	8
Study Day	168-169	252-253	336-337
Study Week	24	36	48
<b>Procedures and Assessments</b>			
Renal Biopsy <sup>c</sup>			X
Skin Biopsy			X
$\alpha$ -Gal A Leukocyte Assay <sup>d</sup>	X	X	X
GL-3 (plasma and urine)	X	X	X

### PK Samples Collection Time:

- Plasma: On Days 1, 14, and 84, PK blood samples were collected at pre-dose and at 0.5, 1, 2, 3, 4, 5, 6, 8, and 10 hours post-dose. In addition, blood samples were collected at 12 hours before the doses on Days 14 and 84.
- Urine: On Days 1, 14, and 84, the complete urine output was collected from each subject for the analysis of AT1001 for the following intervals: -14 to 9 hours (before the dose); 0 to 4 hours post-dose; 4 to 8 hours post-dose; and 8 to 10 hours post-dose.

### **RESULTS:**

#### **PK:**

- Plasma exposure of migalastat increased in approximately dose-proportional manner following a single dose administration on Day 1 and following multiple QOD dose administration on Day 14 and 84 in Fabry patients between 50 mg QOD and 250 mg QOD doses.
- T<sub>max</sub> was 2-3.5 hour following both single dose and multiple dose administration.
- There was no accumulation of migalastat following QOD administration for 12 weeks in Fabry patients.
- For urine recovery, 34 to 60% of the dosed migalastat was recovered in urine as unchanged drug within 10 hours of dosing for 150 mg migalastat HCl and 30 to 39% of the dosed migalastat was recovered in urine as unchanged drug within 10 hours of dosing of 250 mg dose.

*Table 3: AT1001 Plasma PK Parameters Following Administration of a Single Oral AT1001 Dose on Day 1, Day 14, and Day 84*

	<b>AT1001 50 mg QOD n=2</b>	<b>AT1001 150 mg QOD n=4</b>	<b>AT1001 250 mg QOD n=3</b>
<b>Day 1 PK Parameters</b>			
Geometric Mean (CV%)			
AUC <sub>0-4</sub> (ng*h/mL)	2628.9 (100.4)	8941.6 (32.2)	13217.2 (30.2)
C <sub>max</sub> (ng/mL)	519.5 (95.5)	1690.7 (22.0)	2461.4 (43.4)
Median (Range)			
t <sub>max</sub> (h)	2.00 (2.00, 2.00)	3.50 (1.00, 4.00)	3.00 (2.00, 5.00)
<b>Day 14 PK Parameters</b>			
Geometric Mean (CV%)			
AUC <sub>0-10</sub> (ng*h/mL)	3191.6 (64.9)	10637.9 (35.6)	14850.6 (9.6)
C <sub>max</sub> (ng/mL)	600.3 (33.1)	2028.6 (40.0)	2662.7 (22.8)
Median (Range)			
t <sub>max</sub> (h)	3.17 (2.33, 4.00)	2.96 (2.00, 4.00)	3.00 (3.00, 5.00)
<b>Day 84 PK Parameters</b>			
Geometric Mean (CV%)			
AUC <sub>0-4</sub> (ng*h/mL)	2300.3 (79.6)	8581.9 (29.7)	9970.3 (37.8)
C <sub>max</sub> (ng/mL)	500.9 (67.4)	1523.7 (23.3)	1953.8 (49.1)
Median (Range)			
t <sub>max</sub> (h)	2.00 (2.00, 2.00)	3.50 (2.00, 4.00)	3.00 (3.00, 4.00)

#### **PD Endpoints:**

There were no dose-dependent changes in PD markers,  $\alpha$ -Gal A activity in leukocytes, kidney, and skin and GL-3 in urine, kidney, plasma, and skin. Only proof-of-concept was demonstrated where  $\alpha$ -Gal A activity consistently increased compared to baseline in leukocytes at week 48 and in kidney and skin at

week 12. GI-3 levels were too variable to make any meaningful interpretation. Furthermore, there were too few patients in each dose cohort (n=2-4 per dose cohort) to make a meaningful interpretation.

Table 4: Leukocyte  $\alpha$ -Gal A Activity at Selected Visits during AT1001 Treatment

Subject	Dose (QOD)	Leukocyte $\alpha$ -Gal A Activity (nmol 4-MU/hr/mg protein)					
		Base-line	Visit 3 Week 8	Visit 4 Week 12	Visit 6 Week 24	Visit 7 Week 36	Visit 8 Week 48
01-01	50 mg	13.4	1.54 <sup>a</sup>	2.46 <sup>a</sup>	24.6	22.6	26
03-02		24.5	33.1	35.8	43.2	51.5	39.6
01-02	150 mg	25.1	4.45 <sup>a</sup>	4.15 <sup>a</sup>	33.8	40	- <sup>b</sup>
02-01		6.39	18.8	18.4	16.6	5.2 <sup>a</sup>	16.1
02-03		17.3	0.45 <sup>a</sup>	5.85	31.8	21.6	22.2
04-02		24.6	12.1 <sup>a</sup>	12.6 <sup>a</sup>	37.7	43.8	46.5
03-01	250 mg	3.25	8.41	6.56	9.92	7.71	4.83
03-05		14.7	18.9	23.3	34.6	30.4	29.2
06-03		13.1	17.4 <sup>a</sup>	8.86 <sup>a</sup>	23.8	9.93	7.58

Figure 1: Mean (SE) change in Leukocyte  $\alpha$  Gal A activity from baseline at week 12 and week 48

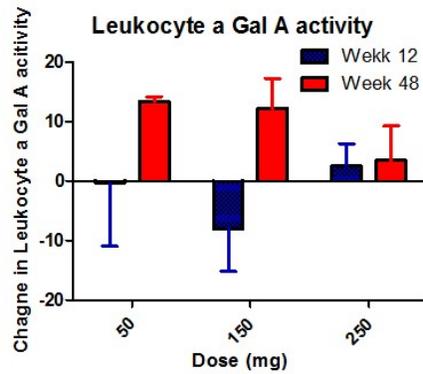


Table 5: Kidney  $\alpha$ -Gal A Activity in Tissue Homogenate

Subject	Dose (QOD)	Kidney $\alpha$ -Gal A Activity (nmol 4-MU/hr/mg protein)	
		Baseline	Visit 4 Week 12
01-01	50 mg	117.9	133.0
03-02		252.9	355.6
01-02	150 mg	86.6	126.2
02-01		80.5	64.1
02-03		125.0	185.8
04-02		87.4	172.7
03-01	250 mg	70.3	181.0
03-05		106.4	217.2
06-03		209.9	398.7

Figure 2: Mean (SE) change in Kidney  $\alpha$  Gal A activity from baseline at week 12

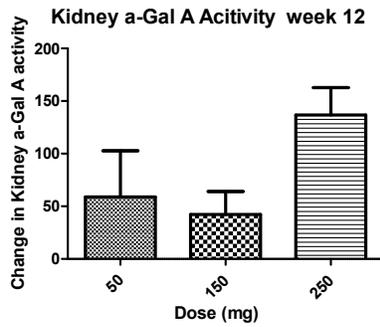


Table 6: Skin  $\alpha$ -Gal A Activity in Tissue Homogenate

Subject	Dose (QOD)	Skin $\alpha$ -Gal A Activity (nmol 4-MU/hr/mg protein)	
		Baseline	Visit 4 Week 12
01-01	50 mg	6.2	111.7
03-02		14.3	237.2
01-02	150 mg	110.0	159.3
02-01		14.2	42.3
02-03		8.2	83.9
04-02	250 mg	125.7	133.4
03-01		22.1	50.7
03-05		12.1	431.9
06-03		64.2	185.8

Figure 3: Mean (SE) change in skin  $\alpha$  Gal A activity from baseline at week 12

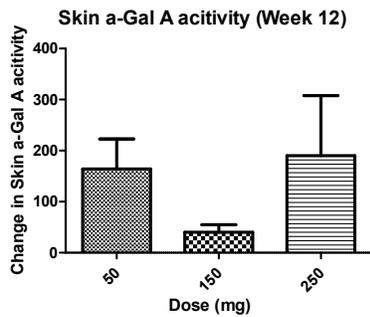


Table 7: Total Urine GL-3 at Selected Visits

Subject	Dose (QOD)	Total <sup>a</sup> Urine GL-3 (pmol/nmol PC)							
		Base-line	Visit 1 Week 2	Visit 2 Week 4	Visit 3 Week 8	Visit 4 Week 12	Visit 6 Week 24	Visit 7 Week 36	Visit 8 Week 48
01-01	50 mg	117.8	103.5	178.4	167.4	196.3	77.2	-	97.9
03-02		28.1	49.5	20.2	47.5	29.4	17.6	22.4	17.3
01-02	150 mg	651.4	372.0	310.9	-	228.9	213.8	327.3	340.8
02-01		502.2	440.4	-	413.7	248.0	373.8	267.8	284.6
02-03		51.7	90.5	177.4	234.3	352.8	151.7	377.5	541.2
04-02		267.0	571.2	779.1	730.3	94.5	289.2	680.0	673.2
03-01	250 mg	408.7	285.5	513.3	486.9	365.3	368.5	-	267.7
03-05		295.5	123.1	219.7	69.3	189.8	162.4	47.0	42.4
06-03		169.7	204.3	372.3	338.1	151.9	3449.3	348.7	97.2

PC = phosphatidylcholine

<sup>a</sup> Total represents the sum of the five isoforms of GL-3 measured (C16:0, C20:0, C22:0, C24:0, and C24:1).

Figure 4: Mean (SE) Change in Urine GL-3 from baseline at week 12 and week 48

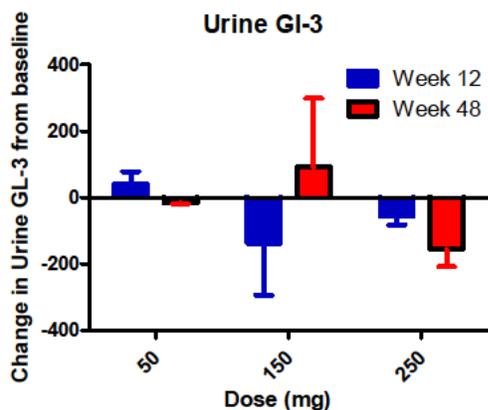


Table 8: Quantitative (Barisoni) Assessment of GL-3 Inclusions in Renal Capillaries

Subject	Dose (QOD)	GL-3 Inclusions in Renal Capillaries (average inclusions/capillary)		
		Baseline	Week 12	Week 48
01-01	50 mg	0.4	0.2	0.1
03-02		0.2	0.1	0.2
01-02	150 mg	0.2	0.4	0.0
02-01		0.3	0.1	0.2
02-03		0.2	0.2	-
04-02		0.2	0.3	0.1
03-01	250 mg	0.1	-	0.0
03-05		0.2	0.0	-
06-03		0.1	0.1	0.1

Figure 5: Mean (SE) Change in Kidney GL-3 from baseline at week 12 and week 48

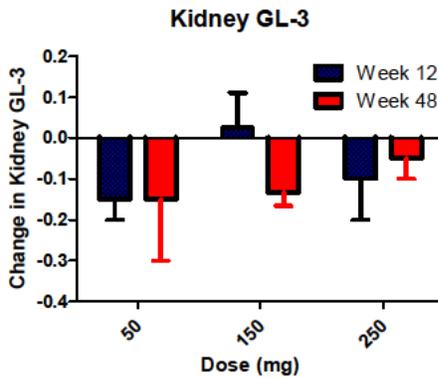
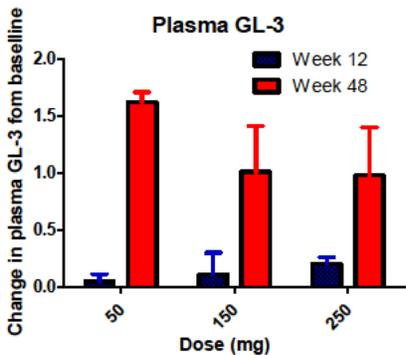


Table 9: Plasma GL-3 at Selected Visits

Subject	Dose (QOD)	Plasma GL-3 (µg/mL)							
		Base-line	Visit 1 Week 2	Visit 2 Week 4	Visit 3 Week 8	Visit 4 Week 12	Visit 6 Week 24	Visit 7 Week 36	Visit 8 Week 48
01-01	50 mg	1.95	2.40	2.11	2.82	1.93	ND	2.63	3.49
03-02		1.29	1.54	1.54	1.53	1.40	1.40	1.50	3.00
01-02	150 mg	1.60	1.29	1.60	1.46	1.37	ND	2.50	2.03
02-01		2.08	2.14	3.44	2.41	2.73	1.67	2.01	ND
02-03		1.32	2.05	1.39	BLQ	1.34	ND	2.25	2.15
04-02	250 mg	1.65	1.45	1.98	1.69	1.64	1.68	3.97	3.43
03-01		1.14	1.03	1.11	BLQ	BLQ	BLQ	2.66	1.39
03-05		1.10	1.39	1.38	1.26	1.36	1.12	2.55	2.07
06-03		1.23	1.24	1.28	1.45	1.37	ND	3.64	2.95

BLQ = below the limit of quantification; ND = not done.

Figure 6: Mean (SE) Change in Plasma GL-3 from baseline at week 12 and week 48



**Safety:** AT1001 was well tolerated up to 250 mg dose in female Fabry disease. There was no death or discontinuation due to AEs in this study. There were two SAEs, one during screening (cardiac tamponade) and one during treatment with 150 mg dose (muscular chest pain). Both were assessed as unrelated to treatment. All subjects reported at least one TEA. A total of 85 TEAEs were reported: 20 TEAEs in the 2 subjects randomized to 50 mg AT1001, 41 TEAEs in the 4 subjects randomized to 150 mg AT1001, and 24 TEAEs in the 3 subjects randomized to 250 mg AT1001. 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.

## In-Vitro Studies:

### 8.11 In-Vitro Metabolism in Hepatocytes

**Title:** Determination of the Metabolic Stability of AT1001 and AT2101 in Cryopreserved Human, Cynomologus Monkey, and Sprague-Dawley Rat Hepatocytes

**Specific Aims:** to evaluate the metabolic stability of AT1001 and AT2101 following incubations with cryopreserved hepatocytes and if necessary, identify and quantify any metabolites produced.

**Note:** Although this study report discusses the result of in vitro metabolism of AT1001 and AT2101 in hepatocytes of rat, monkey and human, this review will only focus on the metabolism of AT1001 in human hepatocytes.

**Test Item:** 1 and 100  $\mu\text{M}$   $^{14}\text{C}$ -AT1001 (MW of free base: 163.17 g/mol)

**Study Method:** 1 or 100  $\mu\text{M}$  AT1001 was incubated in cryopreserved human hepatocytes (pooled from several donors) for 4 hours in cells ( $0.5 \times 10^6$  cells/well) in a total incubation volume of 0.50 mL in a 24-well plate at 37 °C under 5% CO<sub>2</sub> in a humidified incubator. The positive control, 5  $\mu\text{M}$  naloxone, was also incubated to check for metabolic capacity of the cryopreserved hepatocytes. Samples were analyzed with LC-RAD, and positive controls were analyzed with LC-MS. The % of AT1001 metabolized was determined by comparing the response of samples at T=0 hour to the samples at T=1 hour, 2 hour and 4 hours.

#### Results:

There was no detectable amount of naloxone in the metabolic positive control incubation mixtures following incubation for 4 hours.

Following 4 hours of incubation of 1 and 100  $\mu\text{M}$  AT1001 in human hepatocytes, AT1001 was not metabolized in human hepatocytes. 100% of AT1001 remained intact after 4 hours of incubation in human cryopreserved hepatocytes. AT1001 was also stable in rat and monkey hepatocytes.

*Table 1: Metabolic Stability Determination of AT1001 Following Incubation with Cryopreserved Human Hepatocyte Suspensions*

	% of 0 hour Control		
	1 hr	2 hr	4 hr
1 $\mu\text{M}$	101%	104%	105%
100 $\mu\text{M}$	110%	101%	101%

Figure 1: Representative Chromatograms of 1  $\mu$ M AT1001 Incubations with Human Hepatocytes

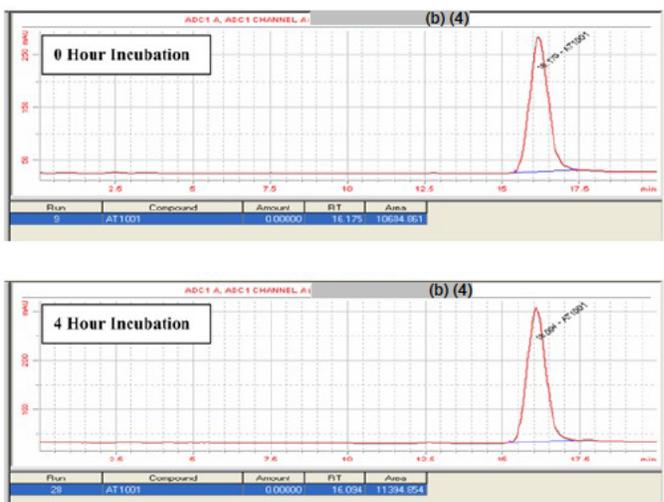
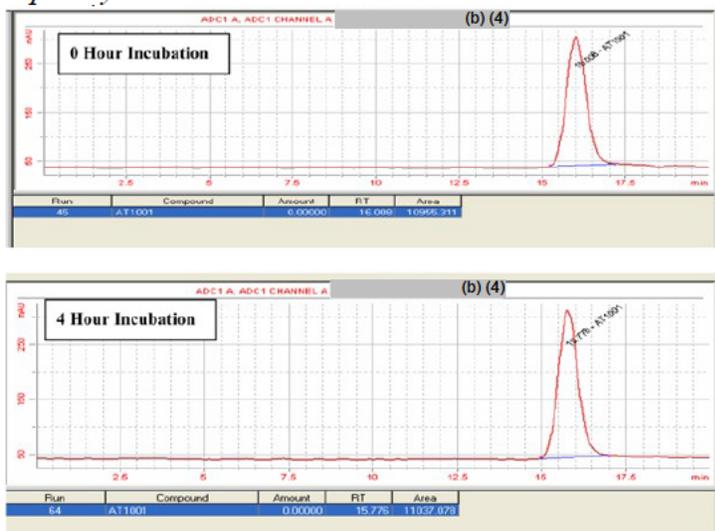


Figure 2: Representative Chromatograms of 100  $\mu$ M AT1001 Incubations with Human Hepatocytes



**Reviewer's Comment:**

1. The concentration of 1 and 100  $\mu$ M of AT1001 in hepatocytes were acceptable as 100 $\mu$ M is approximately 10 fold higher than the expected  $C_{max}$  in human subject at the proposed clinical dose of 150 mg migalastat ( $C_{max}$  at the clinical dose of 150 mg was 1561 ng/mL  $\approx$  9.6  $\mu$ M).
2. AT1001 was stable in human cryopreserved hepatocytes employed in this study after 4 hours of incubation. However, hepatocytes that were employed in this study were not adequately characterized in respect to all possible phase I and phase II enzymes prior to the study. In addition, this study did not have an adequate positive control. The sponsor only had included naloxone as a metabolic positive control. However, it is not clear what metabolic capacity or specific enzyme that naloxone was used as positive control for. According to the Naloxone

label, Naloxone is metabolized primarily by glucuronide conjugation. Therefore, it is difficult to interpret the result of this study in hepatocyte.

## 8.12 In-Vitro CYP Inhibition Study

**Title:** In Vitro Evaluation of Migalastat Hydrochloride as an Inhibitor of Cytochrome P450 (CYP) Enzymes in Human Liver Microsomes

**Report No:** (b) (4) 115084

**Test Site:** (b) (4)

**Specific Aims:** This study was designed to evaluate the ability of migalastat hydrochloride to inhibit the major CYP enzymes in human liver microsomes (namely CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4/5 [using three different substrates]) in vitro with the aim of ascertaining the potential of migalastat to inhibit the metabolism of concomitantly administered drugs.

### Study Design:

Test Item: Migalastat at 0, 0.5, 1.5, 5, 15, 50, 150, 500 uM

Test System: Pooled human liver microsomes (from 16 subjects)

Method: Human liver microsomes from a pool of sixteen individuals were incubated with corresponding selective model substrates in presence and absence of the test compound in duplicate at 37°C. Marker substrate reactions were initiated by the addition of an aliquot of an NADPH-generating system and were automatically terminated at approximately 5 minutes by addition of stop reagent, acetonitrile. The concentrations of marker substrates were based on the *K<sub>m</sub>* or *S<sub>50</sub>* data that were determined previously.

To distinguish between time dependent and metabolism-dependent inhibition, each test article was pre-incubated with human liver microsomes for 30 minutes without and with an NADPH-generating system, respectively, prior to the incubation with the marker substrate. This pre-incubation with NADPH generating system (for metabolism-dependent inhibition) allowed for the generation of intermediates that could inhibit human CYP enzymes. The pre-incubation without NADPH generating system (for time-dependent inhibition) allowed assessment of whether any potential increase in inhibition was dependent upon NADPH (e.g., potentially CYP mediated). Known direct and metabolism-dependent inhibitors of CYP enzymes were included as positive controls in all experiments, as applicable. Solvent controls in absence of test compounds were used as the negative control. The inhibitory effects of test compound on the metabolism of CYP-specific probe substrates were determined by comparing the rate of metabolite formation in the absence and presence of different concentrations of test compound.

**Table 1: Incubation Concentrations of Probe Substrates and Positive Controls**

CYP	Model Substrate	Metabolites	Positive control Direct Inhibition	Positive-Controls Metabolism-dependent inhibition
1A2	Phenacetin (40 uM)	acetaminophen	$\alpha$ -Naphthoflavone (0.5 uM)	Furafylline (1.0 uM)
2A6	Coumarin 7-hydroxylation (0.75 uM)	7-Hydroxycoumarin (umbelliferone)	Nicotine (300 uM)	8-Methoxypsoralen (0.05 uM)
2B6	Efavirenz (3 uM)	8-Hydroxyefavirenz	Orphenadrine (750 uM)	Phencyclidine (30 uM)
2C8	Paclitaxel (10 uM)	6 $\alpha$ -Hydroxypaclitaxel	Montelukast (0.5 uM)	Gemfibrozil glucuronide (15 uM)
2C9	Diclofenac (6 uM)	4'-hydroxydiclofenac	Sulphaphenazole (2.0 uM)	Tienilic acid (0.25 uM)
2C19	S-Mephenytoin (40 uM)	4-hydroxymephenytoin	Modafinil (250 uM)	S-Fluoxetine (20 uM)
2D6	Dextromethorphan (7.5 uM)	Dextrorphan	Quinidine (0.5 uM)	Paroxetine (0.3 uM)
2E1	Chlorzoxazone (30 uM)	6-Hydroxychlorzoxazone	4-Methylpyrazole (15 uM)	3-Amino-1,2,4-triazole (10mM)
3A4	Midazolam (4 uM)	1'-hydroxymidazolam	Ketoconazole (0.075 uM)	Troleandomycin (7.5 uM)
3A4	Nifedipine (10 uM)	Oxidized nifedipine	Ketoconazole (0.15 uM)	Troleandomycin (20 uM)
3A4	Atorvastatin (70 uM)	o-Hydroxyatorvastatin	Ketoconazole (0.15 uM)	Troleandomycin (20 uM)

**Bioanalytical Method:** All metabolite analyses were performed with LC/MS/MS methods.

**Results:**

**Table 2: In vitro evaluation of Migalastat Hydrochloride as an inhibitor of human CYP enzymes**

Enzyme	Enzyme reaction	Direct inhibition		Time-dependent inhibition		Metabolism-dependent inhibition		Potential for metabolism-dependent inhibition <sup>c</sup>
		Zero-minute preincubation		30-minute preincubation without NADPH		30-minute preincubation with NADPH		
		IC <sub>50</sub> (uM) <sup>a</sup>	Inhibition observed at 500 uM (%) <sup>b</sup>	IC <sub>50</sub> (uM) <sup>a</sup>	Inhibition observed at 500 uM (%) <sup>b</sup>	IC <sub>50</sub> (uM) <sup>a</sup>	Inhibition observed at 500 uM (%) <sup>b</sup>	
CYP1A2	Phenacetin O-dealkylation	>500	2.7	>500	3.6	>500	5.5	No
CYP2A6	Coumarin 7-hydroxylation	>500	NA	>500	NA	>500	NA	No
CYP2B6	Efavirenz 8-hydroxylation	>500	NA	>500	NA	>500	3.7	No
CYP2C8	Paclitaxel 6 $\alpha$ -hydroxylation	>500	NA	>500	NA	>500	NA	No
CYP2C9	Diclofenac 4'-hydroxylation	>500	9.3	>500	7.6	>500	11	No
CYP2C19	S-Mephenytoin 4'-hydroxylation	>500	6.3	>500	5.4	>500	6.1	No
CYP2D6	Dextromethorphan O-demethylation	>500	4.8	>500	16	>500	11	No
CYP2E1	Chlorzoxazone 6-hydroxylation	>500	11	>500	14	>500	16	No
CYP3A4/5	Midazolam 1'-hydroxylation	>500	NA	>500	NA	>500	NA	No
CYP3A4/5	Nifedipine oxidation	>500	NA	>500	NA	>500	NA	No
CYP3A4/5	Atorvastatin o-hydroxylation	>500	4.0	>500	NA	>500	4.6	No

a Average data (i.e., percent of control activity) obtained from duplicate samples for each test article concentration were used to calculate IC<sub>50</sub> values.

b Inhibition observed (%) is calculated with the following formula (results are rounded to two significant figures):  
Inhibition observed (%) = 100% - Percent solvent control.

c Metabolism-dependent inhibition was determined by comparison of IC<sub>50</sub> values both with and without preincubation and with and without NADPH-generating system present in the preincubation, by comparison of the observed inhibition (%) for all preincubation conditions and by visual inspection of the IC<sub>50</sub> plots.

NA Not applicable. No value was obtained as the rates at the highest concentration of Migalastat Hydrochloride evaluated (500 uM) were higher than the control rates.

Figure 1: Direct inhibitors: Positive control graphs

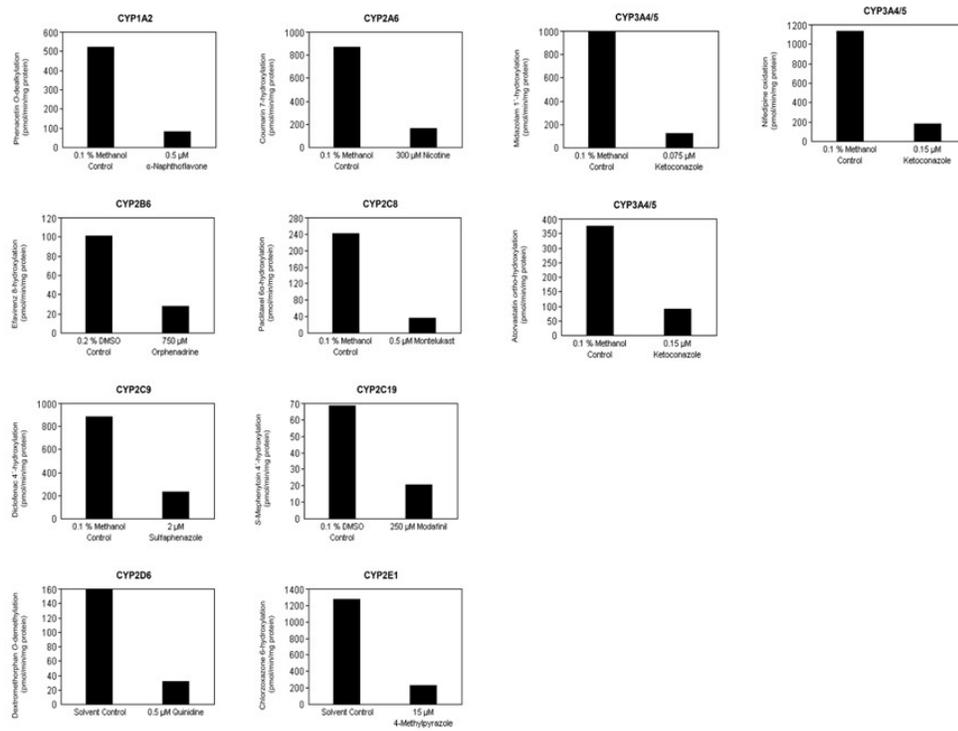
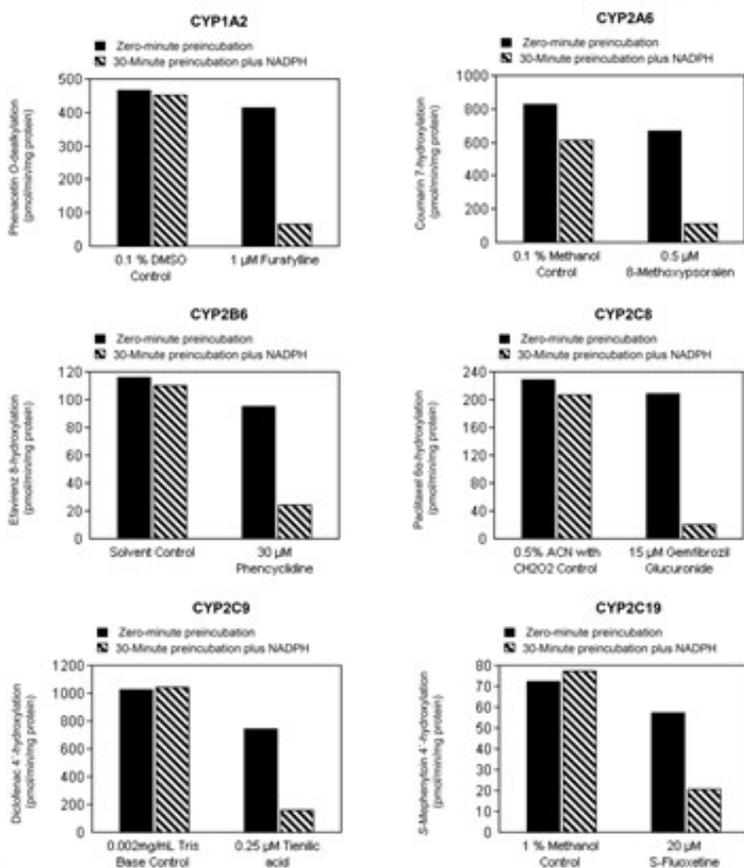


Table 2: Metabolism-dependent inhibitors: Positive control graphs



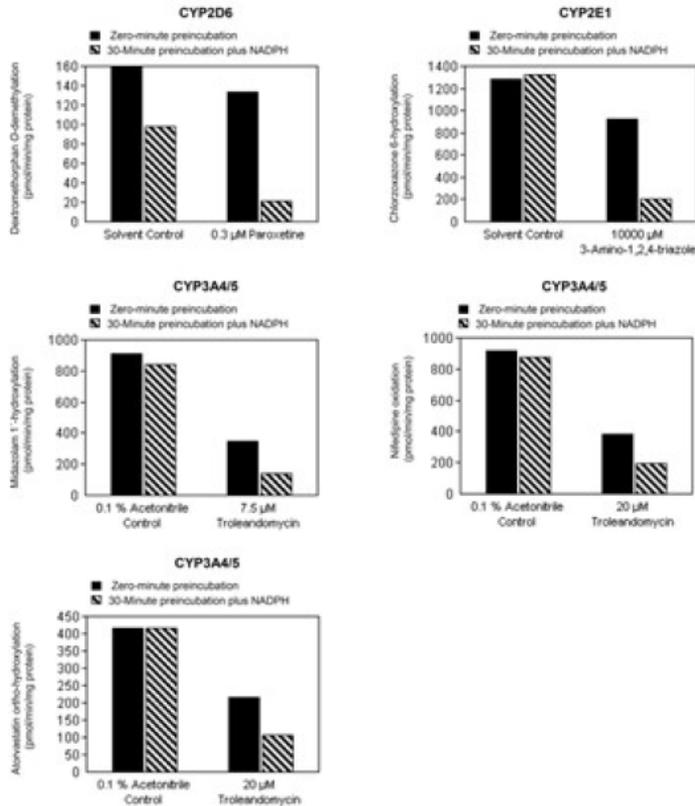


Table 3: Characterization of the test system

## Enzyme activities in a pool of sixteen individuals

Enzyme	Enzyme reaction	Pool of sixteen <sup>a</sup>	
		Observed $V_{max}$ (pmol/mg protein/min)	Observed $K_m$ (μM)
CYP1A2	Phenacetin O-dealkylation <sup>b</sup>	1140 ± 60 <sup>c</sup>	49 ± 15 <sup>c</sup>
CYP2A6	Coumarin 7-hydroxylation <sup>d</sup>	1780 ± 45 <sup>e</sup>	0.75 ± 0.04 <sup>e</sup>
CYP2B6	Efavirenz 8-hydroxylation <sup>b</sup>	250 ± 40 <sup>c</sup>	4.0 ± 0.4 <sup>c</sup>
CYP2C8	Paclitaxel 6α-hydroxylation <sup>d</sup>	650 ± 18 <sup>e</sup>	9.9 ± 0.4 <sup>e</sup>
CYP2C9	Diclofenac 4'-hydroxylation <sup>b</sup>	3240 ± 50 <sup>e</sup>	6.5 ± 0.3 <sup>e</sup>
CYP2C19	S-Mephenytoin 4'-hydroxylation <sup>b</sup>	210 ± 2 <sup>c</sup>	45 ± 4 <sup>c</sup>
CYP2D6	Dextromethorphan O-demethylation <sup>b</sup>	297 ± 6 <sup>e</sup>	8.9 ± 0.4 <sup>e</sup>
CYP2E1	Chlorzoxazone 6-hydroxylation <sup>d</sup>	1960 ± 70 <sup>e,f</sup>	24 ± 2 <sup>e,f</sup>
CYP3A4/5	Midazolam 1'-hydroxylation <sup>b</sup>	1940 ± 50 <sup>c</sup>	2.8 ± 0.1 <sup>c</sup>
CYP3A4/5	Nifedipine oxidation <sup>d</sup>	4980 ± 60 <sup>e</sup>	9.3 ± 0.2 <sup>e</sup>
CYP3A4/5	Atorvastatin o-hydroxylation <sup>b</sup>	810 ± 120 <sup>c,f</sup>	42 ± 9 <sup>c,f</sup>

- a (b) (4) lot number 0810229 is a mixed-gender pool of sixteen individual human liver microsomal samples ( (b) (4) sample code numbers 286, 290, 312, 313, 315, 333, 334, 335, 336, 339, 348, 359, 364, 383, 389 and 390).
- b The kinetic constants for these CYP activities were determined in (b) (4) 089011 (an internal (b) (4) study).
- c Constants are shown ± standard deviation (where constants are rounded to three significant figures for  $V_{max}$  values and two significant figures for  $K_m$  values, and standard errors are rounded to the same degree of accuracy as the constant).
- d The kinetic constants for these CYP activities were determined in (b) (4) 079005 (an internal (b) (4) study).
- e Constants are shown ± standard error (where constants are rounded to three significant figures for  $V_{max}$  values and two significant figures for  $K_m$  values, and standard errors are rounded to the same degree of accuracy as the constant).
- f The values for chlorzoxazone 6 hydroxylation and atorvastatin o-hydroxylation represent the high affinity component of the activity.

**Reviewer's Comment:**

1. The test system of human liver microsomes were well characterized in respect to various CYP enzymes. The observed  $K_m$  for CYP enzymes were within previously reported range.
2. The choices of CYP-specific model substrates and their respective concentrations to evaluate the inhibitory potential test compound on each CYP isoforms were acceptable as those model substrate concentrations are approximately below the respective  $K_m$  value.
3. Choices of model inhibitors as positive controls appears to be reasonable. The positive controls had expected level of inhibition to demonstrate the appropriateness of the test system.
4. The tested concentration of migalastat up to 500  $\mu\text{M}$  are acceptable as they approximately cover the  $C_{\text{max}}$  and 10 times  $C_{\text{max}}$  values to be expected in Fabry patients taking migalastat at the clinical dose of 150 mg QOD at steady states ( $C_{\text{max}}$  at steady state in Fabry patients was approximately 2029 ng/mL = 10  $\mu\text{M}$ ).
5. Under the experimental conditions examined, migalastat did not cause direct, time-dependent or metabolism-dependent inhibition of any CYP enzyme activity investigated at concentrations up to 500  $\mu\text{M}$ .
6. Migalastat did not inhibit any CYP to up 500  $\mu\text{M}$ . Therefore,  $\text{IC}_{50}$  of  $K_i > 500 \mu\text{M}$  (0.5 mM). The likelihood of migalastat to inhibit CYP3A4 in the gut is less likely as
  - a.  $I_{\text{gut}} = \text{Dose}/250\text{mL} = 123 \text{ mg}/250 \text{ mL} = 2.46 \text{ mM}$
  - b.  $R_{1\text{gut}} = 1 + I_{\text{gut}}/\text{IC}_{50} = 1 + 2.46 \text{ mM}/0.5\text{mM} = 5.95 < 11$

### 8.13 In-Vitro CYP Induction Study

**Title:** AT1001: In Vitro Cytochrome P450 Enzyme Induction Study in Human Hepatocytes

**Specific Aims:** This study was conducted to investigate the potential of AT1001 to induce CYP1A2 and CYP3A4 using cryopreserved human hepatocytes.

Report No: 08-64515

Test Site: (b) (4)

#### **Study Design:**

Test Item: AT1001

Test System: cryopreserved human hepatocytes from three individual donors

Study Method: Cryopreserved human hepatocytes (from 3 liver donors) were treated with regular incubation media for 2 days for plating followed by 2 days incubation with either AT1001 at 10, 100, and 1000  $\mu\text{M}$  or known inducers (50  $\mu\text{M}$  omeprazole for CYP1A2 and 25  $\mu\text{M}$  rifampin for CYP3A4) as positive controls. CYP1A2 and CYP3A4 enzyme activities were evaluated on the fifth day of incubation.

After treatment, the cells were harvested to isolate microsomes for the analysis of several human CYP enzymes. The activities of target enzymes CYP1A2 and CYP3A4 were assessed by incubating the microsomes with model probe substrates phenacetin (marker for CYP1A2) and testosterone (marker for CYP3A4/5) for 4 hours and monitoring the appearance rate of their respective enzyme specific metabolites.

*Table 1: Overview of substrates and inducers*

CYP isoenzyme	Model probe Substrate	Metabolite	Known Inducer (positive control)
1A2	Phenacetin (50 $\mu\text{M}$ )	acetaminophen	omeprazole (50 $\mu\text{M}$ )
3A4	Testosterone (50 $\mu\text{M}$ )	6 $\beta$ -Hydroxytestosterone	Rifampicin (25 $\mu\text{M}$ )

Bioanalytical Method: The appearances of metabolites of probe substrates were determined with LC-MS-MS method.

Data Analysis: Fold-increases were determined by dividing the enzymatic rate of each treatment group by that of the vehicle control.

$$\text{Fold Increase} = C_{\text{met}} \div C_{\text{control}} \times 100\%$$

- $C_{\text{met}}$ : Concentration of the enzyme-specific metabolite in the incubations in the presence of AT1001 or selective inducer
- $C_{\text{control}}$ : Concentration of the enzyme-specific metabolite in the incubations in the absence of AT1001 and selective inducers (in the presence of vehicle)

#### **Results:**

AT1001 did not induced CYP1A2 and CYP3A4 at concentration to up 1000 uM. The positive controls under the same condition showed significant fold induction.

*Table 1: Induction of CYP1A2 and CYP3A4 by AT1001 in Cryopreserved Human Hepatocytes*

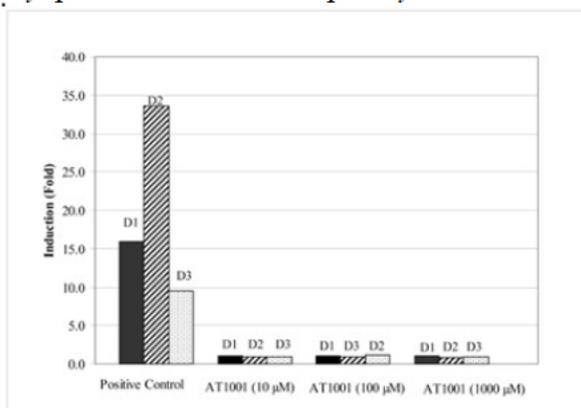
CYPs	Donor	Induction (Fold) <sup>a</sup>			
		10 μM	100 μM	1000 μM	PC <sup>c</sup>
1A2 <sup>b</sup>	1 (b) (6)	1.10	1.10	1.20	42.4
	2	0.89	0.89	0.89	20.0
	3	0.89	1.00	0.89	22.5
3A4 <sup>c</sup>	1	1.00	1.00	1.00	15.9
	2	0.94	0.97	0.87	33.6
	3	0.96	1.20	0.93	9.00

<sup>a</sup> Induction (Fold) = Activity with induced cells/Control activity. Control activity was measured in the absence of respective known inducers and AT1001 (vehicle control). Activity was measured in triplicate.

<sup>b</sup> For positive control activity of CYP1A2, Omeprazole (50 μM) and Phenacetin (50 μM) were used as the inducer and marker substrate, respectively.

<sup>c</sup> PC = positive control. For positive control activity of CYP3A4, Rifampin (25 μM) and Testosterone (50 μM) were used as the inducer and marker substrate, respectively.

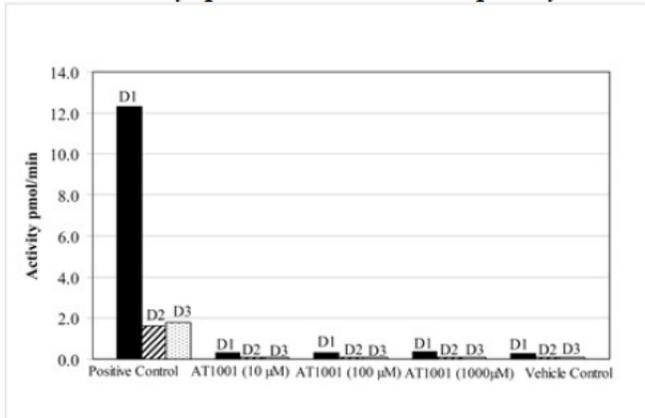
*Figure 1: Fold Increase of CYP1A2 Activity by Known Inducer (Omeprazole) and AT1001 in Cryopreserved Human Hepatocytes*



Positive control: Omeprazole (Inducer) at 50 μM.

D1: Donor 1 (b) (6)  
D2: Donor 2 (b) (6)  
D3: Donor 3

Figure 2: CYP1A2 Activity (pmol/min) by Known Inducer (Omeprazole), AT1001, and Vehicle Control in Cryopreserved Human Hepatocytes



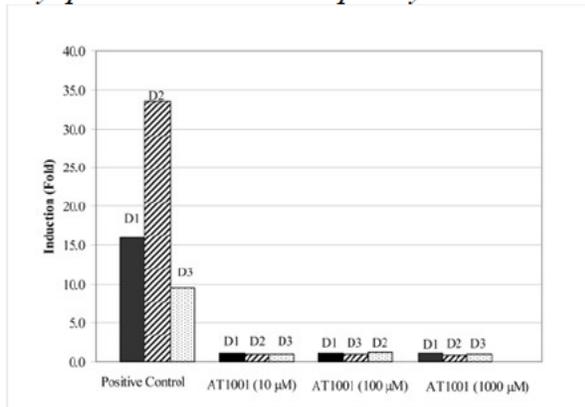
Positive control: Omeprazole (Inducer) at 50 µM.

D1: Donor 1 (b) (6)

D2: Donor 2

D3: Donor 3

Figure 3: Fold Increase of CYP3A4 Activity by Known Inducer (Rifampin) and AT1001 in Cryopreserved Human Hepatocytes



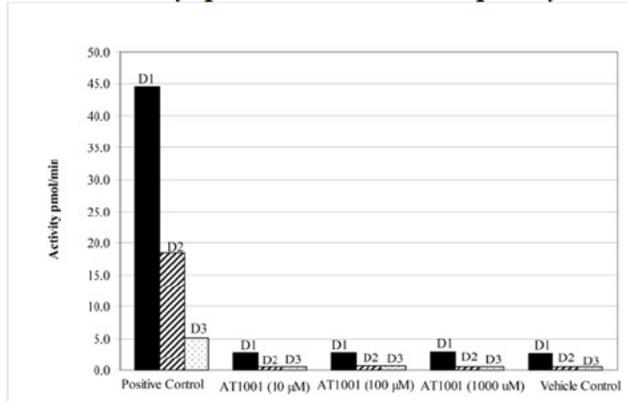
Positive control: Rifampin (Inducer) at 25 µM.

D1: Donor 1 (b) (6)

D2: Donor 2

D3: Donor 3

Figure 4: CYP3A4 Activity (pmol/min) by Known Inducer (Rifampin), AT1001, and Vehicle Control in Cryopreserved Human Hepatocytes



Positive control: Rifampin (Inducer) at 25 µM.

D1: Donor 1 (b) (6)

D2: Donor 2

D3: Donor 3

**Reviewer’s Comment:**

- The tested concentration of AT1001 at 10, 100, and 1000 µM are acceptable as they approximately cover the Cmax and 10 times Cmax values to be expected in Fabry patients taking migalastat at the clinical dose of 150 mg QOD at steady states (Cmax at steady state in Fabry patients was approximately 2029 ng/mL= 10 uM).
- The choice for positive controls (model inducers) and CYP-specific model substrate and their respective concentration to evaluate the CYP enzyme activities were appropriate.
- The test conditions were appropriate for measuring the target enzyme CYP1A2 and CYP3A4 activities as the treatment of human hepatocytes with positive controls CYP inducer caused anticipated and appropriate in CYP activity.

## 8.14 In-Vitro CYP Induction Study

**Title:** AT1001: Migalastat HCl (AT1001): Evaluation of Human Cytochrome P450 2B6 Induction Potential in Human Hepatocytes

**Specific Aims:** To evaluate the potential of migalastat (also known as AT1001) to induce one of the major inducible drug-metabolizing human cytochrome P450 (CYP) isozymes, i.e., CYP2B6, *in vitro* using cryopreserved human hepatocytes (HH).

Report No: RPT03793

Test Site: (b) (4)

### Study Design:

Test Item: AT1001

Test System: Cryopreserved human hepatocytes from three individual donors

Study Method: Cryopreserved human hepatocytes (from 3 liver donors) were treated with regular incubation media for one day for plating followed by 3 days incubation with either AT1001 at 5, 50, and 500  $\mu\text{M}$  or known inducers of CYP2B6 (750  $\mu\text{M}$  phenobarbital) and an known CYP2B6 non-inducer (25  $\mu\text{M}$  flumazenil) as positive and negative controls. At the end of incubation, the RNA of HH was isolated and the mRNA expression levels of CYP2B6 were evaluated by using real-time Polymerase Chain Reaction (qPCR) after being reverse transcribed (RT) to cDNA.

After treatment, the activity of target enzyme CYP2B6 was assessed by incubating the microsomes with model probe substrate bupropion (100  $\mu\text{M}$ ) for 1 hours and monitoring the appearance rate of their respective enzyme specific metabolites.

Bioanalytical Method: The appearances of metabolites of probe substrates were determined with LC-MS-MS method.

Data Analysis: Fold-increases were determined by dividing the enzymatic rate of each treatment group by that of the vehicle control.

Fold Increase =  $C_{\text{met}} \div C_{\text{control}} \times 100\%$

- $C_{\text{met}}$ : Concentration of the enzyme-specific metabolite in the incubations in the presence of AT1001 or selective inducer
- $C_{\text{control}}$ : Concentration of the enzyme-specific metabolite in the incubations in the absence of AT1001 and selective inducers (in the presence of vehicle)

### Results:

Migalastat did not induced CYP2B6 at concentration up to 500  $\mu\text{M}$ . Both mRNA expression levels (0.93-1.51 fold) and CYP activities (0.75-1.08 fold) did not increase significantly relative to untreated hepatocytes. The positive controls under the same condition showed significant fold induction relative to untreated hepatocytes ( $> 7$ -fold in mRNA expression levels and  $> 6$ -fold in CYP activity).

*Table 1: Summary of CYP2B6 mRNA induction by migalastat in human hepatocytes*

Donor	CYP2B6 mRNA Induction Fold <sup>a</sup> after Various Treatments				
	Migalastat 5 $\mu$ M	Migalastat 50 $\mu$ M	Migalastat 500 $\mu$ M	NC <sup>b</sup>	PC <sup>c</sup>
(b) (6)	1.30	1.11	1.01	1.07	7.34
	1.51	1.40	1.42	0.95	9.17
	0.93	0.94	1.16	1.10	10.03

<sup>a</sup> CYP2B6 mRNA induction fold was calculated as described in Section 5.4.4.

<sup>b</sup> NC – negative control, 25  $\mu$ M flumazenil was used as the negative control.

<sup>c</sup> PC – positive control, 750  $\mu$ M phenobarbital was used as the positive control.

Data are calculated from triplicate measurements.

*Table 2: Summary of CYP2B6 activity induction by migalastat in human hepatocytes*

Donor	CYP2B6 Activity Induction Fold <sup>a</sup> after Various Treatments				
	Migalastat 5 $\mu$ M	Migalastat 50 $\mu$ M	Migalastat 500 $\mu$ M	NC <sup>b</sup>	PC <sup>c</sup>
(b) (6)	0.95	1.01	0.77	1.12	9.69
	0.80 <sup>d</sup>	0.75 <sup>d</sup>	0.76	0.88	10.08
	1.08	0.87	1.02 <sup>d</sup>	0.91	6.74

<sup>a</sup> CYP activity induction fold was calculated as described in Section 5.4.5.

<sup>b</sup> NC – negative control, 25  $\mu$ M flumazenil was used as the negative control.

<sup>c</sup> PC – positive control, 750  $\mu$ M phenobarbital was used as the positive control.

<sup>d</sup> Data are calculated from duplicate measurements.

Data are calculated from triplicate measurements except data with footnote <sup>d</sup>.

**Reviewer’s Comment:**

- The tested concentrations of AT1001 at 5, 50 and 500  $\mu$ M are acceptable as they approximately cover the C<sub>max</sub> and 10 times C<sub>max</sub> values to be expected in Fabry patients taking migalastat at the clinical dose of 150 mg QOD at steady states (C<sub>max</sub> at steady state in Fabry patients was approximately 2029 ng/mL= 10  $\mu$ M).
- The choice for positive controls (model inducers) and CYP-specific model substrate and their respective concentrations to evaluate the CYP enzyme activities were appropriate.
- The test conditions were appropriate for measuring the target enzyme CYP2B6 activity as the treatment of human hepatocytes with positive controls CYP inducer caused anticipated and appropriate in CYP activity.

## 8.15 In-Vitro Transporter Substrate Study

**Title:** In vitro Interaction Studies of AT1001 with Human BCRP and MDR1 Efflux (ABC) Transporters, and with Human MATE1, MATE2-K, OAT1, OAT3 and OCT2 Uptake Transporters

**Specific Aims:** The purpose of this study was to evaluate the potential of AT1001 being a substrate of the human ABC (efflux) transporters: BCRP and MDR1; and the human SLC (uptake) transporters: MATE1, MATE2-K, OAT1, OAT3 and OCT2

**Test Site:** (b) (4)

**Test Item:** AT1001 (50 and 100 uM for BCRP and MDR1 and 10 and 100 uM for uptake transporters)

### Efflux Transporters:

**Test Systems:** Vesicular transport assays were performed with inside-out membrane vesicles prepared from cells overexpressing human ABC transporters.

### Study Method:

The uptake of AT1001 into membrane vesicles was determined in triplicates using inside-out membrane vesicles (total protein: 50 µg/well) prepared from cells overexpressing human BCRP or MDR1 as well as from control cells (not expressing BCRP or MDR1). Two incubation time points (2 and 20 min) and two concentrations (50 and 100 µM) of AT1001 were tested in the presence of ATP or AMP, to determine whether AT1001 is actively transported into the vesicles. Reactions were quenched by the addition of 200 µL of ice-cold washing buffer and immediate filtration via glass fiber filters mounted to a 96-well plate (filter plate). The filters were washed 5 × 200 µL of ice-cold washing buffer. Amount of AT1001 accumulated inside the vesicles was determined by LC-MS/MS.

Inhibition of AT1001 accumulation (50 µM) by reference inhibitor (1 µM Ko134 for BCRP and 100 µM verapamil for MDR1) in transporter containing and control vesicles was evaluated in triplicate with ATP or AMP at 2 min (all incubations containing 1% DMSO).

As positive controls, reference inhibitor or 1% DMSO was incubated with membrane vesicle preparations and the probe substrates.

*Table 1: Vesicular transport assay parameters and control incubation conditions*

Transporter	Applying protocol	Protein content/well (µg)	Incubation time (min)	Probe substrate	Reference inhibitor
human BCRP	VT-HTS-BCRP-M-E3S	25	1	E3S (1 µM)	Ko134 (1 µM)
human MDR1	VT-HTS MDR1-K-NMQ	50	3	NMQ (2 µM)	Verapamil (100 µM)

Incubations were carried out in the presence of 4 mM ATP or AMP to distinguish between transporter-mediated uptake and passive diffusion into the vesicles.

## Uptake Transporters:

Test Systems: Uptake experiments were performed using CHO or MDCKII cells stably expressing the respective uptake transporters.

### Study Method:

The uptake of AT1001 was determined in triplicates using cells overexpressing the respective uptake transporter and control cells, at two incubation time points (2 and 20 min) and at two concentrations (10 and 100  $\mu\text{M}$ ) of AT1001 (without DMSO) at  $37 \pm 1$   $^{\circ}\text{C}$  to determine whether or not AT1001 was actively taken up into the cells. In order to confirm the interaction in case of OAT3, the transporter-specific uptake of AT1001 (with 1% DMSO) was determined in the presence of a known OAT3 inhibitor (20  $\mu\text{M}$  probenecid) in transfected and control cell at 20 minutes. The amount of AT1001 accumulated inside the vesicles was determined by LC-MS/MS.

*Table 2: Parameters of uptake transporter assays*

Transporter	Applying assay protocol	Incubation time (min)	Probe substrate	Reference inhibitor
human MATE1	UPT-MDCKII-MATE1-metformin	15	Metformin (10 $\mu\text{M}$ )	Pyrimethamine (1 $\mu\text{M}$ )
human MATE2-K	UPT-MDCKII-MATE2K-metformin	15	Metformin (10 $\mu\text{M}$ )	Pyrimethamine (10 $\mu\text{M}$ )
human OAT1	UPT-CHO-OAT1-Tenofovir	10	Tenofovir (5 $\mu\text{M}$ )	Probenecid (200 $\mu\text{M}$ )
human OAT3	UPT-MDCKII-OAT3-E3S	3	E3S (1 $\mu\text{M}$ )	Probenecid (200 $\mu\text{M}$ )
human OCT2	UPT-CHO-OCT2-Metf	10	Metformin (10 $\mu\text{M}$ )	Verapamil (100 $\mu\text{M}$ )

As positive controls, the uptake of probe substrates was evaluated in presence of reference inhibitor.

### *Controls*

- Incubation with AMP provided background activity values for all data points (efflux transporters).
- Uptake of the probe substrates in control cells provided background activity values for all data points (uptake transporters).
- Incubation with probe substrate (solvent only) provided 100% activity values.
- A reference inhibitor served as positive control for inhibition.

### Statistical Analysis:

- *Efflux Transporters (ATP dependent transport):*

$$ATP - dependent transport = n_{ATP} - n_{AMP}$$

$$Fold\ accumulation = \frac{n_{ATP}}{n_{AMP}}$$

### Legend:

nATP: amount of translocated AT1001 in the presence of 4 mM ATP, in pmol/mg

nAMP: amount of translocated AT1001 in the presence of 4 mM AMP, in pmol/mg

If the ATP-dependent accumulation value is > 2-fold in transporter containing vesicles and can be inhibited by a known inhibitor of the transporter, then the test article (TA) can be considered a substrate of the transporter investigated. In addition, similar ATP-dependent accumulation should not be observed in the control vesicles lacking the transporter of interest.

- *Uptake transporter:*

$$\text{Fold accumulation} = \frac{UPT_{TRP}}{UPT_{CTL}}$$

Legend:

UPTTRP: accumulated amount of AT1001 or probe substrate in transfected cells normalized by protein content [pmol/mg protein]

UPTCTL: accumulated amount of AT1001 or probe substrate in control cells normalized by protein content [pmol/mg protein]

If the accumulation is > 2-fold and can be inhibited by a known inhibitor of the transporter, the TA can be considered to be a substrate of the respective transporter.

## RESULTS:

### Efflux Transporters:

The ATP-dependent accumulation values of AT1001 were 3.53-, 0.78-, 1.01- and 1.05-fold for BCRP and 2.07-, 1.20-, 1.12- and 1.01-fold for MDR1 (P-gp). Even though only one out of the four conditions (50  $\mu$ M and 2 min for both BCRP and MDR1) showed an accumulation value above the threshold of 2-fold for considering a compound as substrate, a follow-up assay was conducted for both transporters. The accumulation values obtained in the follow-up experiment at 50  $\mu$ M and 2 min were 0.87-fold for BCRP and 0.99-fold for MDR1, thus not reproducible in case of both transporters, indicating that AT1001 is unlikely a substrate of BCRP or MDR1. The positive control experiments applying a probe substrate confirmed the function of the transporter in the applied vesicles in all experiments.

### Uptake Transporter:

AT1001 does not appear to be a substrate for MATE1, MATE2, OAT1 and OCT2 as the accumulation of AT1001 was comparable in the MATE1, MATE2-K-, OAT1- and OCT2- expressing cells and the control cell (transporter specific accumulations were <2-fold).

However, as the accumulation in OAT3 was variable, it was difficult to draw a definitive conclusion regarding the potential of AT1001 being a substrate for OAT3.

In all experiments the positive control incubations applying a probe substrate with and without a respective inhibitor for each experiment confirmed the function of the transporter in the applied cells.

Table 3: Summary of the fold accumulation values obtained for all experiments

Vesicular transport substrate assay accumulation values (-fold)						
	BCRP	MDR1				
50 $\mu$ M and 2 minutes	3.53	2.07				
50 $\mu$ M and 20 minutes	0.78	1.20				
100 $\mu$ M and 2 minutes	1.01	1.12				
100 $\mu$ M and 20 minutes	1.05	1.01				
50 $\mu$ M and 2 minutes (without inhibitor in 1% DMSO)	0.87	0.99				
50 $\mu$ M and 2 minutes (with inhibitor in 1% DMSO)	1.64	0.85				
Uptake transport substrate assay accumulation values (-fold)						
	MATE1	MATE2-K	OAT1	OAT3 (04Aug2015)	OAT3 (08Sep2015)	OCT2
10 $\mu$ M and 2 minutes	0.78	1.35	0.91	1.04	1.14	0.88
10 $\mu$ M and 20 minutes	1.19	0.89	0.92	1.96	0.92	0.57
100 $\mu$ M and 2 minutes	1.18	0.77	0.51	1.08	15.65	0.64
100 $\mu$ M and 20 minutes	0.99	1.09	0.76	4.00	4.75	0.73
100 $\mu$ M and 20 minutes (without inhibitor in 1% DMSO)	-	-	-	-	1.00	-
100 $\mu$ M and 20 minutes (with inhibitor in 1% DMSO)	-	-	-	-	0.52	-

**Reviewer's Comment:**

- The tested concentration of AT1001 up to 500  $\mu$ M are acceptable as they approximately cover the C<sub>max</sub> and 10 times C<sub>max</sub> values to be expected in Fabry patients taking migalastat at the clinical dose of 150 mg QOD at steady states (C<sub>max</sub> at steady state in Fabry patients was approximately 2029 ng/mL= 10  $\mu$ M).
- All of the test systems appear to be valid as positive controls with model substrates has significant accumulation in uptake transporter and the uptake were significantly inhibited in presence of model inhibitors (positive controls).

As the result of current study was inconclusive regarding the interaction of migalastat with OAT3, the sponsor had conducted a follow study evaluating the potential of AT1001 being a substrate for OAT3. In this follow-up study, accumulation of AT1001 was similar between the OAT3-over expressing and control cell up to 300  $\mu$ M AT1001 concentration indicating that AT1001 is unlikely a substrate for OAT3 transporter. The test system appear to be valid as positive control with model substrate (1  $\mu$ M E3S) has significant accumulation in uptake transporter (27-fold) and the uptake were significantly inhibited in presence of model inhibitors probenecid (4-fold).

Table 4: Summary of the fold accumulation values for OAT3 Transporter

Transporter and assay type	Conditions	Fold accumulation	Substrate
OAT3 uptake substrate	3 $\mu$ M and 2 minutes	NA	Unlikely
	3 $\mu$ M and 20 minutes	1.19	
	10 $\mu$ M and 2 minutes	1.14	
	10 $\mu$ M and 20 minutes	0.95	
	30 $\mu$ M and 2 minutes	1.25	
	30 $\mu$ M and 20 minutes	1.01	
	100 $\mu$ M and 2 minutes	1.08	
	100 $\mu$ M and 20 minutes	1.03	
	300 $\mu$ M and 2 minutes	1.52	
	300 $\mu$ M and 20 minutes	1.16	

## 8.16 In-Vitro Transporters Inhibition Study

**Title:** In vitro Interaction Studies of AT1001 with human BCRP, BSEP and MDR1 Efflux (ABC) Transporters, and with human MATE1, MATE2-K, OAT1, OAT3, OATP1B1, OATP1B3, OCT1 and OCT2 Uptake Transporters.

**Specific Aims:** The purpose of this study was to provide data on the interaction of AT1001 with the human ABC (efflux) transporters: BCRP (ABCG2/MXR), BSEP (ABCB11/sP-gp) and MDR1 (ABCB1/P-gp); and the human SLC (uptake) transporters: MATE1, MATE2-K, OATP1B1 (OATP2, OATP-C), OATP1B3 (OATP8), OAT1, OAT3, OCT1 and OCT2.

**Test Site:** (b) (4)

**Test Item:** AT1001 (migalastat) at 0.69, 2.06, 6.17, 18.5, 55.5, 166.7 and 500  $\mu\text{M}$

### **Efflux Transporters:**

**Test Systems:** Vesicular transport assays were performed with inside-out membrane vesicles prepared from cells overexpressing human ABC transporters

### **Study Method:**

AT1001 was incubated with membrane vesicle preparations and the probe substrates in triplicates. Incubations were carried out in the presence of 4 mM ATP or AMP to distinguish between transporter-mediated uptake and passive diffusion into the vesicles. AT1001 was added to the reaction mixture in 0.75 (in case of BCRP) or 1.5  $\mu\text{l}$  (in case of MDR1 and BSEP) purified water. Reaction mixtures were pre-incubated for ten minutes at  $37 \pm 1^\circ\text{C}$  (or  $32 \pm 1^\circ\text{C}$  for BCRP). Reactions were initiated by the addition of 25  $\mu\text{l}$  of 12 mM MgATP (or 12 mM AMP in assay buffer as a background control), pre-incubated separately. After the incubation time (1-5 minutes), reactions were quenched by the addition of 200  $\mu\text{l}$  of ice-cold washing buffer and immediate filtration via glass fiber filters mounted to a 96-well plate (filter plate). The filters were washed, dried and the amount of substrate inside the filtered vesicles determined by liquid scintillation counting.

*Table 1: Vesicular transport assay parameters*

Transporter	Applying protocol	Assay buffer	Protein content ( $\mu\text{g}$ )	Incubation time (min)	Probe substrate	Reference inhibitor
human BCRP (ABCG2)	VT-HTS-BCRP-M-E3S	START Mix	25	1	E3S (1 $\mu\text{M}$ )	Ko134 (1 $\mu\text{M}$ )
human BSEP (ABCB11, sP-gp)	VT-HTS-BSEP-S $\beta$ -TC	BSEP Assay Mix	50	5	TC (2 $\mu\text{M}$ )	Cyclosporin A (20 $\mu\text{M}$ )
human MDR1 (ABCB1/P-gp)	VT-HTS MDR1-K-NMQ	START Mix	50	3	NMQ (2 $\mu\text{M}$ )	Verapamil (100 $\mu\text{M}$ )

### **Uptake Transporters:**

**Test Systems:** Uptake experiments were performed using CHO, MDCKII, or HEK293 cells stably expressing the respective uptake transporters.

### Study Method:

Uptake experiments were carried out at  $37 \pm 1$  °C in 50 µl of HK buffer (pH 7.4, pH 8.0 for MATE1 and MATE2-K) containing the probe substrate and AT1001 or solvent in triplicates. After the experiment, cells were washed twice with ice cold, 100 µl of HK buffer and lysed with 50 µl of 0.1 M NaOH. Radiolabelled probe substrate transport was determined by measuring an aliquot (35 µl) from each well for liquid scintillation counting.

Table 2: Parameters of uptake transporter assays

Transporter	Applying assay protocol	Incubation time (min)	Probe substrate	Reference inhibitor
human MATE1	UPT-MDCKII-MATE1-metformin	15	Metformin (10 µM)	Pyrimethamine (1 µM)
human MATE2-K	UPT-MDCKII-MATE2K-metformin	15	Metformin (10 µM)	Pyrimethamine (10 µM)
human OATP1B1	UPT-HEK293-OATP1B1-E <sub>2</sub> 17βG	3	E <sub>2</sub> 17βG (1 µM)	Rifampicin (50 µM)
human OATP1B3	UPT-HEK293-OATP1B3-CCK8	10	CCK-8 (0.1 µM)	Rifampicin (50 µM)
human OAT1	UPT-CHO-OAT1-Tenofovir	10	Tenofovir (5 µM)	Probenecid (200 µM)
human OAT3	UPT-MDCKII-OAT3-E3S	3	E3S (1 µM)	Probenecid (200 µM)
human OCT1	UPT-CHO-OCT1-Metf	20	Metformin (10 µM)	Verapamil (100 µM)
human OCT2	UPT-CHO-OCT2-Metf	10	Metformin (10 µM)	Verapamil (100 µM)

### Controls:

1. Incubation with AMP provided background activity values for all data points (efflux).
2. Uptake of the probe substrate in control cells provided background activity values for all data points (uptake transporters).
3. Incubation without est article (TA) or reference inhibitor (vehicle only) provided 100% activity values.
4. A reference inhibitor served as positive control for inhibition.

### Statistical Analysis:

- *Efflux Transporters:*

$$\text{Activity \%} = \frac{A-B}{C-D} \times 100$$

### Legend:

A: amount of translocated substrate in the presence of TA and ATP

B: amount of translocated substrate in the presence of TA and AMP

C: amount of translocated substrate in the presence of solvent and ATP

D: amount of translocated substrate in the presence of solvent and AMP

- *Uptake transporter:*

$$\text{Activity \%} = \frac{A - B}{C - D} \times 100$$

Legend:

A: amount of translocated substrate in the presence of TA in transfected cells

B: amount of translocated substrate in the presence of TA in control cells

C: amount of translocated substrate in the presence of solvent in transfected cells

D: amount of translocated substrate in the presence of solvent in control cells

## RESULTS:

AT1001 at concentration up to 500 uM does not inhibit BCRP, MDR1(P-gp) and BSEP human efflux transporters and OATP1B1, OATP1B3, OAT1, OAT3, OCT1, OCT2, MATE1 and MATE2-K human uptake transporters.

Table 3: Calculated reaction parameters from vesicular transport inhibition assays

Test article	Transporter	IC <sub>50</sub> (μM)	Maximum inhibition (% of control)
AT1001	BCRP	NA	NIO
	MDR1	NA	NIO
	BSEP	NA	NIO

Table 4: Calculated reaction parameters from uptake transporter inhibition assays

Test article	Transporter	IC <sub>50</sub> (μM)	Maximum inhibition (% of control)
AT1001	OATP1B1	NA	NIO
	OATP1B3	NA	NIO
	OAT1	NA	NIO
	OAT3	NA	NIO
	OCT1	NA	NIO
	OCT2	NA	NIO
	MATE1	NA	NIO
	MATE2-K	NA	NIO

NA: Not applicable, No IC50 was calculate

NIO: No interaction observed, defined as inhibition being less than 20%

Table 5: Effect of AT1001 on the BCRP-mediated transport of E3S measured in the vesicular transport inhibition assay

Compound	Nominal Concentration (μM)	Transporter specific transport (pmol/mg/min)	Relative transport (% of control)
AT1001	500.00	259.27 ± 12.63	106.84 ± 13.08
	166.67	245.17 ± 32.52	101.03 ± 17.56
	55.56	270.97 ± 8.52	111.66 ± 13.02
	18.52	262.84 ± 4.34	108.31 ± 12.29
	6.17	249.78 ± 27.63	102.93 ± 16.22
	2.06	243.02 ± 33.83	100.15 ± 17.91
	0.69	257.96 ± 16.95	106.30 ± 13.83
	H <sub>2</sub> O	242.67 ± 27.25	100.00 ± 15.88
Ko134	1.00	13.15 ± 3.59	4.10 ± 1.25
	DMSO	320.41 ± 43.14	100.00 ± 19.04

Table 6: Effect of AT1001 on the MDR1-mediated transport of NMQ measured in the vesicular transport inhibition assay

Compound	Nominal Concentration ( $\mu\text{M}$ )	Transporter specific transport (pmol/mg/min)	Relative transport (% of control)
AT1001	500.00	518.72 $\pm$ 45.06	108.51 $\pm$ 9.55
	166.67	554.81 $\pm$ 17.33	116.06 $\pm$ 3.99
	55.56	557.19 $\pm$ 7.28	116.56 $\pm$ 2.25
	18.52	564.67 $\pm$ 12.90	118.13 $\pm$ 3.18
	6.17	578.67 $\pm$ 27.58	121.06 $\pm$ 6.02
	2.06	549.02 $\pm$ 14.50	114.85 $\pm$ 3.45
	0.69	509.36 $\pm$ 12.93	106.56 $\pm$ 3.10
	H <sub>2</sub> O	478.02 $\pm$ 6.82	100.00 $\pm$ 2.02
Verapamil	100.00	12.73 $\pm$ 2.73	2.84 $\pm$ 0.61
	DMSO	449.10 $\pm$ 4.64	100.00 $\pm$ 1.46

Table 7: Effect of AT1001 on the BSEP-mediated transport of taurocholate measured in the vesicular transport inhibition assay

Compound	Nominal Concentration ( $\mu\text{M}$ )	Transporter specific transport (pmol/mg/min)	Relative transport (% of control)
AT1001	500.00	25.39 $\pm$ 1.69	103.75 $\pm$ 7.11
	166.67	26.48 $\pm$ 3.07	108.18 $\pm$ 12.66
	55.56	25.54 $\pm$ 0.63	104.34 $\pm$ 3.08
	18.52	26.38 $\pm$ 0.89	107.77 $\pm$ 4.03
	6.17	27.65 $\pm$ 1.47	112.95 $\pm$ 6.28
	2.06	26.76 $\pm$ 1.82	109.31 $\pm$ 7.65
	0.69	27.26 $\pm$ 1.00	111.38 $\pm$ 4.48
	H <sub>2</sub> O	24.48 $\pm$ 0.40	100.00 $\pm$ 2.32
Cyclosporin A	20.00	2.42 $\pm$ 0.70	8.49 $\pm$ 2.50
	DMSO	28.48 $\pm$ 1.39	100.00 $\pm$ 6.89

Table 8: Effect of AT1001 on the OATP1B1-mediated transport of E217 $\beta$ G measured in the uptake transporter inhibition assay

Compound	Nominal Concentration ( $\mu\text{M}$ )	Transporter specific transport (cpm)	Relative transport (% of control)
AT1001	500.00	4168.67 $\pm$ 203.14	102.93 $\pm$ 5.08
	166.67	3876.00 $\pm$ 367.62	95.70 $\pm$ 9.11
	55.56	4084.33 $\pm$ 56.09	100.85 $\pm$ 1.60
	18.52	4062.00 $\pm$ 99.73	100.30 $\pm$ 2.59
	6.17	4162.00 $\pm$ 331.57	102.77 $\pm$ 8.23
	2.06	4038.33 $\pm$ 237.01	99.71 $\pm$ 5.90
	0.69	4078.67 $\pm$ 123.86	100.71 $\pm$ 3.16
	H <sub>2</sub> O	4050.00 $\pm$ 31.81	100.00 $\pm$ 1.11
Rifampicin	50.00	193.33 $\pm$ 13.94	4.61 $\pm$ 0.35
	DMSO	4194.67 $\pm$ 107.73	100.00 $\pm$ 3.63

Table 9: Effect of AT1001 on the OATP1B3-mediated transport of CCK-8 measured in the uptake transporter inhibition assay

Compound	Nominal Concentration ( $\mu\text{M}$ )	Transporter specific transport (cpm)	Relative transport (% of control)
AT1001	500.00	706.67 $\pm$ 50.43	97.25 $\pm$ 7.48
	166.67	682.33 $\pm$ 12.46	93.90 $\pm$ 3.19
	55.56	714.33 $\pm$ 58.74	98.30 $\pm$ 8.56
	18.52	729.67 $\pm$ 24.79	100.41 $\pm$ 4.47
	6.17	719.33 $\pm$ 39.51	98.99 $\pm$ 6.13
	2.06	770.00 $\pm$ 39.52	105.96 $\pm$ 6.23
	0.69	736.00 $\pm$ 25.39	101.28 $\pm$ 4.54
	H <sub>2</sub> O	726.67 $\pm$ 20.85	100.00 $\pm$ 4.06
Rifampicin	50.00	8.33 $\pm$ 9.87	1.24 $\pm$ 1.47
	DMSO	671.67 $\pm$ 31.21	100.00 $\pm$ 6.57

Table 10: Effect of AT1001 on the OAT1-mediated transport of tenofovir measured in the uptake transporter inhibition assay

Compound	Nominal Concentration ( $\mu\text{M}$ )	Transporter specific transport (cpm)	Relative transport (% of control)
AT1001	500.00	1306.00 $\pm$ 110.14	95.91 $\pm$ 8.53
	166.67	1280.67 $\pm$ 108.29	94.05 $\pm$ 8.38
	55.56	1314.33 $\pm$ 102.86	96.52 $\pm$ 8.03
	18.52	1343.33 $\pm$ 46.87	98.65 $\pm$ 4.43
	6.17	1300.67 $\pm$ 96.96	95.52 $\pm$ 7.61
	2.06	1292.67 $\pm$ 42.54	94.93 $\pm$ 4.12
	0.69	1376.67 $\pm$ 108.98	101.10 $\pm$ 8.50
	H <sub>2</sub> O	1361.67 $\pm$ 38.45	100.00 $\pm$ 3.99
Probenecid	200.00	99.33 $\pm$ 19.17	8.00 $\pm$ 1.56
	DMSO	1242.33 $\pm$ 37.86	100.00 $\pm$ 4.31

Table 11: Effect of AT1001 on the OAT3-mediated transport of E3S measured in the uptake transporter inhibition assay

Compound	Nominal Concentration ( $\mu\text{M}$ )	Transporter specific transport (cpm)	Relative transport (% of control)
AT1001	500.00	922.00 $\pm$ 70.57	87.39 $\pm$ 8.12
	166.67	1041.33 $\pm$ 30.62	98.70 $\pm$ 5.95
	55.56	1151.00 $\pm$ 47.31	109.10 $\pm$ 7.28
	18.52	1079.33 $\pm$ 125.06	102.31 $\pm$ 13.02
	6.17	1104.67 $\pm$ 88.22	104.71 $\pm$ 10.01
	2.06	1060.00 $\pm$ 93.80	100.47 $\pm$ 10.34
	0.69	972.33 $\pm$ 24.17	92.16 $\pm$ 5.36
	H <sub>2</sub> O	1055.00 $\pm$ 55.49	100.00 $\pm$ 7.44
Probenecid	200.00	80.00 $\pm$ 24.90	6.51 $\pm$ 2.05
	DMSO	1229.33 $\pm$ 64.03	100.00 $\pm$ 7.37

Table 12: Effect of AT1001 on the OCT1-mediated transport of metformin measured in the uptake transporter inhibition assay

Compound	Nominal Concentration ( $\mu\text{M}$ )	Transporter specific transport (cpm)	Relative transport (% of control)
AT1001	500.00	133.67 $\pm$ 7.96	106.37 $\pm$ 7.73
	166.67	126.00 $\pm$ 11.08	100.27 $\pm$ 9.75
	55.56	129.33 $\pm$ 11.03	102.92 $\pm$ 9.77
	18.52	138.33 $\pm$ 8.68	110.08 $\pm$ 8.29
	6.17	131.00 $\pm$ 20.53	104.24 $\pm$ 16.91
	2.06	134.33 $\pm$ 8.21	106.90 $\pm$ 7.90
	0.69	140.00 $\pm$ 12.41	111.41 $\pm$ 10.91
	H <sub>2</sub> O	125.67 $\pm$ 5.23	100.00 $\pm$ 5.88
Verapamil	100.00	1.00 $\pm$ 1.41	0.84 $\pm$ 1.19
	DMSO	119.33 $\pm$ 14.67	100.00 $\pm$ 17.39

Table 13: Effect of AT1001 on the OCT2-mediated transport of metformin measured in the uptake transporter inhibition assay

Compound	Nominal Concentration ( $\mu\text{M}$ )	Transporter specific transport (cpm)	Relative transport (% of control)
AT1001	500.00	397.33 $\pm$ 40.68	104.20 $\pm$ 11.01
	166.67	401.33 $\pm$ 23.44	105.24 $\pm$ 6.73
	55.56	408.67 $\pm$ 7.96	107.17 $\pm$ 3.48
	18.52	407.00 $\pm$ 9.36	106.73 $\pm$ 3.71
	6.17	396.33 $\pm$ 12.06	103.93 $\pm$ 4.16
	2.06	401.33 $\pm$ 12.86	105.24 $\pm$ 4.34
	0.69	397.67 $\pm$ 4.62	104.28 $\pm$ 2.97
	H <sub>2</sub> O	381.33 $\pm$ 9.92	100.00 $\pm$ 3.68
Verapamil	100.00	9.67 $\pm$ 1.83	3.26 $\pm$ 0.65
	DMSO	296.67 $\pm$ 19.19	100.00 $\pm$ 9.15

Table 14: Effect of AT1001 on the MATE1-mediated transport of metformin measured in the uptake transporter inhibition assay

Compound	Nominal Concentration ( $\mu\text{M}$ )	Transporter specific transport (cpm)	Relative transport (% of control)
AT1001	500.00	482.33 $\pm$ 70.56	84.62 $\pm$ 13.37
	166.67	597.67 $\pm$ 50.27	104.85 $\pm$ 10.82
	55.56	584.67 $\pm$ 61.24	102.57 $\pm$ 12.37
	18.52	580.33 $\pm$ 29.96	101.81 $\pm$ 8.04
	6.17	655.67 $\pm$ 23.20	115.03 $\pm$ 7.99
	2.06	611.00 $\pm$ 7.39	107.19 $\pm$ 6.54
	0.69	618.00 $\pm$ 36.70	108.42 $\pm$ 9.14
	H <sub>2</sub> O	570.00 $\pm$ 34.10	100.00 $\pm$ 8.46
Pyrimethamine	1.00	52.67 $\pm$ 11.30	10.00 $\pm$ 2.15
	DMSO	526.67 $\pm$ 10.46	100.00 $\pm$ 2.81

Table 15: Effect of AT1001 on the MATE2-K-mediated transport of metformin measured in the uptake transporter inhibition assay

Compound	Nominal Concentration (μM)	Transporter specific transport (cpm)	Relative transport (% of control)
AT1001	500.00	231.00 ± 5.63	110.70 ± 5.97
	166.67	210.00 ± 5.54	100.64 ± 5.52
	55.56	228.67 ± 5.42	109.58 ± 5.87
	18.52	218.00 ± 13.37	104.47 ± 8.14
	6.17	246.67 ± 13.17	118.21 ± 8.49
	2.06	239.00 ± 3.56	114.54 ± 5.77
	0.69	234.33 ± 6.06	112.30 ± 6.13
	H <sub>2</sub> O	208.67 ± 10.03	100.00 ± 6.80
Pyrimethamine	10.00	19.33 ± 4.51	9.08 ± 2.23
	DMSO	213.00 ± 16.48	100.00 ± 10.94

Reviewer's Comment:

- AT1001 at concentration up to 500 uM does not inhibit BCRP, MDR1 (P-gp) and BSEP human efflux transporters and OATP1B1, OATP1B3, OAT1, OAT3, OCT1, OCT2, MATE1 and MATE2-K human uptake transporters.
- The test systems appear to be valid as the positive controls with model inhibitors had significant inhibited the uptake of probe substrates compared to vehicle control for all transporters.
- The tested concentration of AT1001 up to 500 μM are acceptable as they approximately cover the C<sub>max</sub> and 10 times C<sub>max</sub> values to be expected in Fabry patients taking migalastat at the clinical dose of 150 mg QOD at steady states (C<sub>max</sub> at steady state in Fabry patients was approximately 2029 ng/mL= 10 uM).
- Migalastat did not inhibit any transporter to up 500 uM. Therefore, IC<sub>50</sub> of K<sub>i</sub> > 500 uM (0.5 mM). The likelihood of migalastat to inhibit P-gp in the gut is less likely as
  - $I_{\text{gut}} = \text{Dose}/250\text{mL} = 123 \text{ mg}/250 \text{ mL} = 2.46 \text{ mM}$
  - $R1_{\text{gut}} = 1 + I_{\text{gut}}/\text{IC}_{50} = 1 + 2.46 \text{ mM}/0.5\text{mM} = 5.95 < 11$

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**This is a representation of an electronic record that was signed electronically. Following this are manifestations of any and all electronic signatures for this electronic record.**

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/s/  
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DILARA JAPPAR  
08/03/2018

PAULA L HYLAND  
08/03/2018

INSOOK KIM on behalf of JAMES L WEAVER  
08/03/2018

JUSTIN C EARP  
08/03/2018

LIAN MA  
08/04/2018

INSOOK KIM  
08/04/2018

MICHAEL A PACANOWSKI  
08/06/2018



My review is complete and has been added to the multidisciplinary review and evaluation document. My review is based on the information currently in the administrative record. If I must review information that is subsequently added to the administrative record, I will update my part of the multidisciplinary review and evaluation document accordingly.

**Study title: AT1001: 104-Week Oral Carcinogenicity Study in Sprague Dawley Rats**

Study no.: G4970  
Study report location: N/A  
Conducting laboratory and location: [REDACTED] (b) (4)  
Date of study initiation: 08/06/2009  
GLP compliance: Yes  
QA statement: Yes  
Drug, lot #, and % purity: AT1001 / 98.7-101.1% purity  
Lot No: 1) F09-03208  
2) CMLW-081/07-AM5  
3) CMLW-037/06-AM5  
4) F10-02433  
5) F11-00767  
6) F11-00766  
7) F11-00765  
8) F11-00692  
9) F11-00768  
10) F10-04862  
CAC concurrence: No (E-CAC meeting held on 06/12/2007)

**Key Study Findings:**

- No drug-related tumors were observed in the 2-year rat carcinogenicity study.



## Methods

Doses: See below  
Frequency of dosing: Twice daily  
Dose volume: 5 ml/kg twice daily  
Route of administration: Oral gavage  
Formulation/Vehicle: Water  
Basis of dose selection: See below  
Species/Strain: Sprague Dawley rats  
Males: 272.02 g  
Females: 183.2 g  
Number/Sex/Group: 50  
Age: 7-8 weeks  
Animal housing: Individually

Dual control employed: Yes  
Interim sacrifice: No  
Satellite groups: 18/sex/group (10/sex/group for control)  
Deviation from study protocol: Deviations did not have a significant impact on the study outcome.

The following doses were given twice daily 6 hours apart (see Applicant's table below).

/

**Main Toxicology groups:**

Group No.	Group	Colour of cage card	Dose (mg/kg Bwt/ day)*	No. of rats	Sex	Rat Numbers	
						From	To
G1	Vehicle Control - 1	White	0	50	M	Ri1721	Ri1770
				50	F	Ri1771	Ri1820
G2	Vehicle Control - 2	White	0	50	M	Ri1821	Ri1870
				50	F	Ri1871	Ri1920
G3	Low Dose	Yellow	50	50	M	Ri1921	Ri1970
				50	F	Ri1971	Ri2020
G4	Mid dose	Green	200	50	M	Ri2021	Ri2070
				50	F	Ri2071	Ri2120
G5	High dose	Pink	800/1200#	50	M	Ri2121	Ri2170
				50	F	Ri2171	Ri2220

**Toxicokinetic groups (TK):**

Group No.	Group	Colour of cage card	Dose (mg/kg Bwt/ day)*	No. of rats	Sex	Rat Numbers	
						From	To
G1TK	Vehicle Control	White	0	10	M	Ri2221	Ri2230
				10	F	Ri2231	Ri2240
G3TK	Low Dose	Yellow	50	18	M	Ri2241	Ri2258
				18	F	Ri2259	Ri2276
G4TK	Mid dose	Green	200	18	M	Ri2277	Ri2294
				18	F	Ri2295	Ri2312
G5TK	High dose	Pink	800/1200#	18	M	Ri2313	Ri2330
				18	F	Ri2331	Ri2348

\*: The daily dose for all groups including controls was administered at an equivolume of 5 ml/kg body weight for each dose administration (total of 10 mL/kg/day) which was administered approximately 6 hours apart each day.

#: G5 rats were treated with 800 mg/kg/day during weeks 1 - 35, and then 1200 mg/kg/day from week 36 till termination.

M: Male; F: Female

The study protocol was presented to the Executive Carcinogenicity Assessment Committee (E-CAC) on June 12, 2007. The proposed doses were (b) (4) based on a pharmacokinetic endpoint. However, the E-CAC did not agree with the Applicant's proposal, and expressed that there was inadequate data to support the dose selection based on exposure levels. This assessment was based on the proposed (b) (4) in the carcinogenicity study, whereas dosing was performed twice daily in the 26-week dose-ranging study. The Applicant initiated the study using the proposed doses without further consultation with the E-CAC. However, rats were



dosed twice daily 6 hours apart, in contrast to the proposed [REDACTED] (b) (4) in the original protocol. The high dose of 800 mg/kg/day was increased to 1200 mg/kg/day during week 36. The estimated rat to human AUC ratio at the high dose of 800 and 1200 mg/kg/day was approximately 23 and 39, respectively, which indicates that the weighted-average AUC multiple was expected to exceed 25-fold. However, the calculated rat to human AUC ratio based on the weighted-average AUC in the high-dose group and the AUC in Fabry disease patients is 24.5 (see section 5.5.3 above). Thus, it appears that the initial estimate of the rat to human exposure multiple was based on the AUC in healthy volunteers, which is lower than that observed in Fabry disease patients.

**Observations and times:**

- **Mortality:** Daily
- **Clinical signs:** Daily
- **Body weights:** Weekly
- **Food consumption:** Weekly
- **Ophthalmology:** Once during the predose phase and at termination
- **Gross pathology:** Animals were necropsied at termination
- **Histopathology:** The following organs or tissues were collected for examination (table was taken from study report).



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Tissue	Collection and Preservation	Microscopic examination
1. Liver	X	X
2. Kidneys	X	X
3. Ureters	X	X
4. Lungs with bronchi (To be inflated with fixative and then immersed in formalin).	X	X
5. Spleen	X	X
6. Heart	X	X
7. Aorta	X	X
8. Thymus	X	X
9. Esophagus	X	X
10. Stomach (glandular and non-glandular)	X	X
11. Duodenum	X	X
12. Ileum with peyer's patch, Jejunum	X	X
13. Cecum, Colon, Rectum	X	X
14. Lymph nodes (mesenteric and mandibular)	X	X
15. Eyes with optic nerve(Collected in Davidson's fluid)	X	X
16. Salivary glands	X	X
17. Thyroid with parathyroids	X	X
18. Adrenals	X	X
19. Urinary Bladder	X	X
20. Urethra	X	X
21. Ovaries with oviducts	X	X



Tissue	Collection and Preservation	Microscopic examination
22. Uterus with cervix, vagina	X	X
23. Testes (collected in modified Davidson's fluid)	X	X
24. Epididymides	X	X
25. Prostate	X	X
26. Seminal vesicles with coagulating glands	X	X
27. Mammary gland	X	X
28. Skin	X	X
29. Pituitary	X	X
30. Spinal cord (cervical, thoracic and lumbar)	X	X
31. Brain (pons, medulla, cerebellum, cerebrum)	X	X
32. Skeletal muscle	X	X
33. Sciatic nerves	X	X
34. Femur with tibial joint, sternum with marrow and bone marrow smear* (femur)	X	X
35. Pancreas	X	X
36. All gross lesions (including all grossly identified tumors)	X	X
37. Animal identification (tail tattoo)#	X	-
38. Harderian gland#	X	X
39. Larynx#	X	X
40. Nasal passage#	X	X
41. Preputial glands#	X	X
42. Clitoral gland#	X	X
43. Tongue#	X	X
44. Trachea#	X	X
45. Pharynx#	X	X

X: Organs collected and preserved and subjected to the microscopic examination.

\*: Collected from all rats and stained with Giemsa.

#: Collected from day 532 onwards.

– **Toxicokinetics:** Blood was collected before dosing and at 0.5, 1, 2, 6, 7, 8, 12, and 24 hours after dosing on day 1, and during weeks 29 and 36.

## Results:

**Mortality:** Treatment with the test article did not significantly affect survival. The mortality data were summarized in the following tables (taken from the study report).



Moribundity/mortality summary table

Sex	Males					Females				
	0	0	50	200	800/1200 <sup>^</sup>	0	0	50	200	800/1200 <sup>^</sup>
Dose (mg/kg Bwt/day)	0	0	50	200	800/1200 <sup>^</sup>	0	0	50	200	800/1200 <sup>^</sup>
Number of animals on study	50	50	50	50	50	50	50	50	50	50
Number of preterminally dead and moribund sacrificed animals	33	33	30	29	28#	25	20	21#	31	23
Number of animals at terminal kill	17	17	20	21	22	25	30	29	19	27
% survival at Week 104	34	34	40	42	44	50	60	58	38	54

<sup>^</sup>: G5 rats were treated with 800 mg/kg/day during Weeks 1 –35, and then 1200 mg/kg/day from Week 36 till termination.

#: which includes 1 rat which died during staggered terminal sacrifice

Sex	Males					Females				
	0	0	50	200	800/1200 <sup>^</sup>	0	0	50	200	800/1200 <sup>^</sup>
Dose (mg/kg Bwt/day)	0	0	50	200	800/1200 <sup>^</sup>	0	0	50	200	800/1200 <sup>^</sup>
Number of Found Dead/Unscheduled Sacrificed	33	33	30	29	28	25	20	21	31	23
Cause of Death										
Could not be determined	12	2	4	5	3	3	2	4	3	1
Neoplasm	4	4	7	6	6	21	16	17	25	21
Abscess	0	0	1	0	0	0	0	0	0	0
Brain - Degenerative changes	1	0	0	1	0	0	0	0	0	0
Urogenital tract infection/inflammation	0	2	1	1	0	0	0	0	2	0
Multiorgan necrosis	0	0	0	0	0	0	0	0	1	0
Nephropathy	16	24	14	14	18	1	1	0	0	1
Nephropathy & renal abscess	0	0	0	0	0	0	1	0	0	0
Splenic abscess	0	0	1	0	0	0	0	0	0	0
Suppurative infection/Suppurative inflammation	0	1	1	1	1	0	0	0	0	0
Suppurative meningitis	0	0	0	1	0	0	0	0	0	0
Suppurative pyelonephritis	0	0	1	0	0	0	0	0	0	0

<sup>^</sup>: G5 rats were treated with 800 mg/kg/day during Weeks 1 –35, and then 1200 mg/kg/day from Week 36 till termination.

**Clinical Signs:** There were no clear treatment-related changes.

**Body Weight:** The initial and final body weights for the control animals were 272 g and 563 g, respectively, for males and 183 g and 370 g, respectively, for females. There were no treatment-related changes.

**Feed Consumption:** There were no treatment-related changes.

**Gross Pathology:** No treatment-related changes were observed.



**Histopathology:**

**Peer Review:** Yes.

**Non-Neoplastic Changes:** No treatment-related changes were observed.

**Premature deaths (found dead or sacrificed in poor condition):**

Sex Groups Dose (mg/kg Bwt/day)	Males					Females				
	VC1	VC2	L	M	H	VC1	VC2	L	M	H
Liver (No. of rats examined)	33	33	30	29	28	25	20	21	31	23
- Basophilic focus(i)	3	1	0	1	1	5	3	2	6	1
- Extramedullary hematopoiesis	0	1	0	0	1	7	4	5	2	4

VC1: Vehicle Control-1; VC 2: Vehicle Control-2; L: Low dose M: Mid dose H: High dose

^: G5 rats were treated with 800 mg/kg/day during Weeks 1 -35, and then 1200 mg/kg/day from Week 36 till termination.



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Sex Groups Dose (mg/kg Bwt/day)	Males					Females				
	VC1	VC2	L	M	H	VC1	VC2	L	M	H
	0	0	50	200	800/1200 <sup>^</sup>	0	0	50	200	800/1200 <sup>^</sup>
Liver (No. of rats examined)	33	33	30	29	28	25	20	21	31	23
- Hepatocellular hypertrophy	2	5	6	4	5	7	7	5	13	6
- Hepatocyte vacuolation	7	15	5	5	8	7	3	6	2	1
- Bile duct proliferation	7	6	6	8	8	2	0	0	1	3
Kidneys (No. of rats examined)	33	33	30	29	27	25	20	21	30	22
- Mineralisation	0	1	0	0	1	5	3	7	5	1
- Nephropathy	32	29	25	28	26	5	7	4	5	6
- Proteinaceous material in tubules	1	3	2	1	0	11	9	8	13	7
- Dilated tubules	0	2	1	0	0	3	1	5	8	3
Lungs with bronchi (No. of rats examined)	33	33	30	29	28	25	20	21	31	23
- Alveolar histiocytosis	7	6	8	5	7	10	10	11	13	8
- Mineralisation-pulmonary vessel	9	6	5	5	4	3	7	3	2	6
Heart (No. of rats examined)	33	33	30	29	28	25	20	21	31	23
- Myocardial fibrosis\degeneration	19	20	11	11	14	2	2	0	1	1
- Myocardial fibrosis\vacuolation	3	1	2	3	6	1	2	1	0	1
Salivary gland (No. of rats examined)	33	33	30	29	28	25	20	21	31	23
- Atrophy	2	5	0	5	3	2	2	1	2	1
- Lobular atrophy	2	2	5	2	1	2	1	1	2	2
Pancreas (No. of rats examined)	33	33	30	29	28	25	20	21	31	23
- Vasculitis	4	6	3	5	7	1	0	1	0	0
Stomach (No. of rats examined)	32	33	29	28	28	25	19	21	31	23
- Distended glands	21	15	9	15	13	15	13	9	11	13

VC1: Vehicle Control-1; VC 2: Vehicle Control-2; L: Low dose M: Mid dose H: High dose

<sup>^</sup>: G5 rats were treated with 800 mg/kg/day during Weeks 1 –35, and then 1200 mg/kg/day from Week 36 till termination.



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Sex Groups Dose (mg/kg Bwt/day)	Males					Females				
	VC1	VC2	L	M	H	VC1	VC2	L	M	H
	0	0	50	200	800/1200^	0	0	50	200	800/1200^
Ileum with peyer's patch (No. of rats examined)	15	20	14	15	17	19	15	20	24	15
- Dilatation	3	4	5	2	6	2	2	4	3	2
Colon (No. of rats examined)	25	27	20	20	19	24	18	20	31	22
- Dilatation	4	4	3	3	2	2	2	3	2	2
Rectum (No. of rats examined)	31	30	26	25	22	25	20	21	31	23
- Dilatation	5	3	2	1	2	3	2	1	1	3
Spleen (No. of rats examined)	33	33	30	29	28	25	20	21	31	23
- Increased extramedullary hematopoiesis	16	13	13	14	12	18	14	16	18	13
- Increased hemosiderosis	21	25	21	19	21	12	15	13	19	15
- Lymphoid depletion marginal zone	7	14	13	4	10	6	8	8	5	4
Thymus (No. of rats examined)	31	32	27	27	24	23	18	18	30	23
- Involution	30	32	27	26	23	22	18	18	29	21
Thyroid gland (No. of rats examined)	33	33	30	28	28	24	20	21	31	23
- C cell hyperplasia focal\diffuse	0	1	2	3	0	0	0	1	1	2
Parathyroid gland (No. of rats examined)	30	32	26	26	25	21	18	21	27	22
- Hyperplasia diffuse	2	4	0	2	2	0	0	0	0	0
Adrenal glands (No. of rats examined)	32	33	30	29	28	25	20	21	31	23
- Corticular hypertrophy focal\multifocal	11	8	11	8	9	7	6	11	4	9
- Corticular hyperplasia focal\multifocal	6	4	6	6	7	5	2	2	4	3
- Medullary hyperplasia focal\multifocal	6	8	5	7	8	5	5	6	8	5

VC1: Vehicle Control-1; VC 2: Vehicle Control-2; L: Low dose M: Mid dose H: High dose  
^: G5 rats were treated with 800 mg/kg/day during Weeks 1 -35, and then 1200 mg/kg/day from Week 36 till termination.



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Sex Groups Dose (mg/kg Bwt/day)	Males					Females				
	VC1	VC2	L	M	H	VC1	VC2	L	M	H
	0	0	50	200	800/1200 <sup>^</sup>	0	0	50	200	800/1200 <sup>^</sup>
Testes (No. of rats examined)	33	33	30	29	28	NA	NA	NA	NA	NA
- Atrophy-seminiferous tubules unilateral\bilateral	17	21	14	15	19	NA	NA	NA	NA	NA
Seminal vesicle (No. of rats examined)	31	33	30	28	25	NA	NA	NA	NA	NA
- Atrophy bilateral	10	14	11	9	10	NA	NA	NA	NA	NA
Coagulating glands (No. of rats examined)	29	28	28	26	25	NA	NA	NA	NA	NA
- Atrophy bilateral	9	10	8	8	10	NA	NA	NA	NA	NA
Prostate (No. of rats examined)	33	33	30	29	28	NA	NA	NA	NA	NA
- Suppurative inflammation	3	6	7	5	3	NA	NA	NA	NA	NA
Ovaries with oviduct (No. of rats examined)	NA	NA	NA	NA	NA	25	20	21	31	22
- No corpus luteum	NA	NA	NA	NA	NA	11	15	12	26	11
- Cyst(s)	NA	NA	NA	NA	NA	5	2	3	4	1
Uterus with cervix (No. of rats examined)	NA	NA	NA	NA	NA	25	20	21	31	23
- Cystic gland(s)	NA	NA	NA	NA	NA	2	4	4	7	5
- Dilatation	NA	NA	NA	NA	NA	1	3	8 <sup>+</sup>	5	2
Urinary bladder (No. of rats examined)	33	33	30	29	28	25	20	21	31	22
- Epithelial hyperplasia diffuse\focal\multifocal	1	3	1	4	2	2	0	1	1	1
Epididymides (No. of rats examined)	33	33	30	29	28	NA	NA	NA	NA	NA
- Cell debris in lumen unilateral\bilateral	15	21	16	9	14	NA	NA	NA	NA	NA
- Oligospermia unilateral\bilateral	9	16	10	7	11	NA	NA	NA	NA	NA

VC1: Vehicle Control-1; VC 2: Vehicle Control-2; L: Low dose M: Mid dose H: High dose  
<sup>^</sup>: G5 rats were treated with 800 mg/kg/day during Weeks 1 –35, and then 1200 mg/kg/day from Week 36 till termination.

+ : Significantly higher than the vehicle control-1 group at P ≤ 0.05.



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Sex Groups Dose (mg/kg Bwt/day)	Males					Females				
	VC1 0	VC2 0	L 50	M 200	H 800/1200 <sup>^</sup>	VC1 0	VC2 0	L 50	M 200	H 800/1200 <sup>^</sup>
Pituitary gland (No. of rats examined)	32	31	29	29	28	25	20	20	31	23
- Hyperplasia focal	4	2	2	3	1	1	0	1	1	0
Spinal cord (No. of rats examined)	33	33	28	29	28	25	20	21	31	22
- Degeneration	7	10	5	6	9	4	2	5	9	4
Sciatic nerve (No. of rats examined)	33	33	30	29	28	25	20	21	21	23
- Axonal degeneration	26	24	20	22	25	12	9	10	9	6
Eyes with optic nerve (No. of rats examined)	33	32	30	28	28	25	20	21	31	23
- Retinal atrophy\unilateral\bilateral	18	15	19	13	12	18	15	17	23	20
- Keratitis unilateral\bilateral	6	4	5	3	4	0	0	1	0	0
- Cataract unilateral\bilateral	2	6	3	2	1	0	0	1	2	2
Mammary gland (No. of rats examined)	28	32	25	27	27	25	20	21	31	23
- Atrophy	6	6	7	5	9	0	0	0	0	2
- Glandular hyperplasia	2	2	1	3	2	15	16	11	22	14
Mandibular lymph nodes (No. of rats examined)	33	33	30	29	28	25	20	21	31	23
- Plasmacytosis	20	14	14	15	14	13	9	8	17	14
Skeletal muscle (No. of rats examined)	33	33	30	29	28	25	20	21	31	23
- Degeneration	0	2	0	1	4	1	0	2	1	1
Preputial gland (No. of rats examined)	28	27	23	24	25	NA	NA	NA	NA	NA
- Ductectasia	8	10	9	12	8	NA	NA	NA	NA	NA
- Inflammation	0	2	3	5	8	NA	NA	NA	NA	NA

VC1: Vehicle Control-1; VC 2: Vehicle Control-2; L: Low dose M: Mid dose H: High dose

<sup>^</sup>: G5 rats were treated with 800 mg/kg/day during Weeks 1 –35, and then 1200 mg/kg/day from Week 36 till termination.



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Sex Groups Dose (mg/kg Bwt/day)	Males					Females				
	VC1 0	VC2 0	L 50	M 200	H 800/1200 <sup>^</sup>	VC1 0	VC2 0	L 50	M 200	H 800/1200 <sup>^</sup>
Clitoral gland (No. of rats examined)	NA	NA	NA	NA	NA	17	13	10	16	13
- Ductectasia	NA	NA	NA	NA	NA	3	4	3	6	3
Sternum with marrow (No. of rats examined)	33	33	30	28	28	25	20	21	30	23
- Hypercellularity marrow	4	6	4	2	2	7	6	9	11	9
- Hypocellularity marrow	5	9	5	1 <sup>a-</sup>	11	0	0	1	1	1
Bone femur with joint (No. of rats examined)	33	33	30	29	28	25	20	21	30	23
- Hypercellularity marrow	1	3	4	2	2	7	6	9	11	9
- Hypocellularity marrow	5	10	5	2	11	0	0	1	1	1
Nasal passage (No. of rats examined)	29	27	25	25	25	19	16	14	23	16
- Eosinophilic inclusion- olfactory epithelium	8	13	11	16	17	13	12	9	15	11

VC1: Vehicle Control-1; VC 2: Vehicle Control-2; L: Low dose M: Mid dose H: High dose

<sup>^</sup>: G5 rats were treated with 800 mg/kg/day during Weeks 1 -35, and then 1200 mg/kg/day from Week 36 till termination.

a-: Significantly lower (a-) than the vehicle control-2 group at P ≤ 0.05.



**Rats sacrificed at study termination:**

Sex Groups Dose (mg/kg Bwt/day)	Males					Females				
	VC1 0	VC2 0	L 50	M 200	H 800/1200 <sup>^</sup>	VC1 0	VC2 0	L 50	M 200	H 800/1200 <sup>^</sup>
Liver (No. of rats examined)	17	17	20	21	22	25	30	29	19	27
- Basophilic focus(t)	11	2	5	6	3	7	5	6	6	12
- Clear cell focus(t)	14	4	6	12	3	2	1	5	3	1
- Extramedullary hematopoiesis	0	0	0	0	0	3	4	2	4	1
- Hepatocellular hypertrophy	0	0	0	0	1	14	8	9	11	6
- Hepatocyte vacuolation	7	4	2	6	7	3	8	3	a-	5
- Bile duct proliferation	3	6	8	4	8	1	2	1	0	2
- Rarefied hepatocyte cytoplasm focal/multifocal	11	7	11	8	3	1	0	2	0	0
Kidneys (No. of rats examined)	17	17	20	21	22	25	30	29	19	27
- Mineralisation	1	0	0	0	0	7	8	12	6	2
- Nephropathy	17	17	20	21	22	5	9	4	2	10
- Proteinaceous material in tubules	0	0	0	0	0	13	16	24	13	13
- Cyst(s)	1	7	5	9	5	1	0	0	0	0
Lungs with bronchi (No. of rats examined)	17	17	20	21	22	25	30	29	19	27
- Inflammatory focus(t)	2	4	4	7	4	4	6	10	5	8

VC1: Vehicle Control-1; VC 2: Vehicle Control-2; L: Low dose M: Mid dose H: High dose.

-: Significantly lower (-) than the vehicle control-1 group at  $P \leq 0.05$ .

a-: Significantly lower (a-) than the vehicle control-2 group at  $P \leq 0.05$ .

<sup>^</sup>: G5 rats were treated with 800 mg/kg/day during Weeks 1 -35, and then 1200 mg/kg/day from Week 36 till termination.



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Sex Groups Dose (mg/kg Bwt/day)	Males					Females				
	VC1 0	VC2 0	L 50	M 200	H 800/1200 <sup>^</sup>	VC1 0	VC2 0	L 50	M 200	H 800/1200 <sup>^</sup>
Heart (No. of rats examined)	17	17	20	21	22	25	30	29	19	27
- Myocardial fibrosis\degeneration	4	3	6	4	7	1	0	1	0	0
- Myocardial fibrosis\vacuolation	2	2	1	0	0	0	1	0	1	0
Salivary gland (No. of rats examined)	17	17	20	21	22	25	30	29	19	27
- Vasculitis	0	2	3	5	2	0	0	0	0	0
Pancreas (No. of rats examined)	17	17	20	21	22	25	30	29	19	27
- Vasculitis	1	3	4	4	6	1	2	0	2	1
Stomach (No. of rats examined)	17	17	20	21	22	25	30	29	19	27
- Distended glands	12	8	13	17	18	18	22	14	7	7
Spleen (No. of rats examined)	17	17	20	21	22	25	30	29	19	27
- Increased extramedullary hematopoiesis	17	13	16	19	16	23	26	23	15	22
- Increased hemosiderosis	12	12	14	14	19	21	23	24	15	20
Thymus (No. of rats examined)	17	17	20	21	22	25	30	29	19	26
- Involution	17	17	20	20	22	24	29	29	19	26
Thyroid gland (No. of rats examined)	17	17	20	21	22	25	30	29	19	27
- C cell hyperplasia focal\multifocal	1	1	0	2	3	2	6	5	0	2
Adrenal glands (No. of rats examined)	17	17	20	21	22	25	30	29	19	26
- Corticular hypertrophy focal\multifocal	14	12	19	18	12	20	17	19	13	24
- Corticular hyperplasia focal\multifocal	9	7	15	13	12	5	8	7	8	10

VC1: Vehicle Control-1; VC 2: Vehicle Control-2; L: Low dose M: Mid dose H: High dose  
<sup>^</sup>: G5 rats were treated with 800 mg/kg/day during Weeks 1 –35, and then 1200 mg/kg/day from Week 36 till termination.



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Sex Groups Dose (mg/kg Bwt/day)	Males					Females				
	VC1 0	VC2 0	L 50	M 200	H 800/1200 <sup>^</sup>	VC1 0	VC2 0	L 50	M 200	H 800/1200 <sup>^</sup>
Adrenal glands (No. of rats examined)	17	17	20	21	22	25	30	29	19	26
- Medullary hyperplasia focal/multifocal	6	6	5	4	7	3	9	8	6	8
Testes (No. of rats examined)	17	17	20	21	22	NA	NA	NA	NA	NA
- Atrophy-seminiferous tubules unilateral/bilateral	5	6	7	9	9	NA	NA	NA	NA	NA
Seminal vesicle (No. of rats examined)	17	17	20	21	22	NA	NA	NA	NA	NA
- Atrophy bilateral	0	2	1	0	6	NA	NA	NA	NA	NA
Coagulating glands (No. of rats examined)	17	17	20	21	22	NA	NA	NA	NA	NA
- Atrophy bilateral	0	2	1	0	5	NA	NA	NA	NA	NA
Ovaries with oviduct (No. of rats examined)	NA	NA	NA	NA	NA	25	29	28	19	27
- No corpus luteum	NA	NA	NA	NA	NA	15	14	18	14	17
- Cyst(s) unilateral/bilateral	NA	NA	NA	NA	NA	5	4	2	3	5
Uterus with cervix (No. of rats examined)	NA	NA	NA	NA	NA	24	29	29	19	27
- Cystic gland(s)	NA	NA	NA	NA	NA	9	6	6	4	4
- Dilatation	NA	NA	NA	NA	NA	2	6	0 <sup>a-</sup>	5	7
- Cystic endometrial hyperplasia	NA	NA	NA	NA	NA	2	6	4	2	6
Epididymides (No. of rats examined)	17	17	20	21	22	NA	NA	NA	NA	NA
- Cell debris in lumen unilateral/bilateral	0	1	3	3	4	NA	NA	NA	NA	NA
- Oligospermia bilateral	1	1	2	0	1	NA	NA	NA	NA	NA
Pituitary gland (No. of rats examined)	17	17	20	21	22	25	30	29	19	26
- Hyperplasia focal/multifocal	1	3	1	4	6	0	1	0	0	0

VC1: Vehicle Control-1; VC 2: Vehicle Control-2; L: Low dose M: Mid dose H: High dose  
<sup>^</sup>: G5 rats were treated with 800 mg/kg/day during Weeks 1 –35, and then 1200 mg/kg/day from Week 36 till termination.

a-: Significantly lower (a-) than the vehicle control-2 group at P ≤ 0.05.



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Sex Groups Dose (mg/kg Bwt/day)	Males					Females				
	VC1 0	VC2 0	L 50	M 200	H 800/1200^	VC1 0	VC2 0	L 50	M 200	H 800/1200^
Sciatic nerve (No. of rats examined)	17	17	20	21	22	25	30	29	19	27
- Axonal degeneration	16	16	20	20	21	24	27	25	14	22
Eyes with optic nerve (No. of rats examined)	17	17	20	21	22	25	30	29	19	27
- Retinal atrophy\unilateral\bilateral	11	9	17	14	14	22	24	25	18	24
- Keratitis unilateral\bilateral	1	3	5	5	4	0	0	0	0	0
- Cataract unilateral\bilateral	2	2	4	0	2	2	2	0	1	6
Mammary gland (No. of rats examined)	12	14	15	16	16	25	30	29	19	27
- Glandular hyperplasia	0	0	0	1	0	17	25	24	14	23
Mandibular lymph nodes (No. of rats examined)	17	17	20	21	22	25	30	29	19	27
- Plasmacytosis	13	12	14	15	12	20	24	21	15	21
Preputial gland (No. of rats examined)	17	17	20	21	21	NA	NA	NA	NA	NA
- Ductectasia with debris	5	8	9	5	5	NA	NA	NA	NA	NA
- Inflammation	0	5	10	10	4	NA	NA	NA	NA	NA
Clitoral gland (No. of rats examined)	NA	NA	NA	NA	NA	25	28	23	16	21
- Ductectasia with debris	NA	NA	NA	NA	NA	9	5	1	3	5
Tongue (No. of rats examined)	17	17	20	21	22	25	30	29	19	27
- Vasculitis	4	5	7	8	10	0	0	1	0	1
Sternum with marrow (No. of rats examined)	17	17	20	21	22	25	30	29	19	27
- Hypercellularity marrow	1	1	1	1	3	8	9	6	8	14
Bone femur with joint (No. of rats examined)	17	17	20	21	22	25	30	29	19	27
- Hypercellularity marrow	0	1	0	0	1	8	9	6	8	13
Nasal passage (No. of rats examined)	17	17	20	21	22	25	30	29	19	27
- Eosinophilic inclusion-olfactory epithelium	7	6	8	6	2	14	5	9	8	14

VC1: Vehicle Control-1; VC 2: Vehicle Control-2; L: Low dose M: Mid dose H: High dose

^: G5 rats were treated with 800 mg/kg/day during Weeks 1 –35, and then 1200 mg/kg/day from Week 36 till termination.

+/-: Significantly higher (+) / lower (-) than the vehicle control-1 group at P ≤ 0.05.



**Neoplastic Changes:** Tumor incidences are summarized in the Applicant's tables below.

Sex Groups Dose (mg/kg Bwt/day)	Males					Females				
	VC1 0	VC2 0	L 50	M 200	H 800/1200 <sup>^</sup>	VC1 0	VC2 0	L 50	M 200	H 800/1200 <sup>^</sup>
<b>Combined fates</b>										
Adrenal glands (No. of rats examined)	49	50	50	50	50	50	50	50	50	49
- Pheochromocytoma (B)	21	-	16	10	a+	5	6	3	4	2
Percentage	43	18	32	2	38	10	12	6	8	4
P values	(0.4073)	(0.0398)			(0.0176)					
- Pheochromocytoma (B+M)	23	-	18	13	a+	5	7	4	4	2
Percentage	47	26	36	26	46	10	14	6	8	4
P values	(0.2875)	(0.0304)			(0.0242)					

VC1: Vehicle Control-1; VC 2: Vehicle Control-2; L: Low dose M: Mid dose  
H: High dose  
<sup>^</sup>: G5 rats were treated with 800 mg/kg/day during Weeks 1 –35, and then 1200 mg/kg/day from Week 36 till termination.  
-: Significantly lower than the vehicle control-1 group at P ≤ 0.05 (Trend test)  
a+: Significantly higher than the vehicle control-2 group at P ≤ 0.05 (Trend test & pair wise)  
B: Benign; M: Malignant

Sex Groups Dose (mg/kg Bwt/day)	Males				Females			
	VC1+2 0	L 50	M 200	H 800/1200 <sup>^</sup>	VC1+2 0	L 50	M 200	H 800/1200 <sup>^</sup>
Adrenal glands (No. of rats examined)	99	50	50	50	100	50	50	49
- Cortical cell adenoma (B)	0	1	1	3	6	0	1	1
Percentage		2	2	6	6		2	2
P values	(0.0222)			(0.0438)				

VC1: Vehicle Control-1; VC 2: Vehicle Control-2; L: Low dose M: Mid dose  
H: High dose  
<sup>^</sup>: G5 rats were treated with 800 mg/kg/day during Weeks 1 –35, and then 1200 mg/kg/day from Week 36 till termination.  
B: Benign;



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Sex Groups Dose (mg/kg Bwt/day)	Males				Females			
	VC1+2 0	L 50	M 200	H 800/1200 <sup>^</sup>	VC1+2 0	L 50	M 200	H 800/1200 <sup>^</sup>
Pancreas (No. of rats examined)	100	50	50	50	100	50	50	50
- Islet cell adenoma + Carcinoma (B+M)	8	7	5	11	3	3	1	1
Percentage	8	14	10	22	3	6	2	2
P values	(0.0143)			(0.0132)				
- Islet cell adenoma (B)	6	6	4	10	3	2	1	1
Percentage	6	12	8	20	3	4	2	2
P values	(0.0079)			(0.0056)				

VC1: Vehicle Control-1; VC 2: Vehicle Control-2; L: Low dose M: Mid dose H: High dose  
<sup>^</sup>: G5 rats were treated with 800 mg/kg/day during Weeks 1 –35, and then 1200 mg/kg/day from Week 36 till termination.

+: Significantly higher than the vehicle control (1+ 2) groups at P ≤ 0.01 (Pair wise)  
 B: Benign; M: Malignant

Sex Groups Dose (mg/kg Bwt/day)	Males				Females			
	VC1+2 0	L 50	M 200	H 800/1200 <sup>^</sup>	VC1+2 0	L 50	M 200	H 800/1200 <sup>^</sup>
Pituitary gland (No. of rats examined)	97	49	50	50	100	49	50	49
- Adenoma (B)	21	13	18	11	55	30	37	30
Percentage					55		74	
P values					(0.4164)	(0.0006)		

VC1: Vehicle Control-1; VC 2: Vehicle Control-2; L: Low dose M: Mid dose H: High dose  
<sup>^</sup>: G5 rats were treated with 800 mg/kg/day during Weeks 1 –35, and then 1200 mg/kg/day from Week 36 till termination.

+: Significantly higher than the vehicle control (1+2) groups at P ≤ 0.05 (Pair wise)  
 B: Benign;



Sex Groups Dose (mg/kg Bwt/day)	Males				Females			
	VC1+2 0	L 50	M 200	H 800/1200 <sup>^</sup>	VC1+2 0	L 50	M 200	H 800/1200 <sup>^</sup>
Thyroid glands (No. of rats examined)	100	50	49	50	99	50	50	50
- C cell adenoma (B)	3	3	1	2	6	+	3	4
Percentage					6	18		
P values					(0.5871)	(0.0079)		
- C cell adenoma + carcinoma (B+M)	8	5	2	3	7	+	3	6
Percentage					7	24		
P values					(0.4443)	(0.0012)		

VC1: Vehicle Control-1; VC 2: Vehicle Control-2; L: Low dose M: Mid dose H: High dose  
<sup>^</sup>: G5 rats were treated with 800 mg/kg/day during Weeks 1 –35, and then 1200 mg/kg/day from Week 36 till termination.  
 +: Significantly higher than the vehicle control (1+2) groups at P ≤ 0.05 (Pair wise)  
 B: Benign; M: Malignant

The FDA statistical review concluded that treatment with AT1001 significantly increased cortical cell adenoma in adrenal glands in the high dose males (pairwise comparison, p = 0.0430 < 0.05 for rare tumors; trend test, p = 0.0190 < 0.025 for rare tumors). Although there was no incidence of cortical cell adenomas in the male control groups (0/99 males), cortical cell adenomas are generally considered as common tumors in male and female SD rats. It is noteworthy that cortical cell adenomas occurred in 6/100 control females. Neither the pairwise comparison nor the trend test show statistical significance when the common tumor significance criteria are used (pairwise comparison, p = 0.0430 > 0.01 for common tumors; trend test, p = 0.0190 > 0.005 for common tumors). Similarly, the combination of cortical cell adenoma and carcinoma in adrenal glands failed to show significance in the pairwise comparison and trend test based on the common tumor significance criteria (pairwise comparison, p = 0.0430 > 0.01 for common tumors; trend test, p = 0.0388 > 0.005 for common tumors). The following table is from the FDA statistical review.



**Table 1: Tumor Types with Statistical Significant Dose Response Relationships and Pairwise Comparisons of Treated Groups and Control**

Animal	Organ name	Tumor name	0 mkd Comb. Controls P - Trend	50 mkd Low (L) P - Comb. C vs. L	200 mkd Mid (M) P - Comb. C vs. M	800/1200 mkd High (H) P - Comb. C vs. H
Male Rats	Adrenal Glands	Cortical Cell Adenoma	0/99 (76) 0.0190 *	1/50 (40) 0.3448	1/50 (39) 0.3391	3/50 (42) 0.0430 **
		C_ Cortical Cell/ Adenoma+ Carcinoma	0/99 (76) 0.0388	2/50 (40) 0.1169	1/50 (39) 0.3391	3/50 (42) 0.0430 **
Female Rats	Pituitary Gland	Adenoma	55/100 (88) 0.2566	30/49 (43) 0.2681	37/50 (43) 0.0040 **	30/49 (42) 0.2117
		Thyroid Gland C_ C-ell/ Adenoma+Carcinoma	7/99 (81) 0.4367	12/50 (42) 0.0050 **	3/50 (37) 0.3846	6/50 (40) 0.2232

Note: X/ZZ (YY): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed. □  
NC = Not calculable.

Note: The p-values marked with an asterisk \* indicate statistically significant dose responses at 0.005 and 0.025 for a common tumor and a rare tumor, respectively. The p-values marked with an asterisk \*\* indicate statistically significant pairwise comparison at 0.01 and 0.05 for a common tumor and a rare tumor, respectively.

The Applicant's analysis showed that the treatment significantly increased the incidence of Islet cell adenoma (p = 0.0056) and combination of adenoma and carcinoma in the high dose males (p = 0.0132). However, FDA statistical analysis of these tumors did not show significance.

**Toxicokinetics:** TK data are summarized in the Applicant's table below.



In-Text Table 1 Summary of AT1001 Toxicokinetic Parameters

	Group/Dose	Sex	AUC <sub>0-inf</sub> ng*hr/mL	AUC <sub>0-last</sub> ng*hr/mL	C <sub>max</sub> ng/mL	t <sub>1/2</sub> hr	T <sub>max</sub> hr	CL/F/kg L/hr/kg	
Day 1	G3TK 50 mg/kg/day	M	NA*	11600	4420	NA	0.5	3.54	
		F	NA	12400	8330	NA	0.5	3.30	
		Overall	NA	12000	6380	NA	0.5	3.42	
	G4TK 200 mg/kg/day	M		32800	32700	10300	2.80	0.5	5.00
		F		NA	34600	14700	NA	0.5	4.72
		Overall		37600	34100	12500	8.34	0.5	4.80
	G5TK 800 mg/kg/day	M		90000	89400	14000	2.80	0.5	7.31
		F		NA	101000	27500	NA	0.5	6.45
		Overall		102000	95400	20800	4.99	0.5	6.85
Week 29	G3TK 50 mg/kg/day	M	NC**	12600	4730	3.28	6.5	3.24	
		F	NC	14900	7030	NA	6.5	2.73	
		Overall	NC	13800	5880	4.46	6.5	2.96	
	G4TK 200 mg/kg/day	M	NC	29500	9970	3.64	6.5	3.64	
		F	NC	37100	14600	6.71	6.5	6.71	
		Overall	NC	33300	12300	5.66	6.5	5.66	
	G5TK 800 mg/kg/day	M	NC	89500	16900	5.61	7.0	5.61	
		F	NC	117000	26600	8.80	6.5	8.80	
		Overall	NC	103000	20900	NA	6.5	NA	
Week 37	G5TK <sup>#</sup> 1200 mg/kg/day	M	NC	184000	27600	4.83	7.0	7.30	
		F	NC	168000	24200	6.29	6.5	5.58	
		Overall	NC	176000	25700	5.48	7.0	6.33	

\*NA= not available

\*\*NC= not calculated

#High dose increased from 800 to 1200 mg/kg/day in Week 36

Note: All values have been rounded to 3 significant figures except for T<sub>max</sub>.

Note: TK parameters are derived as single values from 3 separate mean concentration-time profiles for males, females, and overall.

(AUC<sub>0-last</sub> = AUC<sub>0-24hrs</sub>)

T<sub>1/2</sub> = 5-6 hours during week 37 in animals.

T<sub>1/2</sub> = 2-5 hours in humans

**Dosing Solution Analysis:**

All test drug concentrations were within 90-110% of the nominal values.



**Study title: AT1001: A 26-Week Oral Carcinogenicity Study in Tg.rasH2 Mice**

Study no.: 70576  
Study report location: N/A  
Conducting laboratory and location: (b) (4)  
Date of study initiation: 12/17/2010  
GLP compliance: Yes  
QA statement: Yes  
Drug, lot #, and % purity: AT1001, F09-03208; F10-02433, 98.7%  
CAC concurrence: Yes (E-CAC meeting on 12/14/2010)

**Key Study Findings:**

No drug-related tumors were observed.

**Methods:**



Doses: Male: 100, 300, and 1000 mg/kg/day  
Female: 50, 150, and 500 mg/kg/day (see table below)

Frequency of dosing: Daily  
Dose volume: 10 ml/kg  
Route of administration: Oral gavage  
Formulation/Vehicle: Water  
Basis of dose selection: MTD (see below)  
Species/Strain: Tg.rasH2: CByB6F1-Tg(HRAS)2Jic – Hemizygous  
Non-transgenic littermates: CByB6F1-Tg(HRAS)2Jic –Wild Type  
Males: 20.6 to 29.0 g  
Females: 16.4 to 23.5 g

Number/Sex/Group: 25  
Age: 7-8 weeks  
Animal housing: Individually  
Paradigm for dietary restriction: None  
Dual control employed: No  
Interim sacrifice: No  
Satellite groups: 20/sex/group (5/sex/group for control)  
Deviation from study protocol: Deviations did not have a significant impact on the study outcome.

Group Numbers	Group Designation	Dose Level (mg/kg/day)		Dose Conc. (mg/mL)		Dose Volume (mL/kg)	Main Animals (Tg.rasH2)		Toxicokinetic Animals (CByB6F1) <sup>d</sup>	
		M	F	M	F		M	F	M	F
1	Control <sup>a</sup>	0	0	0	0	10	25	25	5	5
2	Positive Control (Urethane) <sup>b</sup>	1000	1000	100	100	10	25	25	-	-
3	Low Dose <sup>c</sup>	100	50	10	5	10	25	25	20	20
4	Mid Dose <sup>c</sup>	300	150	30	15	10	25	25	20	20
5	High Dose <sup>c</sup>	1000	500	100	50	10	25	25	20	20

M = Males; F = Females

- a - Control (also synonymously referred to as negative control) animals received the control/vehicle article only (deionized water).
- b - The positive Control animals of Group 2 received a total of 3 intraperitoneal injections of Urethane (1000 mg/kg each) at 2-day intervals (on Day 1, 3 and 5).
- c - Doses were based on free base equivalents using a correction factor of 1.22
- d - 2 mice/sex/group served as backup in order to fulfill the required time points, when necessary.



Dose selection was based on the results from a 28-day oral dose range-finding toxicity study in CByB6F1 mice ((b) (4) 70575). In this study, histopathological exam revealed inflammation of the large intestine in females at doses of 1000 and 2000 mg/kg/day and in males at 2000 mg/kg/day. The NOAEL was identified at 500 mg/kg/day for females and 1000 mg/kg/day for males. Therefore, the NOAEL doses (1000 mg/kg/day in males, 500 mg/kg/day in females) were selected as the high dose. The selected doses of 100, 300, and 1000 mg/kg in males, and 50, 150, and 500 mg/kg in females were recommended by the E-CAC (meeting held on 12/14/2010).

**Observations and times:**

- **Clinical signs:** Daily
- **Mortality:** Daily
- **Body weights:** Weekly
- **Food consumption:** Weekly
- **Gross pathology:** Animals were necropsied at termination.
- **Histopathology:** The following organs or tissues were collected for examination (table was taken from study report).



**Text Table 1 Incidence of Found Dead and Pre-terminally Euthanized Animals**

Group Number	Found Dead Animals		Pre-terminally Euthanized Animals		Overall (%)
	Males	Females	Males	Females	
1	0	2	0	0	2 (4%)
2	11	10	13	15	49 (98%)
3	1	0	0	0	1 (2%)
4	0	0	1	1	2 (4%)
5	1	1	1	1	4 (8%)

The deaths of two control females (found dead), one low dose male (found dead), and one mid-dose female (sacrificed) were due to gavage errors. The cause of death in other animals was not clear. It appears that mortality rate was increased in the high dose group (4/25) as compared to the control (0/20, not including the two deaths due to gavage errors).

The positive control article (urethane) significantly increased the mortality rate.

**Clinical Signs:** The major clinical signs of toxicity in the prematurely sacrificed animals included decreased activity, labored respiration, severe swelling of the neck, hypothermia, and distended abdomen.

**Body Weight:** The initial and final body weights for the control animals were 23.7 g and 29.3 g, respectively, in males, and 18.8 g and 23.6 g, respectively, in females. There were no clear treatment-related changes.

**Feed Consumption:** There were no treatment-related changes.

**Gross Pathology:** No treatment-related changes were observed.

### Histopathology

**Peer Review:** Yes

**Non-Neoplastic Changes:** No treatment-related changes were observed.

**Neoplastic Changes:** Tumor incidences were summarized in the Applicant's table below.



**Table 4: Incidence of proliferative lesions**

Sex	Male					Female				
Group	1	2	3	4	5	1	2	3	4	5
Dose level (mg/kg/day)	0	1000 <sup>±</sup>	100	300	100	0	1000 <sup>±</sup>	50	150	500
Total examined	25	25	25	25	25	25	25	25	25	25
<b>Cecum</b>										
<i>Leiomyoma</i>	0	0	0	0	0	0	0	0	0	1
<b>Circulatory System</b>										
<i>Hemangioma</i>	0	3	1	0	0	1	0	0	0	0
<i>Hemangiosarcoma</i>	1	25	2	0	1	0	23	0	0	0
<b>Harderian Gland</b>										
<i>Hyperplasia</i>	0	10	0	0	0	1	8	1	0	1
<i>Adenoma</i>	0	7	0	1	0	0	16	0	0	0
<i>Adenocarcinoma</i>	0	0	0	0	0	0	2	0	0	0
<b>Liver</b>										
<i>Adenoma, hepatocellular</i>	0	1	0	0	0	0	0	0	0	0



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Sex	Male					Female				
Group	1	2	3	4	5	1	2	3	4	5
Dose level (mg/kg/day)	0	1000*	100	300	100	0	1000*	50	150	500
Total examined	25	25	25	25	25	25	25	25	25	25
<b>Lungs</b>										
<i>Hypeplasia, alveolar epithelial</i>	0	4	0	1	0	0	3	0	0	1
<i>Adenoma, bronchiolo-alveolar</i>	1	25	1	0	0	0	25	0	0	2
<i>Carcinoma, bronchiolo-alveolar</i>	0	19	0	1	0	0	21	0	0	0
<b>Hemolymphoreticular System</b>										
<i>Lymphoma, malignant</i>	0	0	0	0	0	0	0	0	0	1
<b>Mammary Gland</b>										
<i>Adenocarcinoma</i>	0	0	0	0	0	0	1	0	0	0
<b>Salivary gland, mandibular</b>										
<i>Adenocarcinoma</i>	0	0	0	0	1	0	1	0	0	0
<b>Skin &amp; Subcutis</b>										
<i>Carcinoma, squamous cell</i>	0	0	0	0	0	0	1	0	0	0
<b>Stomach</b>										
<i>Carcinoma, squamous cell</i>	0	1	0	0	0	0	2	0	0	0
<b>Uterus</b>										
<i>Adenocarcinoma</i>	NA	NA	NA	NA	NA	0	1	0	0	0
<b>Overall Tumor Incidence</b> <sup>^</sup>	<b>2</b>	<b>25</b>	<b>4</b>	<b>2</b>	<b>2</b>	<b>1</b>	<b>25</b>	<b>0</b>	<b>0</b>	<b>4</b>

\* Positive Control (Urethane)

<sup>^</sup> Number of animals in each group with a tumor



**Text Table 3: Incidence of Proliferative Lesions (all neoplasms and selected hyperplastic lesions)**

Group	Sex Male					Sex Female				
	1	2	3	4	5	1	2	3	4	5
Dose level (mg/kg/day)	0	1000*	100	300	1000	0	1000*	50	150	500
Total examined	25	25	25	25	25	25	25	25	25	25
<b>Cecum</b>										
<i>Leiomyoma</i>	0	0	0	0	0	0	0	0	0	1
<b>Circulatory System</b>										
<i>Hemangioma</i>	0	3	1	0	0	1	0	0	0	0
<i>Hemangiosarcoma</i>	1	25	2	0	1	0	23	0	0	0
<b>Harderian Gland</b>										
<i>Hyperplasia</i>	0	10	0	0	0	1	8	1	0	1
<i>Adenoma</i>	0	7	0	1	0	0	16	0	0	0
<i>Adenocarcinoma</i>	0	0	0	0	0	0	2	0	0	0
<b>Liver</b>										
<i>Adenoma, hepatocellular</i>	0	1	0	0	0	0	0	0	0	0
<b>Lungs</b>										
<i>Hyperplasia, alveolar epithelial</i>	0	4	0	1	0	0	3	0	0	1
<i>Adenoma, bronchiolo-alveolar</i>	1	25	1	0	0	0	25	0	0	2
<i>Carcinoma, bronchiolo-alveolar</i>	0	19	0	1	0	0	21	0	0	0
<b>Hemolymphoreticular System</b>										
<i>Lymphoma, malignant</i>	0	0	0	0	0	0	0	0	0	1
<b>Mammary Gland</b>										
<i>Adenocarcinoma</i>	0	0	0	0	0	0	1	0	0	0
<b>Salivary gland, mandibular</b>										
<i>Adenocarcinoma</i>	0	0	0	0	1	0	1	0	0	0
<b>Skin &amp; Subcutis</b>										
<i>Carcinoma, squamous cell</i>	0	0	0	0	0	0	1	0	0	0
<b>Stomach</b>										
<i>Carcinoma, squamous cell</i>	0	1	0	0	0	0	2	0	0	0
<b>Uterus</b>										
<i>Adenocarcinoma</i>	NA	NA	NA	NA	NA	0	1	0	0	0
<b>Overall Tumor Incidence ^</b>	<b>2</b>	<b>25</b>	<b>4</b>	<b>2</b>	<b>2</b>	<b>1</b>	<b>25</b>	<b>0</b>	<b>0</b>	<b>4</b>

\* Positive Control (Urethane)

^Number of animals in each group with a tumor

Treatment with AT1001 did not significantly increase tumor incidence. The positive control article, urethane, produced significant increases in hemangiosarcoma, Harderian gland adenoma, and bronchiolo-alveolar adenoma and carcinoma.

**Toxicokinetics:** TK data were summarized in the Applicant's table below.

**Text Table 2 Summary of AT1001 Toxicokinetic Parameters**

Sex	Dose mg/kg/day	C <sub>max</sub> ng/mL	T <sub>max</sub> hr	AUC <sub>last</sub> ng <sup>±</sup> hr/mL	AUC <sub>0-24</sub> ng <sup>±</sup> hr/mL
Male	100	27900	0.50	25800	NC
	300	27100	0.50	38900	38900
	1000	49600	0.50	121000	121000
Female	50	14400	0.50	13500	NC
	150	23800	0.50	31800	32000
	500	64100	0.50	94400	94400

NC= not calculated

Note: All values have been rounded to 3 significant figures except for T<sub>max</sub>.

Note: TK parameters are derived as single values from 2 separate mean concentration-time profiles for males and females.

### **Dosing Solution Analysis:**

All test drug concentrations were within 90-110% of the nominal values.



**Executive CAC**

**Date of Meeting:** May 15, 2018

**Committee:** Karen Davis Bruno, PhD, OND IO, Chair  
Paul Brown, PhD., OND IO, Member  
Tim McGovern, PhD, OND IO, Member  
Ron Wange, PhD, OND IO, Member  
Newton Woo, PhD, DAAAP, Alternate member  
David Joseph, PhD, DGIEP, Team Leader  
Ke Zhang, PhD, DGIEP, Presenting Reviewer

The following information reflects a brief summary of the Committee discussion and its recommendations.

**NDA#** 208,623

**Drug Name:** Galafold / Migalastat HCl / AT1001

**Sponsor:** Amicus Therapeutics  
Cranbury, NJ

**Background:**

Migalastat is indicated for long-term treatment of Fabry disease, (b) (4). Fabry disease is a rare and progressive X-linked lysosomal storage disease caused by a deficiency in the lysosomal enzyme,  $\alpha$ -galactosidase A ( $\alpha$ -Gal A). Migalastat, an analogue of the terminal galactose of GL-3, binds to the catalytic site of mutated forms of  $\alpha$ -Gal A, thereby stabilizing and enhancing enzyme activity and reducing the levels of GL-3 and lyso-Gb3.

Migalastat HCl was negative in the Ames test, the in vitro mammalian cell mutation assay in L5178Y mouse lymphoma TK<sup>+/-</sup> cells, and the in vivo micronucleus test in rats.

Two carcinogenicity studies were conducted including a 26-week oral carcinogenicity study in Tg.rasH2 mice and a 2-year oral carcinogenicity study in rats.

**Mouse Carcinogenicity Study:**

In the 26-week carcinogenicity study, Tg.rasH2 mice were treated with AT1001 at doses of 100, 300, and 1000 mg/kg/day in males, and 50, 150, and 500 mg/kg/day in females. These doses were recommended by E-CAC (see E-CAC minutes from meeting on 12/14/2010, IND 68,456). Treatment with AT1001 did not increase tumor incidence. The positive control article, urethane, significantly



increased the incidence of hemangiosarcoma, Harderian gland adenoma, and bronchiolo-alveolar adenoma and carcinoma.

### **Rat Carcinogenicity Study:**

In the 2-year carcinogenicity study, Sprague Dawley rats were treated with AT1001 at dose levels of 50, 200, and 800/1200 mg/kg/day. The high dose of 800 mg/kg/day was increased to 1200 mg/kg/day during week 36, to assure that the average AUC over the entire treatment period would be at least 25 times the human AUC at the expected clinical dose. The rat to human AUC ratio at 800 and 1200 mg/kg/day is approximately 23 and 39, respectively.

The study protocol was presented to the E-CAC on June 12, 2007 (IND 68,456). The proposed doses were 50, 200, and 800 mg/kg/day based on a pharmacokinetic endpoint. However, the E-CAC did not agree with the proposed doses, and stated that there was inadequate data to support dose selection based on exposure (AUC). This assessment was based on the proposal of once daily dosing in the carcinogenicity study protocol, whereas twice daily dosing was used in the 26-week dose-ranging study. The sponsor initiated the study using the proposed doses without further consultation with the E-CAC. However, rats were dosed twice daily 6 hours apart in the carcinogenicity study, in contrast to the proposed once daily dosing in the original protocol.

Treatment with AT1001 did not significantly affect the survival rate, body weight, or non-neoplastic findings. However, based on the Agency's statistical analysis, treatment with AT1001 increased the incidence of the cortical cell adenoma in adrenal glands in male rats at the high dose. The trend and pairwise tests were significant ( $p = 0.043 < 0.05$  for rare tumors, pairwise test for adenoma;  $p = 0.0190 < 0.025$  for rare tumors, trend test for adenoma). However, the combination of cortical cell adenoma and carcinoma in adrenal glands was not significant in the trend test (statistical significance of the combination was limited to the pairwise test for high-dose males). The relevant tumor data and analysis are shown in the table below, taken from the FDA statistical review by Dr. Feng Zhou.



**Table 1: Tumor Types with Statistical Significant Dose Response Relationships and Pairwise Comparisons of Treated Groups and Control**

Animal	Organ name	Tumor name	0 mkd Comb.Controls P - Trend	50 mkd Low (L) P - Comb.C vs. L	200 mkd Mid (M) P - Comb.C vs. M	800/1200 mkd High (H) P - Comb.C vs. H
Male Rats	Adrenal Glands	Cortical Cell Adenoma	0/99 (76) 0.0190 *	1/50 (40) 0.3448	1/50 (39) 0.3391	3/50 (42) 0.0430 **
		C_ Cortical Cell/ Adenoma + Carcinoma	0/99 (76) 0.0388	2/50 (40) 0.1169	1/50 (39) 0.3391	3/50 (42) 0.0430 **
Female Rats	Pituitary Gland	Adenoma	55/100 (88) 0.2566	30/49 (43) 0.2681	37/50 (43) 0.0040 **	30/49 (42) 0.2117
		Thyroid Gland C_C-ell/ Adenoma+Carcinoma	7/99 (81) 0.4367	12/50 (42) 0.0050 **	3/50 (37) 0.3846	6/50 (40) 0.2232

Note: X/ZZ (YY): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed. NC = Not calculable.  
 Note: The p-values marked with an asterisk \* indicate statistically significant dose responses at 0.005 and 0.025 for a common tumor and a rare tumor, respectively. The p-values marked with an asterisk \*\* indicate statistically significant pairwise comparison at 0.01 and 0.05 for a common tumor and a rare tumor, respectively.

**Executive CAC Conclusions:**

Mouse study:

- The Committee concurred that the study was adequate, noting prior concurrence with the protocol.
- The Committee concurred that there were no drug-related neoplasms in either males or females in the 6-month transgenic mouse study.

Rat study:

- Although this study was conducted without concurrence from the E-CAC, the Committee concurred that the study was adequate and acceptable.
- The Committee concurred that there were no drug-related neoplasms in the 2-year rat carcinogenicity study.

Karen Davis Bruno, PhD  
Chair, Executive CAC



DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration  
Center for Drug Evaluation and Research  
Silver Spring, MD 20993

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U.S. Department of Health and Human Services  
Food and Drug Administration  
Center for Drug Evaluation and Research  
Office of Translational Sciences  
Office of Biostatistics

## STATISTICAL REVIEW AND EVALUATION CARCINOGENICITY STUDIES

**NDA/BLA #:** NDA 208623

**Drug Name:** Galafold™ (Migalastat HCl, AT1001)

**Indication(s):** Treatment of patients (b) (4) with Fabry disease with amenable mutations

**Applicant:** Amicus Therapeutics, Inc.  
1 Cedar Brook Drive, Cranbury, NJ 08512, USA

**Laboratory:** Test Facility for Mice Study: (b) (4)  
(b) (4)  
Test Facility for Rats Study: (b) (4)  
(b) (4)

**Date(s):** Received 12/13/2017

**Documents Reviewed:** Studies G4970 (rats) and 70576 (mice) were submitted on 12/13/2017 (via S0001) and the electronic tumor.xpt files were submitted on the same time.

**Review Priority:** Priority Review

**Biometrics Division:** Division of Biometrics VI

**Statistical Reviewer:** Feng Zhou, M.S.

**Concurring Reviewers:** Karl Lin, Ph. D., Team Leader

**Medical Division:** Office of Gastroenterology and Inborn Errors Products (DGIEP)

**Nonclinical Team:** Ke Zhang, Ph.D; David Joseph, Ph.D

**Project Manager:** Hong Vu

**Keywords:** Carcinogenicity, Dose response

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## 1 Summary

This review evaluates statistically the data of the 2-year oral carcinogenicity study in rats and the 26-week transgenic mice study of AT1001. The review analyzes the dose-response relationship of tumor incidence and mortality (including tumor-related mortality). The review concludes that AT1001 statistically decreased the survivals in males and females in both species. AT1001 caused statistically significant increases in the incidence of cortical cell adenoma in adrenal glands in male rats when compared with combined controls. See Table 1 for tumor incidences and p-values of those tumor types that had significant dose-response relationships or pairwise comparisons.

The statistical analysis of survival rates and tumor incidence data confirmed that AT1001 did not affect survival or tumor incidence in Tg.rasH2 mice.

**Table 1: Tumor Types with Statistical Significant Dose Response Relationships and Pairwise Comparisons of Treated Groups and Control**

Animal	Organ name	Tumor name	0 mkd	50 mkd	200 mkd	800/1200 mkd
			Comb.Controls P - Trend	Low (L) P - Comb.C vs. L	Mid (M) P - Comb.C vs. M	High (H) P - Comb.C vs. H
Male Rats	Adrenal Glands	Cortical Cell Adenoma	0/99 (76) 0.0190 *	1/50 (40) 0.3448	1/50 (39) 0.3391	3/50 (42) 0.0430 **
		C Cortical Cell/ Adenoma + Carcinoma	0/99 (76) 0.0388	2/50 (40) 0.1169	1/50 (39) 0.3391	3/50 (42) 0.0430 **
		Pituitary Gland Adenoma	55/100 (88) 0.2566	30/49 (43) 0.2681	37/50 (43) 0.0040 **	30/49 (42) 0.2117
Female Rats	Thyroid Gland	C_C-ell/ Adenoma+Carcinoma	7/99 (81) 0.4367	12/50 (42) 0.0050 **	3/50 (37) 0.3846	6/50 (40) 0.2232

Note: X/ZZ (YY): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed. NC = Not calculable.

Note: The p-values marked with an asterisk \* indicate statistically significant dose responses at 0.005 and 0.025 for a common tumor and a rare tumor, respectively. The p-values marked with an asterisk \*\* indicate statistically significant pairwise comparison at 0.01 and 0.05 for a common tumor and a rare tumor, respectively.

**Rat Study:** Rats (50/sex/dose) were dosed by oral gavage with 0 (C1), 0 (C2), 50 (LD), 200 (MD), and 800 (HD)-mg/kg/day AT1001 for males and females twice daily for up to 104 weeks. The high dose group was treated with a dose of 800 mg/kg/day during Weeks 1 to 35, and then the dose was increased to 1200 mg/kg/day effective from treatment day 246 (week 36) as the exposure data on Day 1 and Week 29 were below the target 25-fold of the exposure in humans following a dose of 150 mg every other day (EOD) based on AUCs. Thus, the high dose is represented by weighted average dose of 1066 mg/kg/day for statistical purpose, otherwise; the high dose is represented as 800/1200 mg/kg/day. The two concurrent vehicle control groups received vehicle alone.

The survival analyses didn't show any statistically significant dose response relationship in mortality in males and females. Mortalities in the female MD group were significantly higher than that of the control 2 group and the combined two controls groups ( $P_s < 0.05$ ). The respective survival rates in the C1, C2, LD, MD, or HD groups at the time they were terminated was 34%, 34%, 40%, 42%, or 44% in males and 50%, 60%, 58%, 38%, or 54% in females.

The tumor analysis (Table 1) showed statistically significant positive dose-response relationships in type tumor of adenoma cortical cell in adrenal glands in male rats ( $p=0.0190<0.025$ ) when the combined controls were used in the analysis. The pairwise comparisons of this tumor type incidence rates against the combined-controls was statistically significant for high dosed group ( $p=0.0430<0.05$ ). The pairwise comparison of tumor type of the combination of adenoma and carcinoma cortical cell in adrenal glands against the combined controls was statistically significant for HD male rats ( $p=0.0430<0.05$ ). There was no statistically significant positive dose-response relationship in female rats. The pairwise comparison of tumor type of the adenoma in pituitary gland against the combined controls was statistically significant for MD female rats ( $p=0.004 <0.01$ ). The pairwise comparison of tumor type of the combination of C-cell adenoma and carcinoma in Thyroid gland against the combined controls was statistically significant for LD female rats ( $p=0.005 <0.01$ ).

**Mouse Study:** Mice (25/sex/dose) were dosed by oral gavage with 0 (VC), 100 (LD), 300 (MD), or 1000 mg/kg/day (HD) for male Tg.RasH2 Mice; or 0 (VC), 50 (LD), 150 (MD), or 500 mg/kg/day (HD) for female Tg.RasH2 Mice for at least 26 weeks. Ten male and 25 female mice assigned to the positive control (PC) group and received a total of 3 intraperitoneal injections of Urethane (1000 mg/kg each) at 2-day intervals (i.e., on Day 1, 3 and 5).

The survival analysis didn't show any statistically significant dose response relationship in mortality across controls and treated groups in both sexes. For both males and females, the positive control group showed significantly increasing mortality over the control group ( $p<0.0001$  in all cases). The respective survival rates in the VC, LD, MD, HD, or PC groups at the time they were terminated was 100%, 96%, 96%, 92%, or 4% in males and 92%, 100%, 96%, 92%, or 0% in females.

The tumor analysis did not show any statistically significant dose-response relationship in incidences in all tumor types tested in all dosed male and female groups. The PC group showed statistically significant increases in the incidence of a few tumor types in both males and females ( $p<0.05$ ), when compared against the vehicle control. Those tumor types were listed in following table.

**Tumor Types with P-Values  $\leq 0.05$  for Pairwise Comparisons of VC and PC**

Animals	Organ Name	Tumor Name	0 mg/kg/day VC (N=25)	Urethane (1000 mg/kg) PC (N=10)	P-Value VC vs. PC
Male Mice	Harderian Glands	Adenoma	0/25 (25)	7/25 (25)	0.0048*
	Lungs	Adenoma, Bronchiolo-Alveolar	1/25 (25)	25/25 (25)	<0.001*
		Carcinoma, Bronchiolo-Alveolar	0/25 (25)	19/25 (25)	<0.001*
Female Mice	Harderian Glands	Adenoma	0/25 (25)	16/25 (25)	<0.001*
	Lungs	Adenoma, Bronchiolo-Alveolar	0/25 (25)	25/25 (25)	<0.001*
		Carcinoma, Bronchiolo-Alveolar	0/25 (25)	21/25 (25)	<0.001*

Note: The p-values marked with an asterisk \* indicate statistically significant pairwise comparison at 0.05.

## 2 Background

Migalastat is a first-in-class, and when orally administered, it reduces GL-3 deposition in capillary endothelium of the kidney and in other renal cell types, improves signs and symptoms of diarrhea, reduces left ventricular mass index (LVMI), and stabilizes renal function as measured by glomerular filtration rate (GFR). The sponsor (Amicus) is seeking approval for the use of migalastat for the treatment of (b) (4) Fabry disease with amenable mutations. The sponsor provided the nonclinical study 70576: "A 26-Week Oral Carcinogenicity Study in Tg.RasH2 Mice" and G4970: "A 104-Week Oral Carcinogenicity Study in Sprague Dawley Rats" on 12/13/2017 via submission NDA 208623/S0001. The electronic tumor.xpt data files were submitted on the same time.

The phrase "dose response relationship" refers to the linear component of the effect of treatment, and not necessarily to a strictly increasing or decreasing mortality or tumor incidence rate as dose increases. Results of this review have been discussed with the reviewing pharmacologist Dr. Ke Zhang.

## 3 Rat Study- G4970

**Study Report:** g4970.pdf (statistical report on page 3473)

**SAS data:** tumor.xpt

This study was conducted to evaluate the carcinogenic potential of the test article, AT1001, after twice daily oral gavage administration to male and female Sprague Dawley rats for up to 104 consecutive weeks. Three treatment groups of 50 male or female rats were administered the AT1001 at respective dose levels of 50, 200, or 800 mg/kg/day. The high dose group was treated with a dose of 800 mg/kg/day during Weeks 1 to 35, and then the dose was increased to 1200 mg/kg/day effective from treatment day 246 (week 36) as the exposure data on Day 1 and Week 29 were below the target 25-fold of the exposure in humans following a dose of 150 mg every other day (EOD) based on AUCs. Thus, the high dose is represented by weighted average dose of 1066 mg/kg/day for statistical purpose, other-wise; the high dose is represented as 800/1200 mg/kg/day. The two concurrent vehicle control groups received vehicle alone. Each group consisted of 50 male and 50 female rats. This review refers these dose groups as the control1 (C1), control2 (C2), low (LD), mid (MD), and high (HD) dose groups, respectively.

Assessment of toxicity was based on dose analysis, morbidity, mortality, injury, body weight, food consumption, clinical observations and masses, ophthalmology, clinical pathology, toxicokinetics, macroscopic observations, and microscopic evaluations.

### 3.1 Sponsor's Analyses

#### 3.1.1 Survival Analysis

Intercurrent mortality data were analyzed using the Kaplan-Meier product-limit method. An overall test comparing all groups was conducted using a log-rank test<sup>12</sup>. Any animal with accidental injury that caused death or unscheduled sacrifice was censored in the estimation.

File Name: NDA208623Carcin.doc

In addition, all animals still alive at the end of the experimental period were censored at the following day. If this overall test was significant ( $p < 0.05$ ) and there were more than two groups, then a follow up analysis was done where each treatment group was compared to the control group using a log-rank test. Results of all pair-wise comparisons are reported at the 0.05 and 0.01 significance levels. All endpoints were analyzed using two-tailed tests.

**Sponsor's concluded results:** The total mortalities (dead and moribund sacrifice) in the treated groups were comparable to the concurrent controls for both males and females and for the sexes combined.

The mortality (%) and survival (%) at termination (at 730 days) of the treatment in controls, low, mid and high dose groups were as follows:

Group→	G1		G2		G3		G4		G5	
Dose (mg/kg/day) →	0		0		50		200		800/1200#	
Sex→	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Mortality (%)	66	50	66	40	60	40	58	62	54	46
Survival (%)	34	50	34	60	40	60	42	38	46	54

#: G5 rats were treated with 800 mg/kg/day during weeks 1 - 35, and then 1200 mg/kg/day from week 36 till termination

The mean survival period and percentage (in parenthesis) of the pre-terminally dead and moribund sacrificed rats and life expectancy and percentage (in parenthesis) in each of the five study group is as follows:

Group→	G1		G2		G3		G4		G5	
Dose (mg/kg/day) →	0		0		50		200		800/1200#	
Sex→	Male	Female								
Mean Survival	624 (85)	579 (79)	605 (83)	603 (83)	614 (84)	587 (80)	605 (83)	580 (79)	631 (86)	577 (79)
Life Expectancy	660 (90)	655 (90)	648 (89)	679 (93)	660 (90)	673 (92)	658 (90)	637 (87)	677 (93)	660 (90)

#: G5 rats were treated with 800 mg/kg/day during weeks 1 - 35, and then 1200 mg/kg/day from week 36 till termination

The mean survival of the pre-terminally dead and moribund sacrificed rats and life expectancy were not affected by the treatment in all the test item treated groups for both males and females.

### 3.1.2 Tumor Data Analysis

Neoplastic findings classified as fatal and incidental were processed using the death rate method and the prevalence method, respectively. The processing of incidental tumors was done by creating a single separate interval for the time period following the experimental period (terminal sacrifice period) and by dividing the experimental period into the following fixed intervals [FDA's draft Guidance for industry. 2001]: weeks 1-52, weeks 53-78, weeks 79-92, and over week 92. Using the derived outcomes from the processing of both fatal and incidental tumors, a test statistic was built to perform a global survival-adjusted trend test on tumor data observed in a "mortality dependent" context [Peto et al, 1980].

All p-values are reported using upper-tailed test, unless otherwise indicated. Evaluation criteria (levels of significance) were applied differently for rare tumors (background rate of 1% or less) and common tumors (background rate greater than 1%) The evaluation criteria from the FDA are given in Table F (FDA) <sup>15</sup>.

<b>Table F. Evaluation Criteria for Common and Rare Tumors</b>	
<b>Test for Positive Trends</b>	<b>Control-High Pair-wise Comparisons</b>
Common and rare tumors will be tested at 0.005 and 0.025 significance levels, respectively	Common and rare tumors will be tested at 0.01 and 0.05 significance levels, respectively

**Sponsor's concluded results:** there were no treatment related gross changes in the preterminal dead and moribund sacrificed and terminally sacrificed rats. There was no treatment related non- neoplastic microscopic changes in the preterminal dead and moribund sacrificed and terminally sacrificed rats. There was no treatment related neoplastic microscopic changes in the female rats. The increased incidence of islet cell adenomas of the pancreas in high dose males (800/1200 mg/kg body weights) was considered treatment related. This judgement is based on the incidence of this benign tumor in this group being significantly higher ( $p < 0.01$ ) in a pairwise comparison between combined control groups and above the reported spontaneous rate for Harlan Sprague Dawley rats. However, the trend test for linear dose related increase for this tumor did not reach statistical significance, defined as  $\leq 0.005$  for a common tumor.

## 3.2 Reviewer's Analyses

To verify the sponsor's analyses and to perform additional analyses suggested by the reviewing pharmacologist, this review analyzed the SAS data sets of these studies received on 12/13/2017 via submission NDA 208623/S0001. This study included two controls; and this review analyzed the dose-response relationship of tumor incidence and mortality (including tumor-related mortality) against control 1, control 2, or combined two controls separately.

### 3.2.1 Survival Analysis

The survival distributions of rats in all treatment groups were estimated using the Kaplan-Meier product limit method. For control, low, medium, and high dose groups, the dose response relationship was tested using the likelihood ratio test and the homogeneity of survival distributions was tested using the log-rank test. The Kaplan-Meier curves for survival rates are given in Figures 1A and 1B in the appendix for male and female rats, respectively. The intercurrent mortality data are given in Tables 1A and 1B in the appendix for male and female rats, respectively. Results of the tests for dose response relationship and homogeneity of survivals, are given in Tables 3A and 3B in the appendix for male and female rats, respectively.

**Reviewer's findings:** This reviewer's analysis showed the numbers (percent) of death that occurred prior to termination of the group were 34 (66%), 34 (66%), 30 (60%), 29 (58%), and

28 (56%) in male rats and 25 (50%), 20 (40%), 21 (42%), 31 (62%), and 23 (46%) in female rats in the C1, C2, LD, MD, and HD groups, respectively. The survival analyses didn't show any statistically significant dose response relationship in mortality in males and females. Mortalities in the female MD group were significantly higher than that of the control 2 group and the combined two controls groups ( $P_s < 0.05$ ).

### 3.2.2 Tumor Data Analysis

The tumor data were analyzed for dose response relationships and pairwise comparisons of control group with each of the treated groups. Both the dose response relationship tests and pairwise comparisons were performed using the Poly-k method described in the papers of Bailer and Portier [2] and Bieler and Williams [3]. In this method an animal that lives the full study period ( $w_{\max}$ ) or dies before the terminal sacrifice but develops the tumor type being tested gets a score of  $s_h = 1$ . An animal that dies at week  $w_h$  without developing the tumor before the end of the study gets a score of  $s_h = \left(\frac{w_h}{w_{\max}}\right)^k < 1$ . The adjusted group size is defined as  $\sum s_h$ . As an interpretation, an animal with score  $s_h = 1$  can be considered as a whole animal while an animal with score  $s_h < 1$  can be considered as a partial animal. The adjusted group size  $\sum s_h$  is equal to N (the original group size) if all animals live up to the end of the study or if each animal that dies before the terminal sacrifice develops at least one tumor of the tumor type being tested, otherwise the adjusted group size is less than N. These adjusted group sizes are then used for the dose response relationship (or the pairwise) tests using the Cochran-Armitage test. One critical point for Poly-k test is the choice of the appropriate value of k, which depends on the tumor incidence pattern with the increased dose. For long term 104 week standard rat and mouse studies, a value of k=3 is suggested in the literature. Hence, this reviewer used k=3 for the analysis of this data. For the calculation of p-values the exact permutation method was used.

Multiple testing adjustments currently follow the rule displayed in Table 12.6.<sup>5,6</sup>

**Table 12.6** Recommended decision rules (levels of significance) for controlling the overall false positive rates for various statistical tests performed and submission types

Submission type	Tumor type	Decision rule				
		Trend test alone	Pairwise test alone	Joint test		
				Trend test	Pairwise test	
Standard 2 year study with two sexes and two species	Common	0.005	0.01	0.005	0.05	
	Rare	0.025	0.05	0.025	0.10	
Alternative ICH Studies (One 2-year study in one species and one short- or medium-term alternative study, two sexes)	Two-year study	Common	0.005	0.01	0.005	0.05
		Rare	0.025	0.05	0.025	0.10
	Short- or medium-term alternative study	Common	0.05	0.05	0.05	0.05
		Rare	0.05	0.05	0.05	0.05
Standard 2 year studies with two sexes and one species	Common	0.01	0.025	0.01	0.05	
	Rare	0.05	0.10	0.05	0.10	

The adjusted levels of significance for testing a positive dose response in the 2-year rat study are 0.005 and 0.025 for a common tumor and a rare tumor, respectively. The adjusted levels

of significance for the pairwise comparison in the 2-year rat study are 0.01 and 0.05 for a common tumor and a rare tumor, respectively.

The tumor rates and the p-values of the tested tumor types are listed in Tables 5A-1, 5A-2, 5A-3, 5B-1, 5B-2, and 5B-3 in the appendix for male and female rats, respectively.

**Reviewer’s findings:** Following table displays the tumor types showing p-values less than or equal to 0.05 either for dose response relationships or for pairwise comparisons of treated groups and control.

**Tumor Types with P-Values ≤ 0.05 for Dose Response Relationship or Pairwise Comparisons of Treated Groups and Controls in Male Rats**

Organ name	Tumor name	0 mkd Vehicle (C2) P - Trend	50 mkd Low (L) P - C2 vs. L	200 mkd Mid (M) P - C2 vs. M	800/1200 mkd High (H) P - C2 vs. H
Adrenal Glands	B_Pheochromocytoma	9/50 (39) 0.0564	16/50 (43) 0.1253	10/50 (41) 0.5500	19/50 (45) 0.0514
	M_Pheochromocytoma	5/50 (39) 0.6046	4/50 (40) 0.5164	4/50 (39) 0.5000	4/50 (42) 0.5479
	C_Pheochromocyto/B+M	13/50 (40) 0.0421	18/50 (43) 0.2569	13/50 (41) 0.4361	23/50 (45) 0.0647
Pancreas	Islet Cell Adenoma	2/50 (38) 0.0179	6/50 (40) 0.1486	4/50 (39) 0.3499	10/50 (42) 0.0201
	Islet Cell Carcinoma	1/50 (38) 0.4699	1/50 (40) 0.2597	2/50 (39) 0.5099	1/50 (42) 0.2725
	C_Islet Cell/A+C	3/50 (38) 0.0289	7/50 (40) 0.1769	5/50 (40) 0.3853	11/50 (43) 0.0333
Organ name	Tumor name	0 mkd Comb.Controls P - Trend	50 mkd Low (L) P - Comb.C vs. L	200 mkd Mid (M) P - Comb.C vs. M	800/1200 mkd High (H) P - Comb.C vs. H
Adrenal Glands	Cortical Cell Adenoma	0/99 (76) 0.0190 *	1/50 (40) 0.3448	1/50 (39) 0.3391	3/50 (42) 0.0430 **
	Cortical Cell Carcinoma	0/99 (76) 0.4202	1/50 (40) 0.3448	1/50 (39) 0.3391	0/50 (42) NC
	C_Cortical Cell/A+C	0/99 (76) 0.0388	2/50 (40) 0.1169	1/50 (39) 0.3391	3/50 (42) 0.0430 **
Pancreas	Adenoma	2/100 (78) 0.1917	0/50 (40) 0.5650	1/50 (39) NC	2/50 (42) 0.4374
	Hemangiosarcoma	1/100 (77) 0.6111	0/50 (40) 0.3419	0/50 (39) 0.3362	0/50 (42) 0.3529
	Islet Cell Adenoma	6/100 (77) 0.0143	6/50 (40) 0.1833	4/50 (39) 0.4489	10/50 (42) 0.0167
	Islet Cell Carcinoma	2/100 (77) 0.4420	1/50 (40) 0.2689	2/50 (39) 0.4126	1/50 (42) 0.2840
	C_Islet Cell_A+C	8/100 (77) 0.0244	7/50 (40) 0.2098	5/50 (40) 0.4758	11/50 (43) 0.0289

Note: X/ZZ (YY): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed.  
NC = Not calculable.

Note: The p-values marked with an asterisk \* indicate statistically significant dose responses at 0.005 and 0.025 for a common tumor and a rare tumor, respectively. The p-values marked with an asterisk \*\* indicate statistically significant pairwise comparison at 0.01 and 0.05 for a common tumor and a rare tumor, respectively.

**Tumor Types with P-Values  $\leq 0.05$  for Dose Response Relationship or Pairwise Comparisons of Treated Groups and Controls in Female Rats**

Organ name	Tumor name	0 mkd	50 mkd	200 mkd	800/1200 mkd
		Comb.Controls P - Trend	Low (L) P - Comb.C vs. L	Mid (M) P - Comb.C vs. M	High (H) P - Comb.C vs. H
Pituitary Gland	Adenoma	55/100 (88)	30/49 (43)	37/50 (43)	30/49 (42)
		0.2566	0.2681	0.0040 **	0.2117
Thyroid Gland	C-Cell Adenoma	6/99 (81)	9/50 (42)	3/50 (37)	4/50 (40)
		0.5659	0.0272	0.5787	0.4329
		7/99 (81)	12/50 (42)	3/50 (37)	6/50 (40)
	Adenoma+Carcinoma	0.4367	0.0050 **	0.3846	0.2232

Note: X/ZZ (YY): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed. NC = Not calculable.

Note: The p-values marked with an asterisk \* indicate statistically significant dose responses at 0.005 and 0.025 for a common tumor and a rare tumor, respectively. The p-values marked with an asterisk \*\* indicate statistically significant pairwise comparison at 0.01 and 0.05 for a common tumor and a rare tumor, respectively.

Based on the criteria of adjustment for multiple testing discussed above, adenoma cortical cell in adrenal glands in male rats was considered as a rare tumor and it has a statistically significant positive dose response relationship ( $p=0.0190 < 0.025$ ) when the combined controls were used in the analysis. The pairwise comparisons of this tumor type incidence rates against the combined-controls was statistically significant for high dosed group ( $p=0.0430 < 0.05$ ). The pairwise comparison of tumor type of the combination of adenoma and carcinoma cortical cell in adrenal glands against the combined controls was statistically significant for HD male rats ( $p=0.0430 < 0.05$ ).

There was no statistically significant positive dose-response relationship in female rats. The pairwise comparison of tumor type of the adenoma in pituitary gland against the combined controls was statistically significant for MD female rats ( $p=0.004 < 0.01$ ). The pairwise comparison of tumor type of the combination of C-cell adenoma and carcinoma in Thyroid gland against the combined controls was statistically significant for LD female rats ( $p=0.005 < 0.01$ ).

## 4 Mouse Study- 70576

**Study Report:** 70576.pdf (statistical report on page 1251)

**SAS data:** tumor.xpt

This study was conducted to evaluate the carcinogenic potential of the test article, AT1001, when administered once daily by oral gavage for 26 consecutive weeks to hemizygous Tg.rasH2 mice. The test material (AT1001) was administered daily via oral gavage once daily at doses of 0, 100, 300, or 1000 mg/kg/day for male mice; or 0, 50, 150, or 500 mg/kg/day for female mice for at least 26 weeks. This review refers these dose groups as the vehicle control (VC), low (LD), mid (MD), or high (HD) dose groups, respectively. There were 25 mice/sex/dose. Ten male and 25 female mice assigned to the positive control (PC) group and received a total of 3 intraperitoneal injections of Urethane (1000 mg/kg each) at 2-day intervals (i.e., on Day 1, 3 and 5). Terminal sacrifice occurred on week 27 for animals in all groups. The analyses summarized herein do not include the data from the positive control group.

Assessment of toxicity was based on dose analysis, morbidity, mortality, injury, body weight, food consumption, clinical observations and masses, ophthalmology, clinical pathology, toxicokinetics, macroscopic observations, and microscopic evaluations.

## 4.1 Sponsor's Analyses

### 4.1.1 *Survival Analysis*

The sponsor used the same survival analysis methods for the rat study in this mouse study.

**Sponsor's concluded results:** No treatment related mortality for AT1001 treated groups. As expected, treatment with the positive control article (Urethane), resulted in the death or pre-terminal euthanasia of the vast majority of vehicle control group between Weeks 8 and 26 of the study. As such, 24/25 males (96%) and 25/25 females (100%) were found dead, or were pre-terminally euthanized over the course of the study. Mortality data obtained from the vehicle control group were compared to those obtained from the positive control group using a Peto two-sided test. The results obtained from this test revealed a statistically significant ( $p < 0.0001$ ) increase in mortality rate in the Urethane-treated animals of both sexes in comparison to the vehicle control.

### 4.1.2 *Tumor Data Analysis*

The sponsor used the same tumor data analysis methods for the rat study in this mouse study.

**Sponsor's findings:** There were no AT1001-related carcinogenic findings. There were no statistically significant increases in the incidence of neoplasms in the test article dosed groups compared to vehicle controls. The results for positive control (Urethane) indicate that the transgenic mice used in this study responded to this positive control carcinogen as expected and consistent with historical data generated by (b) (4) and the literature.

## 4.2 Reviewer's Analyses

To verify the sponsor's analyses and to perform additional analyses suggested by the reviewing pharmacologist, this review analyzed the SAS data sets of these studies received on 12/13/2017 via submission NDA 208623/S0001.

### 4.2.1 *Survival Analysis*

The Kaplan-Meier curves for survival rates of all treatment groups are given in Figures 2A and 2B in the appendix for male and female mice, respectively. The intercurrent mortality data of all treatment groups are given in Tables 2A and 2B in the appendix for male and female mice, respectively. Results of the tests for dose response relationship and homogeneity of survivals for control, low, medium, and high dose groups are given in Tables 4A and 4B in the appendix for male and female mice, respectively.

**Reviewer's findings:** This reviewer's analysis showed the numbers (percent) of death that occurred prior to termination of the group were 0, 1 (4%), 1 (4%), 1 (8%), and 24 (96%) in male mice and 2 (8%), 0, 1 (4%), 2 (8%), or 25 (100%) in female mice in the VC, LD, MD, HD, or PC groups, respectively. The tests didn't show any statistically significant dose response relationship in mortality across controls and treated groups in both sexes. For both males and females, the positive control group showed significantly increasing mortality over the control group ( $p < 0.0001$  in all cases).

#### 4.2.2 Tumor Data Analysis

The tumor data were analyzed for dose response relationships and pairwise comparisons of the water control group and the vehicle control group separately with each of the treated groups using the same method that was used for the rat study. The tumor rates and the p-values of the tested tumor types are listed in Tables 6A, and 6B in the appendix for male and female mice, respectively.

**Reviewer's findings:** Because of the small group size and short study duration used in transgenic mouse studies, based on the statistical guideline for transgenic mouse studies, the significance level of 0.05 was used in the tests for dose response and pairwise comparisons in tumor incidences of both rare and common tumors.

Based on the criteria of adjustment for multiple testing discussed above, the tumor analysis did not show any statistically significant dose-response relationship in incidences in all tumor types tested in all dosed male and female groups. The PC group showed statistically significant increases in the incidence of a few tumor types in both males and females ( $p < 0.05$ ), when compared against the vehicle control. Those tumor types were listed in following table.

**Tumor Types with P-Values  $\leq 0.05$  for Pairwise Comparisons of VC and PC**

Animals	Organ Name	Tumor Name	0 mkd VC (N=25)	Urethane (1000 mg/kg) PC (N=10)	P-Value VC vs. PC
<b>Male Mice</b>	Harderian Glands	Adenoma	0/25 (25)	7/25 (25)	0.0048*
	Lungs	Adenoma, Bronchiolo-Alveolar	1/25 (25)	25/25 (25)	<0.001*
		Carcinoma, Bronchiolo-Alveolar	0/25 (25)	19/25 (25)	<0.001*
<b>Female Mice</b>	Harderian Glands	Adenoma	0/25 (25)	16/25 (25)	<0.001*
	Lungs	Adenoma, Bronchiolo-Alveolar	0/25 (25)	25/25 (25)	<0.001*
		Carcinoma, Bronchiolo-Alveolar	0/25 (25)	21/25 (25)	<0.001*

Note: The p-values marked with an asterisk \* indicate statistically significant pairwise comparison at 0.05.

Feng Zhou  
Mathematical Statistician

Concurring Reviewer: Karl Lin, Ph.D., Team Leader, Biometrics-6  
cc:

Dr. Ke Zhang  
Dr. David Joseph  
Dr. Yi Tsong  
Dr. Karl Lin  
Ms. Patrician

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## 5 Appendix

**Table 1A: Intercurrent Mortality Rate in Male Rats**

Week / Type of Death	Control 1		Control 2		Low		Mid		High	
	No. of Death	Cum %								
0 - 52	1	2.00	2	4.00	1	2.00			1	2.00
53 - 78	4	10.00	6	16.00	5	12.00	7	14.00	2	6.00
79 - 92	9	28.00	9	34.00	8	28.00	11	36.00	11	28.00
93 - 104	19	66.00	16	66.00	16	60.00	11	58.00	14	56.00
Terminal sacrifice	17	34.00	17	34.00	20	40.00	21	42.00	22	44.00
Total	50		50		50		50		50	

# All Cum. %Cumulative Percentage except for Terminal sacrifice

**Table 1B: Intercurrent Mortality Rate in Female Rats**

Week / Type of Death	Control 1		Control 2		Low		Mid		High	
	No. of Death	Cum %								
0 - 52	2	4.00	1	2.00			1	2.00	1	2.00
53 - 78	6	16.00	3	8.00	8	16.00	9	20.00	8	18.00
79 - 92	7	30.00	8	24.00	7	30.00	11	42.00	6	30.00
93 - 104	10	50.00	8	40.00	6	42.00	10	62.00	8	46.00
Terminal sacrifice	25	50.00	30	60.00	29	58.00	19	38.00	27	54.00
Total	50		50		50		50		50	

# All Cum. %Cumulative Percentage except for Terminal sacrifice

**Table 2A: Intercurrent Mortality Rate in Male Mice**

Week / Type of Death	Vehicle Control		Low		Mid		High		Positive Control	
	No. of Death	Cum %	No. of Death	Cum %						
0 - 13			1	4.00					1	4.00
14 - 26					1	4.00	2	8.00	23	96.00
Terminal sacrifice	25	100.00	24	96.00	24	96.00	23	92.00	1	4.00
Total	25		25		25		25		25	

# All Cum. %Cumulative Percentage except for Terminal sacrifice

**Table 2B: Intercurrent Mortality Rate in Female Mice**

Week / Type of Death	Vehicle Control		Low		Mid		High		Positive Control	
	No. of Death	Cum %	No. of Death	Cum %						
0 - 13									1	4.00
14 - 26	2	8.00			1	4.00	2	8.00	24	100.00
Terminal sacrifice	23	92.00	25	100.00	24	96.00	23	92.00		
Total	25		25		25		25		25	

# All Cum. %Cumulative Percentage except for Terminal sacrifice

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**Table 3A: Intercurrent Mortality Comparison in Male Rats**

Test	All Dose Groups	Vehicle Control 1 vs. Low	Vehicle Control 1 vs. Mid	Vehicle Control 1 vs. High
Dose-Response (Likelihood Ratio)	0.3514	0.5903	0.6478	0.2822
Homogeneity (Log-Rank)	0.7661	0.5843	0.6433	0.2733
Test	All Dose Groups	Vehicle Control 2 vs. Low	Vehicle Control 2 vs. Mid	Vehicle Control 2 vs. High
Dose-Response (Likelihood Ratio)	0.2881	0.4482	0.4573	0.1817
Homogeneity (Log-Rank)	0.6142	0.4419	0.4523	0.1750
Test	All Dose Groups	Comb.Controls vs. Low	Comb.Controls vs. Mid	Comb.Controls vs. High
Dose-Response (Likelihood Ratio)	0.2290	0.4478	0.4801	0.1623
Homogeneity (Log-Rank)	0.5578	0.4448	0.4780	0.1621

#All Cum. % Cumulative Percentage except for Terminal sacrifice;

\* = Significant at 5% level; \*\* = Significant at 1% level.

**Table 3B: Intercurrent Mortality Comparison in Female Rats**

Test	All Dose Groups	Vehicle Control 1 vs. Low	Vehicle Control 1 vs. Mid	Vehicle Control 1 vs. High
Dose-Response (Likelihood Ratio)	0.7443	0.4027	0.2166	0.7112
Homogeneity (Log-Rank)	0.1693	0.3997	0.2123	0.7090
Test	All Dose Groups	Vehicle Control 2 vs. Low	Vehicle Control 2 vs. Mid	Vehicle Control 2 vs. High
Dose-Response (Likelihood Ratio)	0.8099	0.8092	0.0208 *	0.4867
Homogeneity (Log-Rank)	0.0663	0.8075	0.0194	0.4825
Test	All Dose Groups	Comb.Controls vs. Low	Comb.Controls vs. Mid	Comb.Controls vs. High
Dose-Response (Likelihood Ratio)	0.9023	0.7294	0.0405 *	0.8564
Homogeneity (Log-Rank)	0.1063	0.7289	0.0335	0.8548

#All Cum. % Cumulative Percentage except for Terminal sacrifice;

\* = Significant at 5% level; \*\* = Significant at 1% level.

**Table 4A: Intercurrent Mortality Comparison in Male Mice**

Test	All Dose Groups	Control vs. Low	Control vs. Mid	Control vs. High	Control vs. Positive Control
Dose-Response (Likelihood Ratio)	0.2247	0.2390	0.2390	0.0935	<.0001
Homogeneity (Log-Rank)	0.5723	0.3173	0.3173	0.1531	<.0001

#All Cum. % Cumulative Percentage except for Terminal sacrifice;

\* = Significant at 5% level; \*\* = Significant at 1% level.

**Table 4B: Intercurrent Mortality Comparison in Female Mice**

Test	All Dose Groups	Control vs. Low	Control vs. Mid	Control vs. High	Control vs. Positive Control
Dose-Response (Likelihood Ratio)	0.5429	0.0935	0.5678	0.9915	<.0001
Homogeneity (Log-Rank)	0.5251	0.1531	0.5717	0.9915	<.0001

#All Cum. % Cumulative Percentage except for Terminal sacrifice;

\* = Significant at 5% level; \*\* = Significant at 1% level.

**Table 5A-1: Tumor Rates and P-Values for Dose Response Relationship and Pairwise Comparisons with Control 1 – Male Rats**

Organ name	Tumor name	0 mkd Vehicle (C1) P - Trend	50 mkd Low (L) P – C1 vs. L	200 mkd Mid (M) P – C1 vs. M	800/1200 mkd High (H) P – C1 vs. H
Adrenal Glands	Benign Pheochromocytoma	21/49 (42)	16/50 (43)	10/50 (41)	19/50 (45)
		0.4206	0.8340	0.9860	0.6956
	Cortical Cell Adenoma	0/49 (39)	1/50 (40)	1/50 (39)	3/50 (42)
		0.0493	0.5063	0.5000	0.1346
Cortical Cell Carcinoma	0/49 (39)	1/50 (40)	1/50 (39)	0/50 (42)	
	0.5156	0.5063	0.5000	NC	
Malignant Pheochromocytoma	4/49 (39)	4/50 (40)	4/50 (39)	4/50 (42)	
	0.5197	0.3703	NC	0.3989	
Brain Cerebrum	Oligodendroglioma	0/50 (39)	1/50 (40)	0/50 (39)	0/50 (42)
		0.5062	0.5063	NC	NC
Brain- Medulla/Pons	Benign Glioma	0/50 (39)	0/50 (40)	0/50 (39)	1/50 (42)
		0.2625	NC	NC	0.5185
Gland, Preputial	Hemangioma	0/45 (38)	1/43 (37)	0/45 (38)	0/46 (40)
		0.5098	0.4933	NC	NC
	Squamous Cell Carcinoma	1/45 (38)	0/43 (37)	1/45 (38)	0/46 (40)
		0.6372	0.4933	NC	0.5128
Hemolymphoreticular System	Large Granular Lymphocyte Lymphoma	0/50 (39)	0/50 (40)	1/50 (39)	0/50 (42)
		0.2625	NC	0.5000	NC
	Lymphoma	0/50 (39)	0/50 (40)	1/50 (39)	0/50 (42)
		0.2625	NC	0.5000	NC
Jejunum	Cystadenoma	0/35 (29)	1/36 (30)	0/39 (33)	0/37 (32)
		0.5242	0.5085	NC	NC
Kidneys	Liposarcoma	0/50 (39)	1/50 (40)	0/50 (39)	0/49 (42)
		0.5062	0.5063	NC	NC
Liver	Hepatocellular Adenoma	0/50 (39)	0/50 (40)	0/50 (39)	2/50 (42)
		0.0677	NC	NC	0.2657
Lungs With Bronchi	Bronchiolar Alveolar Adenoma	1/50 (40)	0/50 (40)	0/50 (39)	1/50 (42)
		0.4549	0.5000	0.4937	0.2593
Lymph Node, Mesenteric	Hemangioma	0/49 (39)	0/50 (40)	0/48 (38)	1/49 (42)
		0.2642	NC	NC	0.5185

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Organ name	Tumor name	0 mkd Vehicle (C1) P - Trend	50 mkd Low (L) P - C1 vs. L	200 mkd Mid (M) P - C1 vs. M	800/1200 mkd High (H) P - C1 vs. H
Mammary Glands	Fibroadenoma	0/40 (31) 0.4252	1/40 (31) 0.5000	3/43 (33) 0.1310	1/43 (36) 0.5373
	Fibrosarcoma	0/40 (31) 0.2748	0/40 (31) NC	0/43 (33) NC	1/43 (36) 0.5373
Mesentery	Hemangioma	0/2 (1) 0.5385	0/3 (2) NC	1/3 (3) 0.7500	0/8 (7) NC
Pancreas	Adenoma	1/50 (39) 0.1809	0/50 (40) 0.5063	1/50 (39) NC	2/50 (42) 0.5281
	Islet Cell Adenoma	4/50 (40) 0.0406	6/50 (40) 0.3685	4/50 (39) 0.6297	10/50 (42) 0.0848
	Islet Cell Carcinoma	1/50 (40) 0.4594	1/50 (40) NC	2/50 (39) 0.4904	1/50 (42) 0.2593
Pituitary Gland	Adenoma	12/49 (40) 0.8205	13/49 (40) 0.5000	18/50 (42) 0.1639	11/50 (43) 0.5812
Rectum	Adenocarcinoma	0/48 (39) 0.4901	1/46 (38) 0.4935	0/46 (37) NC	0/44 (37) NC
Skin/Subcutis	Fibroma	0/50 (39) 0.2625	0/50 (40) NC	0/50 (39) NC	1/50 (42) 0.5185
	Keratoacanthoma	2/50 (39) 0.7142	2/50 (40) 0.3173	2/50 (39) NC	1/50 (42) 0.5281
	Malignant Schwannoma	0/50 (39) 0.2625	0/50 (40) NC	0/50 (39) NC	1/50 (42) 0.5185
	Squamous Cell Carcinoma	0/50 (39) 0.2625	0/50 (40) NC	0/50 (39) NC	1/50 (42) 0.5185
	Squamous Cell Papilloma	0/50 (39) 0.7578	2/50 (40) 0.2532	0/50 (39) NC	0/50 (42) NC
Stomach	Squamous Cell Carcinoma	0/49 (39) 0.7609	2/49 (39) 0.2468	0/49 (39) NC	0/50 (42) NC
Testes	Leidig Cell Tumour	1/50 (39) 0.4573	0/50 (40) 0.5063	0/50 (39) 0.5000	1/50 (42) 0.2657
Thyroid Gland	C-Cell Adenoma	1/50 (39) 0.4746	3/50 (40) 0.3173	1/49 (39) NC	2/50 (42) 0.5281
	C-Cell Carcinoma	2/50 (39) 0.7206	2/50 (40) 0.3173	1/49 (39) 0.5000	1/50 (42) 0.5281
	Follicular Cell Adenoma	0/50 (39) 0.2625	0/50 (40) NC	1/49 (39) 0.5000	0/50 (42) NC
	Follicular Cell Carcinoma	1/50 (39) 0.7562	0/50 (40) 0.5063	0/49 (39) 0.5000	0/50 (42) 0.5185

Note: X/ZZ (YY): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed.  
 NC = Not calculable.  
 Note: The p-values marked with an asterisk \* indicate statistically significant dose responses at 0.005 and 0.025 for a common tumor and a rare tumor, respectively. The p-values marked with an asterisk \*\* indicate statistically significant pairwise comparison at 0.01 and 0.05 for a common tumor and a rare tumor, respectively.

**Table 5A-2: Tumor Rates and P-Values for Dose Response Relationship and Pairwise Comparisons with Control 2 – Male Rats**

Organ name	Tumor name	0 mkd Vehicle (C2) P - Trend	50 mkd Low (L) P – C2 vs. L	200 mkd Mid (M) P - C2 vs. M	800/1200 mkd High (H) P – C2 vs. H
Adrenal Glands	Benign Pheochromocytoma	9/50 (39)	16/50 (43)	10/50 (41)	19/50 (45)
		0.0564	0.1253	0.5500	0.0514
	Malignant Pheochromocytoma	5/50 (39)	4/50 (40)	4/50 (39)	4/50 (42)
		0.6046	0.5164	0.5000	0.5479
	C_Pheochromocytoma/B+M	13/50 (40)	18/50 (43)	13/50 (41)	23/50 (45)
		0.0421	0.2569	0.4361	0.0647
	Cortical Cell Adenoma	0/50 (38)	1/50 (40)	1/50 (39)	3/50 (42)
	0.0508	0.5128	0.5065	0.1397	
C_Cortical Cell/A+C	Cortical Cell Carcinoma	0/50 (38)	1/50 (40)	1/50 (39)	0/50 (42)
		0.5187	0.5128	0.5065	NC
	C_Cortical Cell/A+C	0/50 (38)	2/50 (40)	1/50 (39)	3/50 (42)
	0.0972	0.2597	0.5065	0.1397	
Brain Cerebrum	Oligodendroglioma	0/50 (38)	1/50 (40)	0/50 (39)	0/50 (42)
		0.5094	0.5128	NC	NC
Brain- Medulla/Pons	Benign Glioma	0/50 (38)	0/50 (40)	0/50 (39)	1/50 (42)
		0.2642	NC	NC	0.5250
Duodenum	Adenocarcinoma	1/38 (30)	0/38 (31)	0/38 (31)	0/40 (34)
		0.7619	0.5082	0.5082	0.5313
Gland, Preputial	Hemangioma	0/44 (36)	1/43 (37)	0/45 (38)	0/46 (40)
		0.5166	0.5068	NC	NC
	Squamous Cell Carcinoma	0/44 (36)	0/43 (37)	1/45 (38)	0/46 (40)
		0.2649	NC	0.5135	NC
Harderian Glands	Adenoma	1/44 (36)	0/45 (38)	0/45 (38)	0/47 (41)
		0.7647	0.5135	0.5135	0.5325
Heart	Benign Schwannoma	1/50 (38)	0/50 (40)	0/50 (39)	0/50 (42)
		0.7610	0.5128	0.5065	0.5250
Hemolymphoreticular System	Large Granular Lymphocyte Lymphoma	1/50 (38)	0/50 (40)	1/50 (39)	0/50 (42)
		0.6429	0.5128	0.2532	0.5250
	Lymphoma	0/50 (38)	0/50 (40)	1/50 (39)	0/50 (42)
		0.2642	NC	0.5065	NC
Jejunum	Cystadenoma	0/37 (29)	1/36 (30)	0/39 (33)	0/37 (32)
		0.5242	0.5085	NC	NC
Kidneys	Liposarcoma	0/50 (38)	1/50 (40)	0/50 (39)	0/49 (42)
		0.5094	0.5128	NC	NC
Liver	Hepatocellular Adenoma	0/50 (38)	0/50 (40)	0/50 (39)	2/50 (42)
		0.0685	NC	NC	0.2725
Lungs sith Bronchi	Bronchiolar Alveolar Adenoma	0/50 (38)	0/50 (40)	0/50 (39)	1/50 (42)
		0.2642	NC	NC	0.5250

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Organ name	Tumor name	0 mkd Vehicle (C2) P - Trend	50 mkd Low (L) P - C2 vs. L	200 mkd Mid (M) P - C2 vs. M	800/1200 mkd High (H) P - C2 vs. H
Lymph Node, Mesenteric	Hemangioma	0/50 (38) 0.2658	0/50 (40) NC	0/48 (38) NC	1/49 (42) 0.5250
	Hemangiosarcoma	1/50 (38) 0.7595	0/50 (40) 0.5128	0/48 (38) 0.5000	0/49 (42) 0.5250
Mammary Glands	Fibroadenoma	1/46 (34) 0.5400	1/40 (31) 0.7303	3/43 (33) 0.2954	1/43 (36) 0.2609
	Fibrosarcoma	0/46 (34) 0.2687	0/40 (31) NC	0/43 (33) NC	1/43 (36) 0.5143
Mesentery	Hemangioma	0/5 (4) 0.4375	0/3 (2) NC	1/3 (3) 0.4286	0/8 (7) NC
Pancreas	Adenoma	1/50 (38) 0.1838	0/50 (40) 0.5128	1/50 (39) 0.2532	2/50 (42) 0.5380
	Hemangiosarcoma	1/50 (38) 0.7610	0/50 (40) 0.5128	0/50 (39) 0.5065	0/50 (42) 0.5250
	Islet Cell Adenoma	2/50 (38) 0.0179	6/50 (40) 0.1486	4/50 (39) 0.3499	10/50 (42) 0.0201
	Islet Cell Carcinoma	1/50 (38) 0.4699	1/50 (40) 0.2597	2/50 (39) 0.5099	1/50 (42) 0.2725
	C_Islet Cell/A+C	3/50 (38) 0.0289	7/50 (40) 0.1769	5/50 (40) 0.3853	11/50 (43) 0.0333
	Pituitary Gland	Adenoma	9/48 (36) 0.7591	13/49 (40) 0.3213	18/50 (42) 0.0781
Rectum	Adenocarcinoma	0/47 (36) 0.5000	1/46 (38) 0.5135	0/46 (37) NC	0/44 (37) NC
Skin/Subcutis	Fibroma	0/50 (38) 0.2642	0/50 (40) NC	0/50 (39) NC	1/50 (42) 0.5250
	Keratoacanthoma	0/50 (38) 0.4394	2/50 (40) 0.2597	2/50 (39) 0.2532	1/50 (42) 0.5250
	Malignant Schwannoma	1/50 (38) 0.4598	0/50 (40) 0.5128	0/50 (39) 0.5065	1/50 (42) 0.2725
	Squamous Cell Carcinoma	0/50 (38) 0.2642	0/50 (40) NC	0/50 (39) NC	1/50 (42) 0.5250
	Squamous Cell Papilloma	0/50 (38) 0.7609	2/50 (40) 0.2597	0/50 (39) NC	0/50 (42) NC
	C_Squamous Cell/C+P	0/50 (38) 0.4622	2/50 (40) 0.2597	0/50 (39) NC	1/50 (42) 0.5250
Stomach	Squamous Cell Carcinoma	0/50 (38) 0.7641	2/49 (39) 0.2532	0/49 (39) NC	0/50 (42) NC
Testes	Leidig Cell Tumour	1/50 (38) 0.4598	0/50 (40) 0.5128	0/50 (39) 0.5065	1/50 (42) 0.2725
	Mesothelioma	1/50 (38) 0.7610	0/50 (40) 0.5128	0/50 (39) 0.5065	0/50 (42) 0.5250
Thyroid Gland	Adenoma	1/50 (38) 0.7610	0/50 (40) 0.5128	0/49 (39) 0.5065	0/50 (42) 0.5250
	C-Cell Adenoma	2/50 (38) 0.5802	3/50 (40) 0.5247	1/49 (39) 0.5099	2/50 (42) 0.3466

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Organ name	Tumor name	0 mkd	50 mkd	200 mkd	800/1200 mkd
		Vehicle (C2) P - Trend	Low (L) P - C2 vs. L	Mid (M) P - C2 vs. M	High (H) P - C2 vs. H
	C-Cell Carcinoma	3/50 (38) 0.8277	2/50 (40) 0.5247	1/49 (39) 0.7023	1/50 (42) 0.7293
	C_C-Cell/A+C	5/50 (38) 0.7895	5/50 (40) 0.4021	2/49 (39) 0.7953	3/50 (42) 0.6992
	Follicular Cell Adenoma	0/50 (38) 0.2642	0/50 (40) NC	1/49 (39) 0.5065	0/50 (42) NC

Note: X/ZZ (YY): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed. NC = Not calculable.

Note: The p-values marked with an asterisk \* indicate statistically significant dose responses at 0.005 and 0.025 for a common tumor and a rare tumor, respectively. The p-values marked with an asterisk \*\* indicate statistically significant pairwise comparison at 0.01 and 0.05 for a common tumor and a rare tumor, respectively.

**Table 5A-3: Tumor Rates and P-Values for Dose Response Relationship and Pairwise Comparisons with Combined Controls – Male Rats**

Organ name	Tumor name	0 mkd	50 mkd	200 mkd	800/1200 mkd
		Comb.Controls P - Trend	Low (L) P - Comb.C vs. L	Mid (M) P - Comb.C vs. M	High (H) P - Comb.C vs. H
Adrenal Glands	B_Pheochromocytoma	30/99 (81) 0.2214	16/50 (43) 0.5679	10/50 (41) 0.8860	19/50 (45) 0.3503
	M_Pheochromocytoma	9/99 (79) 0.5784	4/50 (40) 0.4570	4/50 (39) 0.4386	4/50 (42) 0.4928
	C_Pheochromocyto/B+M	36/99 (82) 0.1484	18/50 (43) 0.5104	13/50 (41) 0.8661	23/50 (45) 0.2764
	Cortical Cell Adenoma	0/99 (76) 0.0190 \$	1/50 (40) 0.3448	1/50 (39) 0.3391	3/50 (42) 0.0430 \$
	Cortical Cell Carcinoma	0/99 (76) 0.4202	1/50 (40) 0.3448	1/50 (39) 0.3391	0/50 (42) NC
	C_Cortical Cell/A+C	0/99 (76) 0.0388	2/50 (40) 0.1169	1/50 (39) 0.3391	3/50 (42) 0.0430 \$
Brain Cerebrum	Oligodendroglioma	0/100 (77) 0.4091	1/50 (40) 0.3419	0/50 (39) NC	0/50 (42) NC
		Brain- Medulla/Pons	Benign Glioma	0/100 (77) 0.2121	0/50 (40) NC
Duodenum	Adenocarcinoma	1/75 (61) 0.6115	0/38 (31) 0.3370	0/38 (31) 0.3370	0/40 (34) 0.3579
		Gland, Preputial	Hemangioma	0/89 (74) 0.4127	1/43 (37) 0.3333
Squamous Cell Carcinoma	1/89 (74) 0.4981			0/43 (37) 0.3333	1/45 (38) 0.5655
	Harderian Glands	Adenoma	1/90 (75) 0.6094	0/45 (38) 0.3363	0/45 (38) 0.3363
Heart	Benign Schwannoma	1/100 (77) 0.6111	0/50 (40) 0.3419	0/50 (39) 0.3362	0/50 (42) 0.3529
		Hemolymphoreticular System	Large Granular Lymphocyte Lymphoma	1/100 (77) 0.4981	0/50 (40) 0.3419
Lymphoma	0/100 (77)			0/50 (40)	1/50 (39)

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Organ name	Tumor name	0 mkd Comb.Controls P - Trend	50 mkd Low (L) P - Comb.C vs. L	200 mkd Mid (M) P - Comb.C vs. M	800/1200 mkd High (H) P - Comb.C vs. H
		0.2121	NC	0.3362	NC
Jejunum	Cystadenoma	0/72 (58) 0.4248	1/36 (30) 0.3409	0/39 (33) NC	0/37 (32) NC
Kidneys	Liposarcoma	0/100 (77) 0.4091	1/50 (40) 0.3419	0/50 (39) NC	0/49 (42) NC
Liver	Hepatocellular Adenoma	0/100 (77) 0.0441	0/50 (40) NC	0/50 (39) NC	2/50 (42) 0.1226
Lungs with Bronchi	Bronchiolar Alveolar Adenoma	1/100 (77) 0.3801	0/50 (40) 0.3419	0/50 (39) 0.3362	1/50 (42) 0.5833
Lymph Node, Mesenteric	Hemangioma	0/99 (77) 0.2132	0/50 (40) NC	0/48 (38) NC	1/49 (42) 0.3529
	Hemangiosarcoma	1/99 (77) 0.6091	0/50 (40) 0.3419	0/48 (38) 0.3304	0/49 (42) 0.3529
Mammary Glands	Fibroadenoma	1/86 (65) 0.4148	1/40 (31) 0.5439	3/43 (33) 0.1095	1/43 (36) 0.5881
	Fibrosarcoma	0/86 (65) 0.2182	0/40 (31) NC	0/43 (33) NC	1/43 (36) 0.3564
Mesentery	Hemangioma	0/7 (4) 0.4375	0/3 (2) NC	1/3 (3) 0.4286	0/8 (7) NC
Pancreas	Adenoma	2/100 (78) 0.1917	0/50 (40) 0.5650	1/50 (39) NC	2/50 (42) 0.4374
	Hemangiosarcoma	1/100 (77) 0.6111	0/50 (40) 0.3419	0/50 (39) 0.3362	0/50 (42) 0.3529
	Islet Cell Adenoma	6/100 (77) 0.0143	6/50 (40) 0.1833	4/50 (39) 0.4489	10/50 (42) 0.0167
	Islet Cell Carcinoma	2/100 (77) 0.4420	1/50 (40) 0.2689	2/50 (39) 0.4126	1/50 (42) 0.2840
	C_Islet Cell_A+C	8/100 (77) 0.0244	7/50 (40) 0.2098	5/50 (40) 0.4758	11/50 (43) 0.0289
Pituitary Gland	Adenoma	21/97 (77) 0.7123	13/49 (40) 0.3506	18/50 (42) 0.0642	11/50 (43) 0.4904
Rectum	Adenocarcinoma	0/95 (75) 0.3957	1/46 (38) 0.3363	0/46 (37) NC	0/44 (37) NC
Skin/Subcutis	Fibroma	0/100 (77) 0.2121	0/50 (40) NC	0/50 (39) NC	1/50 (42) 0.3529
	Keratoacanthoma	2/100 (77) 0.5301	2/50 (40) 0.4228	2/50 (39) 0.4126	1/50 (42) 0.2840
	Malignant Schwannoma	1/100 (77) 0.3801	0/50 (40) 0.3419	0/50 (39) 0.3362	1/50 (42) 0.5833
	Squamous Cell Carcinoma	0/100 (77) 0.2121	0/50 (40) NC	0/50 (39) NC	1/50 (42) 0.3529
	Squamous Cell Papilloma	0/100 (77) 0.6521	2/50 (40) 0.1149	0/50 (39) NC	0/50 (42) NC
	C_Squamous Cell/C+P	0/100 (77)	2/50 (40)	0/50 (39)	1/50 (42)

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Organ name	Tumor name	0 mkd	50 mkd	200 mkd	800/1200 mkd
		Comb.Controls P - Trend	Low (L) P - Comb.C vs. L	Mid (M) P - Comb.C vs. M	High (H) P - Comb.C vs. H
		0.3150	0.1149	NC	0.3529
Stomach	Squamous Cell Carcinoma	0/99 (77) 0.6545	2/49 (39) 0.1111	0/49 (39) NC	0/50 (42) NC
Testes	Leidig Cell Tumour	2/100 (77) 0.5129	0/50 (40) 0.5688	0/50 (39) 0.5613	1/50 (42) 0.2840
	Mesothelioma	1/100 (77) 0.6111	0/50 (40) 0.3419	0/50 (39) 0.3362	0/50 (42) 0.3529
Thyroid Gland	Adenoma	1/100 (77) 0.6111	0/50 (40) 0.3419	0/49 (39) 0.3362	0/50 (42) 0.3529
	C-Cell Adenoma	3/100 (78) 0.4950	3/50 (40) 0.3281	1/49 (39) 0.4074	2/50 (42) 0.5749
	C-Cell Carcinoma	5/100 (77) 0.8205	2/50 (40) 0.4495	1/49 (39) 0.6604	1/50 (42) 0.6925
	C_C-Cell/A+C	8/100 (78) 0.7351	5/50 (40) 0.4664	2/49 (39) 0.7114	3/50 (42) 0.5806
	Follicular Cell Adenoma	0/100 (77) 0.2121	0/50 (40) NC	1/49 (39) 0.3362	0/50 (42) NC
	Follicular Cell Carcinoma	1/100 (77) 0.6111	0/50 (40) 0.3419	0/49 (39) 0.3362	0/50 (42) 0.3529
	C_Follicular Cell/A+C	1/100 (77) 0.4981	0/50 (40) 0.3419	1/49 (39) 0.5613	0/50 (42) 0.3529

Note: X/ZZ (YY): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed. NC = Not calculable.

Note: The p-values marked with an asterisk \* indicate statistically significant dose responses at 0.005 and 0.025 for a common tumor and a rare tumor, respectively. The p-values marked with an asterisk \*\* indicate statistically significant pairwise comparison at 0.01 and 0.05 for a common tumor and a rare tumor, respectively.

**Table 5B-1: Tumor Rates and P-Values for Dose Response Relationship and Pairwise Comparisons with Control 1 – Female Rats**

Organ name	Tumor name	0 mkd	50 mkd	200 mkd	800/1200 mkd
		Vehicle (C1) P - Trend	Low (L) P – C1 vs. L	Mid (M) P – C1 vs. M	High (H) P – C1 vs. H
Adrenal Glands	Benign Pheochromocytoma	5/50 (41) 0.8018	3/50 (42) 0.6574	4/50 (37) 0.4336	2/49 (39) 0.7629
	Cortical Cell Adenoma	4/50 (40) 0.7641	0/50 (42) 0.9477	1/50 (36) 0.7863	1/49 (39) 0.8127
	Cortical Cell Carcinoma	0/50 (39) 0.4808	1/50 (42) 0.5185	0/50 (36) NC	0/49 (39) NC
	Malignant Pheochromocytoma	0/50 (39) 0.4808	1/50 (42) 0.5185	0/50 (36) NC	0/49 (39) NC
Brain Cerebellum	Malignant Glioma	1/50 (40) 0.7468	0/50 (42) 0.5122	0/50 (36) 0.4737	0/50 (40) 0.5000
Brain Cerebrum	Malignant Glioma	1/50 (40) 0.7468	0/50 (42) 0.5122	0/50 (36) 0.4737	0/50 (40) 0.5000
	Meningeal Tumour	1/50 (39) 0.7516	0/50 (42) 0.5185	0/50 (36) 0.4800	0/50 (40) 0.5063
Brain- Medulla/Pons	Malignant Glioma	1/50 (40) 0.7468	0/50 (42) 0.5122	0/50 (36) 0.4737	0/50 (40) 0.5000
	Meningioma	1/50 (39) 0.7468	0/50 (42) 0.5122	0/50 (36) 0.4737	0/50 (40) 0.5000

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Organ name	Tumor name	0 mkd Vehicle (C1) P - Trend	50 mkd Low (L) P - C1 vs. L	200 mkd Mid (M) P - C1 vs. M	800/1200 mkd High (H) P - C1 vs. H
		0.7516	0.5185	0.4800	0.5063
Eyes With Optic Nerves	Leiomyoma	0/50 (39) 0.2548	0/50 (42) NC	0/50 (36) NC	1/50 (40) 0.5063
	Malignant Schwannoma	0/50 (39) 0.4841	1/50 (42) 0.5185	0/50 (36) NC	0/50 (40) NC
Gland, Clitoral	Squamous Cell Carcinoma	1/42 (37) 0.7633	1/33 (30) 0.6988	0/32 (26) 0.4127	0/34 (30) 0.4478
	Squamous Cell Papilloma	1/42 (37) 0.7633	1/33 (30) 0.6988	0/32 (26) 0.4127	0/34 (30) 0.4478
Hemolymphoreticular System	Histiocytic Sarcoma	0/50 (39) 0.2548	0/50 (42) NC	0/50 (36) NC	1/50 (40) 0.5063
	Large Granular Lymphocyte Lymphoma	0/50 (39) 0.2595	0/50 (42) NC	0/50 (36) NC	1/50 (41) 0.5125
	Lymphoma	1/50 (39) 0.6208	0/50 (42) 0.5185	1/50 (36) 0.7330	0/50 (40) 0.5063
Liver	Adenoma	1/50 (39) 0.7516	0/50 (42) 0.5185	0/50 (36) 0.4800	0/50 (40) 0.5063
	Cholangioma	0/50 (39) 0.4841	1/50 (42) 0.5185	0/50 (36) NC	0/50 (40) NC
	Hepatocellular Adenoma	0/50 (39) 0.4971	1/50 (42) 0.5185	1/50 (37) 0.4868	0/50 (40) NC
	Hepatocellular Carcinoma	0/50 (39) 0.4841	1/50 (42) 0.5185	0/50 (36) NC	0/50 (40) NC
Lymph Node, Mesenteric	Hemangioma	1/49 (38) 0.6189	0/50 (42) 0.5250	1/49 (35) 0.7325	0/49 (39) 0.5065
Mammary Glands	Adenocarcinoma	7/50 (41) 0.0691	3/50 (42) 0.8536	3/50 (37) 0.7994	9/50 (42) 0.4117
	Adenoma	1/50 (39) 0.2646	1/50 (42) 0.2657	2/50 (36) 0.4696	2/50 (40) 0.5096
	Fibroadenoma	22/50 (43) 0.0729	20/50 (45) 0.6616	26/50 (44) 0.2989	30/50 (48) 0.1898
	Fibroma	0/50 (39) 0.2548	0/50 (42) NC	0/50 (36) NC	1/50 (40) 0.5063
	Hemangiosarcoma	0/50 (39) 0.2532	0/50 (42) NC	1/50 (37) 0.4868	0/50 (40) NC
	Squamous Cell Carcinoma	1/50 (40) 0.8017	1/50 (42) 0.2593	0/50 (36) 0.4737	0/50 (40) 0.5000
Ovaries With Oviduct	Benign Granulosa Cell Tumour	1/50 (39) 0.7484	0/49 (41) 0.5125	0/50 (36) 0.4800	0/49 (39) 0.5000
	Luteoma	0/50 (39) 0.7352	2/49 (41) 0.2595	0/50 (36) NC	0/49 (39) NC
	Malignant Granulosa Cell Tumor	1/50 (40) 0.7436	0/49 (41) 0.5062	0/50 (36) 0.4737	0/49 (39) 0.4937
	Sex Chord Stromal Tumour	1/50 (39) 0.4411	0/49 (41) 0.5125	0/50 (36) 0.4800	1/49 (39) NC
Pancreas	Islet Cell Adenoma	1/50 (39)	2/50 (42)	1/50 (37)	1/50 (40)

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Organ name	Tumor name	0 mkd Vehicle (C1) P - Trend	50 mkd Low (L) P - C1 vs. L	200 mkd Mid (M) P - C1 vs. M	800/1200 mkd High (H) P - C1 vs. H
Pituitary Gland	Islet Cell Carcinoma	0.5443	0.5281	0.7400	0.2532
		0/50 (39)	1/50 (42)	0/50 (36)	0/50 (40)
	Adenocarcinoma	0.4841	0.5185	NC	NC
		1/50 (39)	0/49 (41)	1/50 (37)	1/49 (39)
		0.3823	0.5125	0.7400	NC
		25/50 (42)	30/49 (43)	37/50 (43)	30/49 (42)
Adenoma	0.3464	0.2234	0.0056 **	0.1794	
Skin/Subcutis	Fibroma	1/50 (40)	0/50 (42)	0/50 (36)	0/50 (40)
		0.7468	0.5122	0.4737	0.5000
	Squamous Cell Carcinoma	0/50 (39)	2/50 (42)	0/50 (36)	0/50 (40)
		0.7354	0.2657	NC	NC
Spleen	Hemangiopericytoma	1/50 (40)	0/50 (42)	0/50 (36)	0/50 (40)
		0.7468	0.5122	0.4737	0.5000
Thyroid Gland	C-Cell Adenoma	1/49 (39)	9/50 (42)	3/50 (37)	4/50 (40)
		0.5427	0.0100 **	0.2877	0.1873
	C-Cell Carcinoma	1/49 (39)	3/50 (42)	0/50 (36)	2/50 (40)
		0.4339	0.3364	0.4800	0.5096
	Follicular Cell Adenoma	1/49 (39)	0/50 (42)	0/50 (36)	0/50 (40)
		0.7516	0.5185	0.4800	0.5063
	Follicular Cell Carcinoma	0/49 (39)	1/50 (42)	0/50 (36)	0/50 (40)
		0.4841	0.5185	NC	NC
Uterus With Cervix	Adenocarcinoma	1/49 (38)	0/50 (42)	0/50 (36)	0/50 (40)
		0.7564	0.5250	0.4865	0.5128
	Endometrial Adenocarcinoma	4/49 (39)	4/50 (42)	1/50 (36)	6/50 (40)
		0.1418	0.3989	0.7951	0.3850
	Granular Cell Tumour	0/49 (38)	0/50 (42)	0/50 (36)	1/50 (40)
		0.2564	NC	NC	0.5128
	Hemangioma	0/49 (38)	0/50 (42)	1/50 (36)	0/50 (40)
		0.2564	NC	0.4865	NC
	Malignant Schwannoma	0/49 (38)	2/50 (42)	0/50 (36)	1/50 (40)
		0.4434	0.2725	NC	0.5128
	Papilloma	0/49 (38)	0/50 (42)	1/50 (36)	0/50 (40)
		0.2564	NC	0.4865	NC
	Polyp	5/49 (38)	6/50 (43)	5/50 (37)	9/50 (41)
		0.1121	0.5887	0.6146	0.2343
Squamous Cell Carcinoma	0/49 (38)	2/50 (42)	1/50 (36)	2/50 (40)	
	0.2026	0.2725	0.4865	0.2597	
Squamous Cell Papilloma	0/49 (38)	0/50 (42)	1/50 (37)	1/50 (40)	
	0.1846	NC	0.4933	0.5128	

Note: X/ZZ (YY): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed.  
 NC = Not calculable.  
 Note: The p-values marked with an asterisk \* indicate statistically significant dose responses at 0.005 and 0.025 for a common tumor and a rare tumor, respectively. The p-values marked with an asterisk \*\* indicate statistically significant pairwise comparison at 0.01 and 0.05 for a common tumor and a rare tumor, respectively.

**Table 5B-2: Tumor Rates and P-Values for Dose Response Relationship and Pairwise Comparisons with Control 2 – Female Rats**

Organ name	Tumor name	0 mkd Vehicle (C2) P - Trend	50 mkd Low (L) P - C2 vs. L	200 mkd Mid (M) P - C2 vs. M	800/1200 mkd High (H) P - C2 vs. H
Adrenal Glands	Benign Pheochromocytoma	6/50 (42)	3/50 (42)	4/50 (37)	2/49 (39)

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Organ name	Tumor name	0 mkd Vehicle (C2) P - Trend	50 mkd Low (L) P - C2 vs. L	200 mkd Mid (M) P - C2 vs. M	800/1200 mkd High (H) P - C2 vs. H
		0.8501	0.7586	0.5470	0.8428
	Cortical Cell Adenoma	2/50 (43)	0/50 (42)	1/50 (36)	1/49 (39)
		0.4822	0.7471	0.4329	0.4630
	Cortical Cell Carcinoma	0/50 (42)	1/50 (42)	0/50 (36)	0/49 (39)
		0.4717	0.5000	NC	NC
	Malignant Pheochromocytoma	1/50 (43)	1/50 (42)	0/50 (36)	0/49 (39)
		0.7870	0.7471	0.4557	0.4756
Eyes with Optic Nerves	Leiomyoma	0/50 (42)	0/50 (42)	0/50 (36)	1/50 (40)
		0.2500	NC	NC	0.4878
	Malignant Schwannoma	0/50 (42)	1/50 (42)	0/50 (36)	0/50 (40)
		0.4750	0.5000	NC	NC
Gland, Clitoral	Squamous Cell Carcinoma	1/41 (37)	1/33 (30)	0/32 (26)	0/34 (30)
		0.7633	0.6988	0.4127	0.4478
	Squamous Cell Papilloma	0/41 (37)	1/33 (30)	0/32 (26)	0/34 (30)
		0.4553	0.4478	NC	NC
Hemolymphoreticular System	Histiocytic Sarcoma	1/50 (43)	0/50 (42)	0/50 (36)	1/50 (40)
		0.4363	0.4941	0.4557	0.7346
	Large Granular Lymphocyte Lymphoma	0/50 (42)	0/50 (42)	0/50 (36)	1/50 (41)
		0.2547	NC	NC	0.4940
	Lymphoma	0/50 (42)	0/50 (42)	1/50 (36)	0/50 (40)
		0.2500	NC	0.4615	NC
Liver	Cholangioma	0/50 (42)	1/50 (42)	0/50 (36)	0/50 (40)
		0.4750	0.5000	NC	NC
	Hepatocellular Adenoma	0/50 (42)	1/50 (42)	1/50 (37)	0/50 (40)
		0.4880	0.5000	0.4684	NC
	Hepatocellular Carcinoma	1/50 (42)	1/50 (42)	0/50 (36)	0/50 (40)
		0.7936	NC	0.4615	0.4878
Lymph Node, Mesenteric	Hemangioma	0/48 (41)	0/50 (42)	1/49 (35)	0/49 (39)
		0.2484	NC	0.4605	NC
Mammary Glands	Adenocarcinoma	4/50 (43)	3/50 (42)	3/50 (37)	9/50 (42)
		0.0174	0.4866	0.4152	0.1050
	Adenoma	1/50 (42)	1/50 (42)	2/50 (36)	2/50 (40)
		0.2507	NC	0.4417	0.4815
	Fibroadenoma	24/50 (45)	20/50 (45)	26/50 (44)	30/50 (48)
		0.0893	0.7364	0.3695	0.2467
	Fibroma	0/50 (42)	0/50 (42)	0/50 (36)	1/50 (40)
		0.2500	NC	NC	0.4878
	Hemangiosarcoma	0/50 (42)	0/50 (42)	1/50 (37)	0/50 (40)
		0.2484	NC	0.4684	NC
	Squamous Cell Carcinoma	0/50 (42)	1/50 (42)	0/50 (36)	0/50 (40)
		0.4750	0.5000	NC	NC
Ovaries with Oviduct	Luteoma	0/49 (41)	2/49 (41)	0/50 (36)	0/49 (39)
		0.7288	0.2469	NC	NC
	Sex Chord Stromal Tumour	0/49 (41)	0/49 (41)	0/50 (36)	1/49 (39)
		0.2484	NC	NC	0.4875
	Thecoma	1/49 (41)	0/49 (41)	0/50 (36)	0/49 (39)
		0.7389	0.5000	0.4675	0.4875

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Organ name	Tumor name	0 mkd	50 mkd	200 mkd	800/1200 mkd
		Vehicle (C2) P - Trend	Low (L) P - C2 vs. L	Mid (M) P - C2 vs. M	High (H) P - C2 vs. H
Pancreas	Islet Cell Adenoma	2/50 (43) 0.6739	2/50 (42) 0.6831	1/50 (37) 0.4431	1/50 (40) 0.4726
	Islet Cell Carcinoma	0/50 (42) 0.4750	1/50 (42) 0.5000	0/50 (36) NC	0/50 (40) NC
Pituitary Gland	Adenocarcinoma	0/50 (42) 0.1739	0/49 (41) NC	1/50 (37) 0.4684	1/49 (39) 0.4815
	Adenoma	30/50 (47) 0.4151	30/49 (43) 0.3551	37/50 (43) 0.0140	30/49 (42) 0.2962
Skin/Subcutis	Squamous Cell Carcinoma	0/50 (42) 0.7259	2/50 (42) 0.2470	0/50 (36) NC	0/50 (40) NC
Thyroid Gland	C-Cell Adenoma	5/50 (43) 0.7786	9/50 (42) 0.1776	3/50 (37) 0.5558	4/50 (40) 0.4533
	C-Cell Carcinoma	0/50 (42) 0.2875	3/50 (42) 0.1205	0/50 (36) NC	2/50 (40) 0.2349
	Follicular Cell Adenoma	2/50 (42) 0.9323	0/50 (42) 0.7530	0/50 (36) 0.7133	0/50 (40) 0.7407
	Follicular Cell Carcinoma	0/50 (42) 0.4750	1/50 (42) 0.5000	0/50 (36) NC	0/50 (40) NC
Uterus With Cervix	Adenocarcinoma	1/49 (41) 0.7421	0/50 (42) 0.5060	0/50 (36) 0.4675	0/50 (40) 0.4938
	Adenoma	1/49 (42) 0.7375	0/50 (42) 0.5000	0/50 (36) 0.4615	0/50 (40) 0.4878
	Adenosquamous Carcinoma	1/49 (42) 0.7375	0/50 (42) 0.5000	0/50 (36) 0.4615	0/50 (40) 0.4878
	Endometrial Adenocarcinoma	2/49 (42) 0.0591	4/50 (42) 0.3379	1/50 (36) 0.4417	6/50 (40) 0.1169
	Granular Cell Tumour	0/49 (41) 0.2516	0/50 (42) NC	0/50 (36) NC	1/50 (40) 0.4938
	Hemangioma	0/49 (41) 0.2516	0/50 (42) NC	1/50 (36) 0.4675	0/50 (40) NC
	Malignant Schwannoma	0/49 (41) 0.4288	2/50 (42) 0.2530	0/50 (36) NC	1/50 (40) 0.4938
	Papilloma	0/49 (41) 0.2516	0/50 (42) NC	1/50 (36) 0.4675	0/50 (40) NC
	Polyp	7/49 (42) 0.1655	6/50 (43) 0.5186	5/50 (37) 0.5280	9/50 (41) 0.3702
	Squamous Cell Carcinoma	0/49 (41) 0.1905	2/50 (42) 0.2530	1/50 (36) 0.4675	2/50 (40) 0.2407
	Squamous Cell Papilloma	0/49 (41) 0.1777	0/50 (42) NC	1/50 (37) 0.4744	1/50 (40) 0.4938

Note: X/ZZ (YY): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed.  
 NC = Not calculable.

Note: The p-values marked with an asterisk \* indicate statistically significant dose responses at 0.005 and 0.025 for a common tumor and a rare tumor, respectively. The p-values marked with an asterisk \*\* indicate statistically significant pairwise comparison at 0.01 and 0.05 for a common tumor and a rare tumor, respectively.

**Table 5B-3: Tumor Rates and P-Values for Dose Response Relationship and Pairwise Comparisons with Combined Controls – Female Rats**

Organ name	Tumor name	0 mkd	50 mkd	200 mkd	800/1200 mkd
		Comb.Controls P - Trend	Low (L) P - Comb.C vs. L	Mid (M) P - Comb.C vs. M	High (H) P - Comb.C vs. H

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Organ name	Tumor name	0 mkd	50 mkd	200 mkd	800/1200 mkd
		Comb.Controls P - Trend	Low (L) P - Comb.C vs. L	Mid (M) P - Comb.C vs. M	High (H) P - Comb.C vs. H
Adrenal Glands	B_Pheochromocytoma	11/100 (83) 0.8736	3/50 (42) 0.7604	4/50 (37) 0.5178	2/49 (39) 0.8519
	M_Pheochromocytoma	1/100 (82) 0.6566	1/50 (42) 0.5645	0/50 (36) 0.3051	0/49 (39) 0.3223
	C_Pheochromocyto/B+M	12/100 (83) 0.9174	4/50 (42) 0.6830	4/50 (37) 0.5887	2/49 (39) 0.8889
	Cortical Cell Adenoma	6/100 (82) 0.7691	0/50 (42) 0.9216	1/50 (36) 0.6888	1/49 (39) 0.7235
	Cortical Cell Carcinoma	0/100 (82) 0.3769	1/50 (42) 0.3387	0/50 (36) NC	0/49 (39) NC
	C_Cortical Cell/A+C	6/100 (82) 0.8052	1/50 (42) 0.7541	1/50 (36) 0.6888	1/49 (39) 0.7235
	Brain Cerebellum	Malignant Glioma	1/100 (82) 0.5900	0/50 (42) 0.3387	0/50 (36) 0.3051
Brain Cerebrum		Malignant Glioma	1/100 (82) 0.5900	0/50 (42) 0.3387	0/50 (36) 0.3051
		Meningeal Tumour	1/100 (82) 0.5900	0/50 (42) 0.3387	0/50 (36) 0.3051
Brain- Medulla/Pons	Malignant Glioma	1/100 (82) 0.5900	0/50 (42) 0.3387	0/50 (36) 0.3051	0/50 (40) 0.3279
	Meningioma	1/100 (82) 0.5900	0/50 (42) 0.3387	0/50 (36) 0.3051	0/50 (40) 0.3279
Eyes with Optic Nerves	Leiomyoma	0/100 (82) 0.2000	0/50 (42) NC	0/50 (36) NC	1/50 (40) 0.3279
	Malignant Schwannoma	0/100 (82) 0.3800	1/50 (42) 0.3387	0/50 (36) NC	0/50 (40) NC
Gland, Clitoral	Squamous Cell Carcinoma	2/83 (74) 0.7823	1/33 (30) 0.6440	0/32 (26) 0.4543	0/34 (30) 0.4957
	Squamous Cell Papilloma	1/83 (74) 0.6131	1/33 (30) 0.4957	0/32 (26) 0.2600	0/34 (30) 0.2885
Hemolymphoreticular System	Histiocytic Sarcoma	1/100 (82) 0.3608	0/50 (42) 0.3387	0/50 (36) 0.3051	1/50 (40) 0.5501
	Large Granular Lymphocyte Lymphoma	0/100 (82) 0.2040	0/50 (42) NC	0/50 (36) NC	1/50 (41) 0.3333
	Lymphoma	1/100 (82) 0.4684	0/50 (42) 0.3387	1/50 (36) 0.5189	0/50 (40) 0.3279
Liver	Adenoma	1/100 (82) 0.5900	0/50 (42) 0.3387	0/50 (36) 0.3051	0/50 (40) 0.3279
	Cholangioma	0/100 (82) 0.3800	1/50 (42) 0.3387	0/50 (36) NC	0/50 (40) NC
	Hepatocellular Adenoma	0/100 (82) 0.3923	1/50 (42) 0.3387	1/50 (37) 0.3109	0/50 (40) NC
	Hepatocellular Carcinoma	1/100 (82) 0.6601	1/50 (42) 0.5645	0/50 (36) 0.3051	0/50 (40) 0.3279
Lymph Node, Mesenteric	Hemangioma	1/97 (79) 0.4700	0/50 (42) 0.3471	1/49 (35) 0.5217	0/49 (39) 0.3305

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Organ name	Tumor name	0 mkd Comb.Controls P - Trend	50 mkd Low (L) P - Comb.C vs. L	200 mkd Mid (M) P - Comb.C vs. M	800/1200 mkd High (H) P - Comb.C vs. H	
Mammary Glands	Adenocarcinoma	11/100 (83) 0.0499	3/50 (42) 0.7604	3/50 (37) 0.6831	9/50 (42) 0.1782	
	Adenoma	2/100 (82) 0.2134	1/50 (42) 0.2647	2/50 (36) 0.3566	2/50 (40) 0.3976	
	Fibroadenoma	46/100 (88) 0.0783	20/50 (45) 0.7488	26/50 (44) 0.2896	30/50 (48) 0.1668	
	Fibroma	0/100 (82) 0.2000	0/50 (42) NC	0/50 (36) NC	1/50 (40) 0.3279	
	Hemangiomasarcoma	0/100 (82) 0.1990	0/50 (42) NC	1/50 (37) 0.3109	0/50 (40) NC	
	Squamous Cell Carcinoma	1/100 (82) 0.6601	1/50 (42) 0.5645	0/50 (36) 0.3051	0/50 (40) 0.3279	
	Ovaries with Oviduct	Benign Granulosa Cell Tumour	1/99 (81) 0.5888	0/49 (41) 0.3361	0/50 (36) 0.3077	0/49 (39) 0.3250
Luteoma		0/99 (81) 0.6177	2/49 (41) 0.1111	0/50 (36) NC	0/49 (39) NC	
Malignant Granulosa Cell Tumor		1/99 (81) 0.5888	0/49 (41) 0.3361	0/50 (36) 0.3077	0/49 (39) 0.3250	
Sex Chord Stromal Tumour		1/99 (81) 0.3576	0/49 (41) 0.3361	0/50 (36) 0.3077	1/49 (39) 0.5462	
Thecoma		1/99 (81) 0.5888	0/49 (41) 0.3361	0/50 (36) 0.3077	0/49 (39) 0.3250	
Pancreas		Islet Cell Adenoma	3/100 (82) 0.6078	2/50 (42) 0.5525	1/50 (37) 0.3671	1/50 (40) 0.3976
		Islet Cell Carcinoma	0/100 (82) 0.3800	1/50 (42) 0.3387	0/50 (36) NC	0/50 (40) NC
	C_Islet Cell/A+C	3/100 (82) 0.6804	3/50 (42) 0.3277	1/50 (37) 0.3671	1/50 (40) 0.3976	
Pituitary Gland	Adenocarcinoma	1/100 (82) 0.2560	0/49 (41) 0.3333	1/50 (37) 0.5270	1/49 (39) 0.5426	
	Adenoma	55/100 (88) 0.2566	30/49 (43) 0.2681	37/50 (43) 0.0040 **	30/49 (42) 0.2117	
Skin/Subcutis	Fibroma	1/100 (82) 0.5900	0/50 (42) 0.3387	0/50 (36) 0.3051	0/50 (40) 0.3279	
	Squamous Cell Carcinoma	0/100 (82) 0.6168	2/50 (42) 0.1129	0/50 (36) NC	0/50 (40) NC	
Spleen	Hemangiopericytoma	1/100 (83) 0.5871	0/50 (42) 0.3360	0/50 (36) 0.3025	0/50 (40) 0.3252	
Thyroid Gland	C-Cell Adenoma	6/99 (81) 0.5659	9/50 (42) 0.0272	3/50 (37) 0.5787	4/50 (40) 0.4329	
	C-Cell Carcinoma	1/99 (81) 0.2687	3/50 (42) 0.1148	0/50 (36) 0.3077	2/50 (40) 0.2537	
	C_C-ell/A+C	7/99 (81) 0.4367	12/50 (42) 0.0050 **	3/50 (37) 0.3846	6/50 (40) 0.2232	
	Follicular Cell Adenoma	3/99 (81) 0.9340	0/50 (42) 0.7181	0/50 (36) 0.6720	0/50 (40) 0.7037	

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Organ name	Tumor name	0 mkd	50 mkd	200 mkd	800/1200 mkd
		Comb.Controls P - Trend	Low (L) P - Comb.C vs. L	Mid (M) P - Comb.C vs. M	High (H) P - Comb.C vs. H
Uterus with Cervix	Follicular Cell Carcinoma	0/99 (81) 0.3819	1/50 (42) 0.3415	0/50 (36) NC	0/50 (40) NC
	C_Follicular Cell/A+C	3/99 (81) 0.9172	1/50 (42) 0.4220	0/50 (36) 0.6720	0/50 (40) 0.7037
	Adenocarcinoma	2/98 (80) 0.8380	0/50 (42) 0.5719	0/50 (36) 0.5262	0/50 (40) 0.5574
	Adenoma	1/98 (80) 0.5960	0/50 (42) 0.3443	0/50 (36) 0.3103	0/50 (40) 0.3333
	Adenosquamous Carcinoma	1/98 (80) 0.5960	0/50 (42) 0.3443	0/50 (36) 0.3103	0/50 (40) 0.3333
	Endometrial Adenocarcinoma	6/98 (81) 0.0884	4/50 (42) 0.4637	1/50 (36) 0.6943	6/50 (40) 0.1604
	Granular Cell Tumor	0/98 (80) 0.2020	0/50 (42) NC	0/50 (36) NC	1/50 (40) 0.3333
	Hemangioma	0/98 (80) 0.2020	0/50 (42) NC	1/50 (36) 0.3103	0/50 (40) NC
	Malignant Schwannoma	0/98 (80) 0.2892	2/50 (42) 0.1167	0/50 (36) NC	1/50 (40) 0.3333
	Papilloma	0/98 (80) 0.2020	0/50 (42) NC	1/50 (36) 0.3103	0/50 (40) NC
	Polyp	12/98 (80) 0.1360	6/50 (43) 0.4484	5/50 (37) 0.4623	9/50 (41) 0.2388
	Squamous Cell Carcinoma	0/98 (80) 0.0943	2/50 (42) 0.1167	1/50 (36) 0.3103	2/50 (40) 0.1092
	Squamous Cell Papilloma	0/98 (80) 0.1147	0/50 (42) NC	1/50 (37) 0.3162	1/50 (40) 0.3333

Note: X/ZZ (YY): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed.  
 NC = Not calculable.  
 Note: The p-values marked with an asterisk \* indicate statistically significant dose responses at 0.005 and 0.025 for a common tumor and a rare tumor, respectively. The p-values marked with an asterisk \*\* indicate statistically significant pairwise comparison at 0.01 and 0.05 for a common tumor and a rare tumor, respectively.

**Table 6A: Tumor Rates and P-Values for Dose Response Relationship and Pairwise Comparisons with Control in Male mice**

Organ name	Tumor name	0 mkd	100 mkd	300 mkd	1000 mkd	1000 mg/kg
		Control (C) P - Trend	Low (L) P - C vs. L	Mid (M) P - C vs. M	High (H) P - C vs. H	Urethane (PC) P - C vs. PC
Harderian Glands	Adenoma	0/25 (25) 0.5000	0/25 (25) NC	1/25 (25) 0.5000	0/25 (25) NC	7/25 (25) 0.0048 **
Liver	Adenoma, Hepatocellular	0/25 (25) NC	0/25 (25) NC	0/25 (25) NC	0/25 (25) NC	1/25 (25) 0.5000
Lungs	Adenoma, Bronchiolo-Alveolar	1/25 (25) 0.9394	1/25 (25) NC	0/25 (25) 1.0000	0/25 (25) 1.0000	25/25 (25) 0.0000 **
	Carcinoma, Bronchiolo-Alveolar	0/25 (25) 0.5000	0/25 (25) NC	1/25 (25) 0.5000	0/25 (25) NC	19/25 (25) 0.0000 **
Sg. Mandibular	Adenocarcinoma	0/25 (25) 0.2500	0/25 (25) NC	0/25 (25) NC	1/25 (25) 0.5000	0/25 (25) NC
Stomach	Carcinoma, Squamous Cell	0/25 (25) NC	0/25 (25) NC	0/25 (25) NC	0/25 (25) NC	1/25 (25) 0.5000

Organ name	Tumor name	0 mkd Control (C) P - Trend	100 mkd Low (L) P - C vs. L	300 mkd Mid (M) P - C vs. M	1000 mkd High (H) P - C vs. H	1000 mg/kg Urethane (PC) P - C vs. PC
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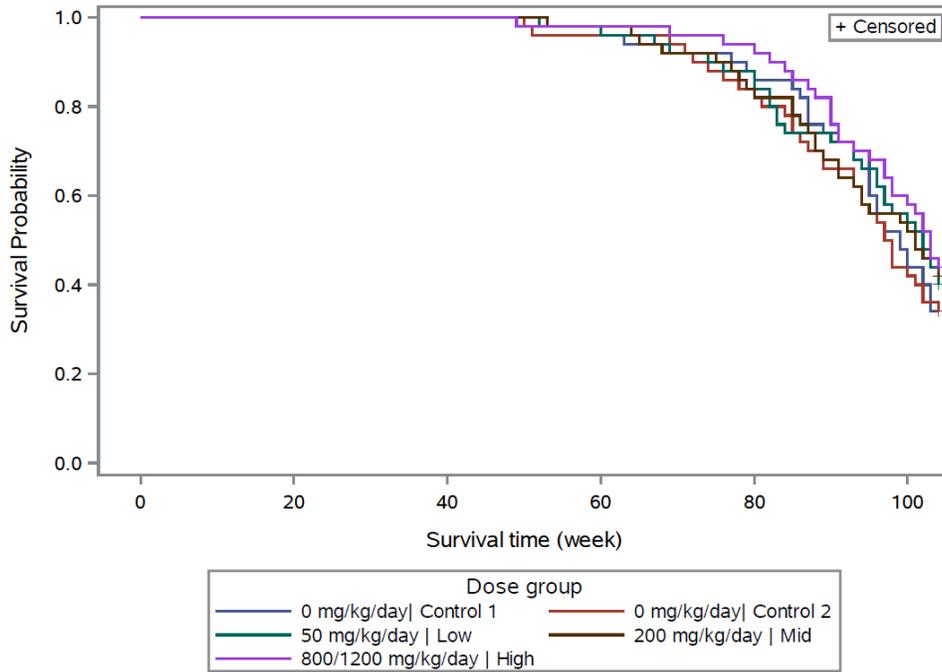
& X/YY (ZZ): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed;  
 NC = Not calculable.

**Table 6B: Tumor Rates and P-Values for Dose Response Relationship and Pairwise Comparisons with Control in Female mice**

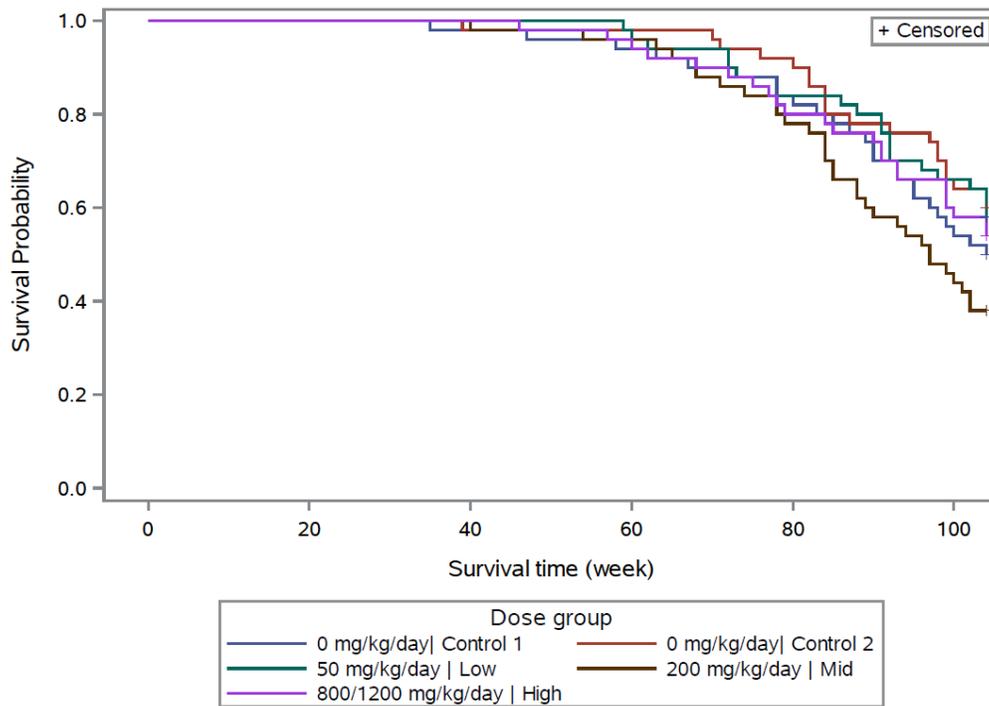
Organ name	Tumor name	0 mkd Control (C) P - Trend	50 mkd Low (L) P - C vs. L	150 mkd Mid (M) P - C vs. M	500 mkd High (H) P - C vs. H	1000 mg/kg Urethane (PC) P - C vs. PC
Cecum	Leiomyoma	0/25 (25) 0.2500	0/25 (25) NC	0/25 (25) NC	1/25 (25) 0.5000	0/25 (25) NC
Harderian Glands	Adenocarcinoma	0/25 (25) NC	0/25 (25) NC	0/25 (25) NC	0/25 (25) NC	2/25 (25) 0.2449
	Adenoma	0/25 (25) NC	0/25 (25) NC	0/25 (25) NC	0/25 (25) NC	16/25 (25) 0.0000 **
Lungs	Adenoma, Bronchiolo-Alveolar	0/25 (25) 0.0606	0/25 (25) NC	0/25 (25) NC	2/25 (25) 0.2449	25/25 (25) 0.0000 \$
	Carcinoma, Bronchiolo-Alveolar	0/25 (25) NC	0/25 (25) NC	0/25 (25) NC	0/25 (25) NC	21/25 (25) 0.0000 **
Mammary Gland	Adenocarcinoma	0/25 (25) NC	0/25 (25) NC	0/25 (25) NC	0/25 (25) NC	1/25 (25) 0.5000
Sg. Mandibular	Adenocarcinoma	0/25 (25) NC	0/25 (25) NC	0/25 (25) NC	0/25 (25) NC	1/25 (25) 0.5000
Skin & Subcutis	Carcinoma, Squamous Cell	0/25 (25) NC	0/25 (25) NC	0/25 (25) NC	0/25 (25) NC	1/25 (25) 0.5000
Stomach	Carcinoma, Squamous Cell	0/25 (25) NC	0/25 (25) NC	0/25 (25) NC	0/25 (25) NC	2/25 (25) 0.2449
Uterus	Adenocarcinoma	0/25 (25) NC	0/25 (25) NC	0/25 (25) NC	0/25 (25) NC	1/25 (25) 0.5000

& X/YY (ZZ): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed;  
 NC = Not calculable.

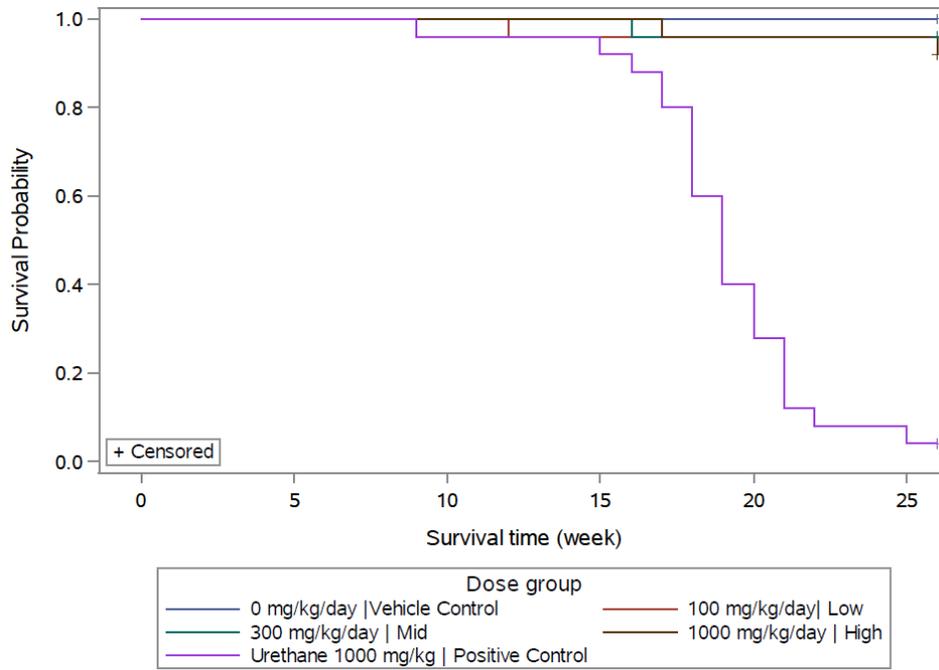
**Figure 1A: Kaplan-Meier Survival Functions for Male Rats**



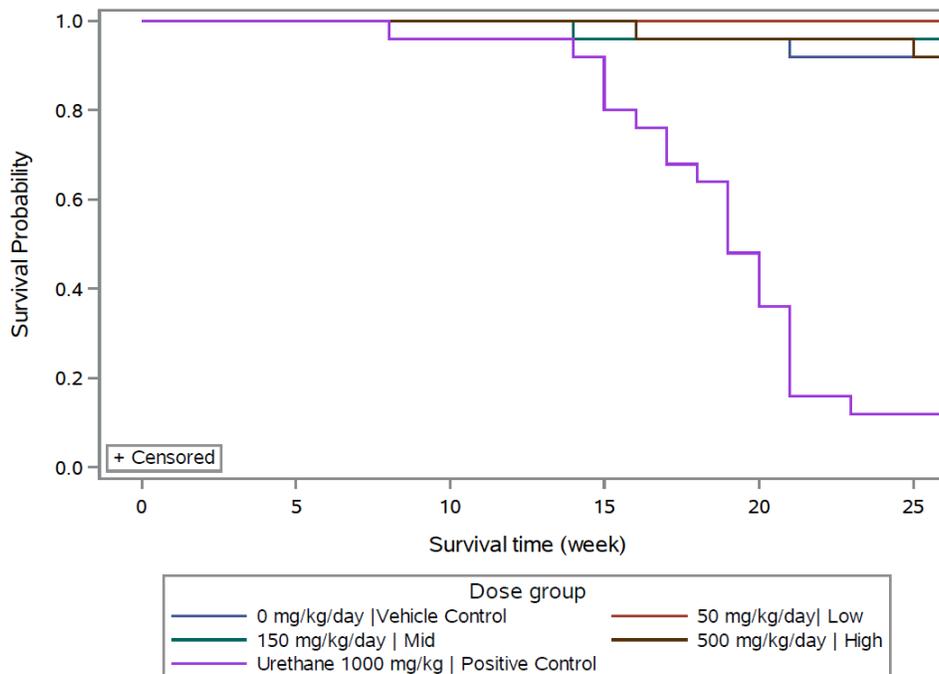
**Figure 1B: Kaplan-Meier Survival Functions for Female Rats**



**Figure 2A: Kaplan-Meier Survival Functions for Male Mice**



**Figure 2B: Kaplan-Meier Survival Functions for Female Mice**



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12. (b) (4) Historical Control Neoplastic Data, CD-1 Mouse, 2 Year

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/s/  
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FENG ZHOU  
05/17/2018

KARL K LIN  
05/21/2018  
Concur with review.

**Date:** May 2, 2018

**From:** Kimberly Smith, Medical Officer  
Division of Cardiovascular and Renal Products

**Through:** Aliza Thompson, Team Leader  
Norman Stockbridge, Director  
Division of Cardiovascular and Renal Products

**To:** Hong Vu, Regulatory Project Manager  
Division of Gastroenterology and Inborn Errors Products

**Subject:** Review of renal function analyses for migalastat (NDA 208623; IND 68456)

### **Background**

Migalastat is a small molecule pharmacological chaperone designed to bind selectively and reversibly to the active site of alpha-galactosidase A enzyme. On December 13, 2017, the Division of Gastroenterology and Inborn Errors Products (DGIEP) received an NDA for migalastat for the treatment of Fabry disease in subjects with amenable mutations.

Fabry Disease is a rare lysosomal storage disease that results from an X-linked mutation in the *GLA* gene causing a deficiency of the enzyme  $\alpha$ -galactosidase A, a lysosomal enzyme that catalyzes the hydrolysis of the terminal glycolipid globotriaosylceramide (Gb3 or GL-3) to galactose and lactosylceramide. In Fabry disease, GL-3 accumulates in tissues leading to systemic manifestations including progressive renal impairment, hypertrophic cardiomyopathy, cardiac conduction system abnormalities, myocardial infarction, stroke, painful neuropathy, and gastrointestinal disturbances. The only therapy currently approved in the U.S. is agalsidase beta (Fabrazyme), an enzyme replacement therapy (ERT) approved in 2003 via accelerated approval “for use in patients with Fabry disease. Fabrazyme reduces globotriaosylceramide (GL-3) deposition in capillary endothelium of the kidney and certain other cell types.” A post-marketing confirmatory trial failed to confirm a clinical benefit on renal function.

DGIEP has requested input from the Division of Cardiovascular and Renal Products regarding the following text in Section 14 of the label related to analyses of renal function data:



In addition, DGIEP has requested comment on whether any post-marketing studies should be required.

### **Regulatory History**

Amicus has completed two phase 3 trials of migalastat in patients with Fabry disease (AT1001-011 and -012) and, on September 9, 2015, met with the DGIEP to discuss the content and format of an NDA (minutes dated September 17, 2015). The applicant proposed to use reduction in GL-3 deposition in renal interstitial capillaries as a surrogate endpoint and basis for Subpart H approval and to conduct a phase 4 [REDACTED] <sup>(b) (4)</sup> and a GI trial to verify the clinical benefit. DGIEP did not agree with their proposed plan, noting

that Study 011 did not reach statistical significance for the pre-specified study endpoints and that Study 012 was not powered to test a specific statistical hypothesis. Given the Fabrazyme experience, the Division also noted that the applicant had not provided sufficient evidence to support treatment effects on GL-3 deposition in renal interstitial capillaries as a surrogate endpoint to support traditional or accelerated approval. The Division of Cardiovascular and Renal Products (DCRP) was asked to provide input on the renal function findings (see appended consult dated December 1, 2015).

On October 26, 2016, Amicus again met with DGIEP to discuss new analyses of the existing trial data and their intention to submit an NDA for Subpart H approval (minutes dated November 24, 2016). DGIEP noted that the additional analyses were considered exploratory and should be used for hypothesis generation only. The meeting focused on the design of a new trial that could be used to establish efficacy based on gastrointestinal symptoms including diarrhea. This was followed by additional discussions on November 21, 2016. On March 21, 2017, Amicus submitted a feasibility report via email that concluded that the trial would take 5-7 years to conduct and therefore is not feasible.

The applicant subsequently had interactions with CDER leadership in March 2017. As a result, on April 24, 2017, Amicus requested that, based on “the collective body of evidence,” DGIEP confirm that no additional Phase 3 studies were required for submission and that the FDA will agree to review an NDA for migalastat. DCRP was consulted at that time to evaluate additional analyses of eGFR data provided with the submission (see consult dated June 2, 2017). The applicant’s request was discussed with the Medical Policy Council on July 5, 2017 and, in a letter dated July 7, 2017, DGIEP confirmed that the applicant could submit an NDA for migalastat.

### **Materials Reviewed**

1. Summary of Clinical Efficacy
2. Proposed draft labeling submitted March 16, 2018
3. Protocol AT1001-011 dated November 20, 2012
4. Protocol AT1001-012 dated March 13, 2013
5. Statistical Analysis Plan for AT1001-012 dated June 26, 2014
6. DCRP consults dated December 1, 2015 and June 2, 2017 (appended).

### **Overview of Phase 3 Trials**

As previously noted, Amicus has completed two phase 3 trials in patients with Fabry disease, AT1001-011 and -012 (Table 1). In brief, Study 011 provides placebo-controlled data on the effect of migalastat on GL-3 inclusions in the renal interstitial capillary over a 6-month period. Study 012 compared open-label migalastat to ERT. Subjects could continue treatment with migalastat in open label extension studies AT1001-041 and -042.

**Table 1: Summary of Phase 3 Trials**

Study Number	Study Design	Study Objective(s)	Subjects (Type, N, Mean Age [Range]) and Number of Sites
AT1001-011	6-month, DB, randomized, placebo-controlled study (Stage 1) followed by 6-month open-label treatment (Stage 2) and an optional 12-month OLE: Total of 24 months	Stage 1: Compare the effect of migalastat HCl versus placebo on kidney GL-3 as assessed by histological scoring of the number of kidney IC GL-3 inclusions Stage 2: Assess the efficacy, safety, and PK of migalastat HCl	Subjects with Fabry disease with migalastat-responsive <i>GLA</i> mutations who were ERT-naïve or ERT-free ≥6 months N=67 (24M/43F) Migalastat-migalastat: N=34 (12M/22F) Placebo-migalastat: N=33 (12M/21F) 42.2 (16 to 68) years 36 sites
AT1001-012	18-month active-controlled, randomized, open-label multinational study with optional 12-month OLE: Total of 30 months	Compare the efficacy and safety of migalastat HCl to ERT in subjects with Fabry disease who were currently receiving ERT and had migalastat-responsive mutations in <i>GLA</i>	Subjects with Fabry disease with migalastat-responsive <i>GLA</i> mutations who were receiving ERT N=57 (25M/32F) Migalastat: N=36 (16M/20F) ERT: N=21 (9M/12F) 48.9 (18 to 72) years 25 sites

Source: Applicant, Summary of Clinical Efficacy, Table 6.

## Renal Findings

### Study AT1001-011

#### Baseline Characteristics

The mean age of patients enrolled in 011 was 42.2 (12) years and 64% were female. Two thirds of patients had baseline proteinuria >150 mg/24 hours, and one third had baseline proteinuria >300 mg/24 hours. The mean baseline eGFR<sub>MDRD</sub> was ~89 mL/min/1.73m<sup>2</sup>. A total of 28% of patients were on a RAS blocker at baseline.

#### Results of Primary Endpoint

Study 011 failed to meet its prespecified primary endpoint, the proportion of subjects with a ≥50% reduction from baseline to Month 6 in the average number of GL-3 inclusions per renal interstitial capillary. In post-hoc analyses of data from patients with “amenable mutations” defined by a GLP HEK assay rather than a preliminary assay used for enrollment, the applicant reports a nominally statistically significant reduction in the average number of GL-3 inclusions per interstitial capillary in the migalastat group compared with placebo through Month 6 (nominal p=0.008).

#### Change in Renal Function

Pre-specified secondary endpoints related to renal function included a comparison of annualized eGFR<sub>MDRD</sub> slope from baseline to month 6 and mean change in mGFR<sub>iohexol</sub> from baseline to month 6 in the overall study population. Neither was successful in either the overall study population or in the population with amenable mutations defined post hoc (data not shown).

(b) (4) the applicant provided post-hoc analyses of the annualized rate of change in eGFR at later timepoints in patients with amenable mutations treated with migalastat in Studies 011 and 041. These analyses were amongst several related to changes in renal function and proteinuria that were conducted without a plan to control the overall type 1 error rate. For Study 011, the applicant asserts that renal function remained stable over 18 to 24 months of treatment with migalastat with an annualized rate of change in eGFR<sub>CKD-EPI</sub> of -0.30 (0.66) mL/min/1.73m<sup>2</sup> (Table 2). The point estimates for eGFR<sub>MDRD</sub> and eGFR<sub>iohexol</sub> vary somewhat, but the confidence intervals are wide and overlapping. Similarly, for Study 041, the applicant asserts that renal function remained stable over an average of 3.5 years (range 1.5 to 4.9 years) in 41 patients that continued migalastat (-0.75 mL/min/1.73m<sup>2</sup>/year [95% CI -1.89, 0.4]).

**Table 2: Annualized change in GFR through month 18 to 24 in migalastat-treated subjects with amenable mutations (Study 011)**

GFR Method	N	Mean (±SD) [95% CI]
eGFR <sub>CKD-EPI</sub>	41	-0.3 (4.25) [-1.65, 1.04]
eGFR <sub>MDRD</sub>	41	0.8 (6.58) [-1.28, 2.87]
mGFR <sub>iohexol</sub>	37	-1.5 (8.07) [-4.20, 1.18]

Source: Applicant, Summary of Clinical Efficacy, Table 20.

The applicant believes the rate of decline in eGFR in patients treated with migalastat is similar to published rates for healthy adults (Levey 2009) and that they compare favorably to rates reported in three published studies of renal function in untreated patients with Fabry disease described below. The applicant selected these studies because they reported annualized rate of change in eGFR by sex and/or baseline proteinuria, key risk factors for progression, and included an observation period of at least 1 year.

- Schiffmann et al. reported data from a retrospective review of records from 447 untreated patients (279 male, 168 female) with Fabry disease diagnosed during life or at the time of death between 1944 to 2002. The applicant reports the annualized rate of change in eGFR stratified by baseline proteinuria for 61 (22%) male and 29 (17%) female patients who had three or more eGFR assessments and an assessment of baseline proteinuria (Table 3).
- Schwarting et al. reported data from the Fabry Outcome Survey, a European database for patients with Fabry disease who are receiving or are candidates for ERT. At the time of publication, 608 patients had been enrolled, including 20 patients with eGFR values 1 year before, at the start of ERT, and up to 1 year after. Of these patients, the applicant provided data on the annualized rate of change in eGFR stratified by baseline proteinuria for 6 male and 13 female patients (Table 3).
- Wanner et al. reported data from the Fabry Registry for 462 (121 male, 341 female) untreated patients with at least two eGFR values over ≥12 months before starting ERT and an assessment of proteinuria. The applicant provided data on the annualized rate of change in eGFR for males vs. females (Table 3). The Fabry registry is a voluntary registry available to any patient with Fabry disease that began enrolling in April 2001.

**Table 3: Annualized eGFR slopes stratified by sex and 24-hour urine protein**

Sex	24-hour Urine Protein (g)	AT1001-011 Migalastat eGFR <sub>MDRD</sub>	Schiffmann 2009 Untreated eGFR <sub>MDRD</sub>	Schwartzing 2006 Untreated eGFR <sub>MDRD</sub>	Wanner 2010 Untreated eGFR <sub>CKD-EPI</sub>
Males	<0.1 (Low)	No subjects	-1.6 (1.5) n=18, age=23 BL eGFR=138	-16.0 (NC) n=1, age=30 BL eGFR=99	-2.53 (0.21) n=121, age=33 BL eGFR=93
	0.1-1.0 (Moderate)	+1.0 (1.4) n=12, age=45 BL eGFR=80	-3.3 (1.8) n=21, age=36 BL eGFR=85	-11.3 (8.3) n=3, age=37 BL eGFR=66	
	>1.0 (High)	-5.9 (1.8) n=2 age=51 BL eGFR=65	-6.9 (1.5) n=22, age=39 BL eGFR=59	-8.0 (2.0) n=2, age=45 BL eGFR=56	
Females	<0.1 (Low)	+0.3 (1.4) n=7, age=42 BL eGFR=78	-0.6 (2.6) n=7, age=39 BL eGFR=92	-8.0 (2.0) n=2, age=45 BL eGFR=56	--
	0.1-1.0 (Moderate)	+1.8 (2.0) n=18, age=44 BL eGFR=88	-2.2 (2.2) n=17, age=42 BL eGFR=90	-7.7 (3.3) n=9, age=52 BL eGFR=73	-0.52 (0.68) n=341, age=39 BL eGFR=95
	>1.0 (High)	-1.3 (2.8) n=2, age=32 BL eGFR=103	-4.6 (2.3) n=5, age=47 BL eGFR=63	-2.5 (3.5) n=2, age=25 BL eGFR=59	--

Source: Applicant, Summary of Clinical Efficacy, Table 22.

As we have noted in previous consults, the cited external controls may not be sufficiently similar to patients treated with migalastat in Study 011 to serve as appropriate controls for the trial population:

- For the Schiffman paper, only a subset of patients had the required minimum of three follow-up creatinine measurements. It is possible patients with milder forms of disease who did not need frequent renal function monitoring were excluded from the analyses, raising concern that the estimates of disease progression may be biased.
- The data were collected from an earlier time period (back as far as 1944) and it is not clear that the patients are similar to patients diagnosed with Fabry disease today. Patients diagnosed with Fabry disease at a time before treatment was available may have been more severely affected. In fact, the manuscript states that the majority of women in the study were “symptomatic with moderate to severe manifestations of Fabry disease.”
- The applicant’s analyses focus on a subset of patients with Fabry disease and “amenable” HEK mutations. It is unclear whether rates of disease progression in this subset differ from patients without “amenable” mutations.
- Describing results based on gender and broad categories of proteinuria is unlikely to account for important covariates that affect disease progression. While both gender and proteinuria levels are important predictors of disease progression, other factors, identified and unidentified, may play a role in disease progression.

Study AT1001-012*Baseline Characteristics*

The mean age of patients enrolled in 012 was 48.9 (14.2) years and 56% were female. The mean 24-hour urine protein at baseline was 301 (529) mg. The mean baseline eGFR<sub>CKD-EPI</sub> was 91.9 mL/min/1.73m<sup>2</sup>. A total of 47% of subjects were on a RAS blocker at baseline.

*Change in Renal Function*

According to the protocol, “no formal hypothesis test” was defined for Study 012. (b) (4)  
 (b) (4) the applicant provided post hoc analyses of the annualized rate of change in eGFR<sub>CKD-EPI</sub> in patients with amenable mutations treated with migalastat or ERT for 18 months (Table 4).<sup>1</sup> The applicant also provided analyses of eGFR<sub>MDRD</sub> and mGFR<sub>iohexol</sub>. The analyses included patients with amenable mutations who received at least one dose of study drug and had both baseline and post-baseline values who were treated with migalastat (n=34 of 36) vs. ERT (n=18 of 24). Although, there were no obvious differences between the treatment arms, sample sizes were small and the confidence intervals were wide. As previously noted, ERT has not been shown to slow the decline in renal function. The applicant also asserts that renal function remained “stable” through month 30 in 31 subjects who continued migalastat treatment (-1.7 mL/min/1.73m<sup>2</sup>/year [95% CI -2.65, -0.78]).

**Table 4: Annualized rate of change in eGFR in patients with amenable mutations (LSmean [95% CI])**

	<b>Migalastat n=34</b>	<b>ERT n=18</b>
eGFR <sub>CKD-EPI</sub>	-0.4 (-2.2, 1.5)	-1.0 (-3.6, 1.6)
eGFR <sub>MDRD</sub>	-1.5 (-3.4, 0.4)	-1.5 (-4.2, 1.1)
mGFR <sub>iohexol</sub>	-4.4 (-7.7, -1.1)	-3.2 (-7.8, 1.3)

Source: Applicant, Summary of Clinical Efficacy, Table 27.

*Composite Clinical Events*

Study 012 included a composite endpoint of renal, cerebrovascular, and cardiac events and death. Renal events included a decrease in eGFR<sub>CKD-EPI</sub> ≥15 mL/min/1.73 m<sup>2</sup> relative to baseline to a value <90 mL/min/1.73 m<sup>2</sup> or an increase in 24-hour urine protein ≥33% relative to baseline to a value ≥300 mg. The protocol does not specify that the values had to be confirmed by a second measurement. In the migalstat arm, eight (24%) subjects experienced a renal event compared with six (33%) ERT-treated subjects. The applicant proposes (b) (4)

(b) (4)

**Consult Questions**

1. Please review and provide input (b) (4)

(b) (4)

(b) (4)



*In addition, the applicant proposes to include the following text in Section 14 of the label:*

(b) (4)



*We note that Section 14 of the Fabrazyme label states that no difference in renal function was observed between groups in Study 1. It also notes that the reduction in GL-3 inclusions suggests that Fabrazyme may ameliorate disease expression; however, the relationship of GL-3 inclusion reduction to specific clinical manifestations of Fabry disease has not been established. Should migalastat be improved, we believe it is important to include similar statements in the migalastat label.*

2. Are there any PMCs or PMRs that should be considered?

*DCRP Response: We do not believe a renal postmarketing safety study is needed. It is our understanding that, should migalastat receive accelerated approval (b) (4), clinical benefit will be confirmed in a post-marketing trial (u) (4).*



**Food and Drug Administration  
Center for Drug Evaluation and Research  
Division of Cardiovascular and Renal Products**

**Date:** December 1, 2015

**Drug Name:** Migalastat

**IND:** 68,456

**Sponsor:** Amicus

**From:** Aliza Thompson, Medical Officer, Division of Cardiovascular and Renal Products

**Through:** Norman Stockbridge, Director, Division of Cardiovascular and Renal Products

**To:** Jessica Benjamin, Regulatory Project Manager, Division of Gastroenterology and Inborn Errors Products (DGIEP)

**Subject:** Consult from DGIEP regarding the renal findings reported in the migalastat development program (IND 68456)

### **Background**

Migalastat, a low molecular weight iminosugar that is an analogue of the terminal galactose of globotriaosylceramide (GL-3), is being developed by Amicus for the treatment of Fabry disease in patients who have an amenable mutation in the  $\alpha$ -galactosidase A gene.

Fabry Disease is a rare, progressive, genetic (X-linked) lysosomal storage disease. The disease is caused by mutations in the  $\alpha$ -galactosidase A gene resulting in deficient or absent activity of the lysosomal enzyme. The absence of significant  $\alpha$ -galactosidase A activity results in accumulation of GL-3 in various tissues, resulting in the clinical complications of the disease. Clinical manifestations of Fabry disease include pain (i.e., acroparesthesias), progressive renal impairment, hypertrophic cardiomyopathy, cardiac conduction abnormalities, cerebrovascular events, angiokeratomas, anhidrosis as well as other complications. At present, the only enzyme replacement therapy licensed in the U.S. for the treatment of Fabry disease is agalsidase beta (Fabrazyme). Fabrazyme was licensed in April 2003 under the accelerated approval regulations and has the following indication: "Fabrazyme® (agalsidase beta) is indicated for use in patients with Fabry disease. Fabrazyme reduces globotriaosylceramide (GL-3) deposition in capillary endothelium of the kidney and certain other cell types."

Migalastat is a competitive inhibitor of  $\alpha$ -Gal A enzyme that reversibly binds to the active site of the enzyme, imparting stability and helping restore proper trafficking from the endoplasmic reticulum to lysosomes. It is reported to dissociate at the low pH of lysosomes in the presence of excess substrate, allowing catalytically-competent forms of  $\alpha$ -Gal A to catalyze the degradation of GL-3.

Amicus has completed two phase 3 trials in patients with Fabry disease and in July 2015, the sponsor requested a pre-NDA meeting to discuss the content and format of an NDA for migalastat. In their briefing document for the meeting, the sponsor proposed to use reduction in GL-3 deposition in kidney interstitial capillaries as a surrogate endpoint and basis for Subpart H approval of migalastat. Amicus also proposed to conduct a phase 4 (b) (4) (as

well as a GI trial) to verify the benefit of migalastat. In a consult dated August 27, 2015, DGIEP requested “informal input on the cardiorenal endpoints proposed in this program” and, specifically, that a nephrologist and cardiologist from the Division of Cardiovascular and Renal Products (DCRP) “attend the meetings in case any questions regarding what DCRP finds acceptable as endpoints come up during the discussion.” Members of DCRP attended the internal meeting but did not attend the sponsor meeting, because it was agreed that DCRP did not need to attend the latter meeting.

Following the pre-NDA meeting with Amicus, DGIEP requested that DCRP “evaluate the summary renal data presented in the background package from studies -011 and -012 and provide input on the cardiorenal endpoints proposed in this program” (see consult request dated September 14, 2015). (b) (4)

The issues raised in the second consult will be addressed in a separate cardiology consult; accordingly, this memo will focus on renal-related issues.

#### **Materials used for review**

- Pre-NDA Briefing Document for migalastat dated August 7, 2015
- Pre-NDA Meeting Minutes for migalastat dated September 17, 2015

(b) (4)

#### **Overview of phase 3 trials**

As previously noted, Amicus has completed two phase 3 trials in patients with Fabry disease; the designs of these trials, AT1001-011 and AT1001-012, are summarized in the table below. In brief, Study AT1001-011 provides placebo-controlled data on the effect of migalastat on GL-3 inclusions in the renal interstitial capillary over the short-term. Data on migalastat’s effect on renal function are provided by the two 3 phase trials and an extension study, AT1001-041.

**Table 1: Overview of phase 3 trials**

Design	Study population	Primary efficacy endpoint
<b>AT1001-011</b>		
Placebo-controlled, randomized (1:1), international trial. Study consisted of two 6-month stages (stage 1 was double-blind and placebo-controlled; in stage 2, all patients were treated with migalastat) and a 12-month open-label extension phase	67 ERT-naïve patients with Fabry disease who had migalastat-responsive GLA mutations based on a preliminary HEK assay	Stage 1: proportion of subjects with a $\geq 50\%$ reduction from baseline to Month 6 in the average number of GL-3 inclusions per renal interstitial capillary (IC GL-3)
<b>AT1001-012</b>		
Active-controlled, randomized, open-label international trial comparing migalastat to ERT (agalsidase beta or agalsidase alfa**). Study consisted of an 18-month randomized period followed by an optional 12-month open-label extension phase*	60 patients with Fabry disease who were receiving ERT prior to study entry and who had migalastat-responsive GLA mutations based on a preliminary HEK assay	“Primary efficacy parameters”: annualized change in measured glomerular filtration rate AND annualized change in estimated GFR (as assessed by plasma clearance of iohexol and the CKD-EPI equation, respectively) from Baseline through Month 18

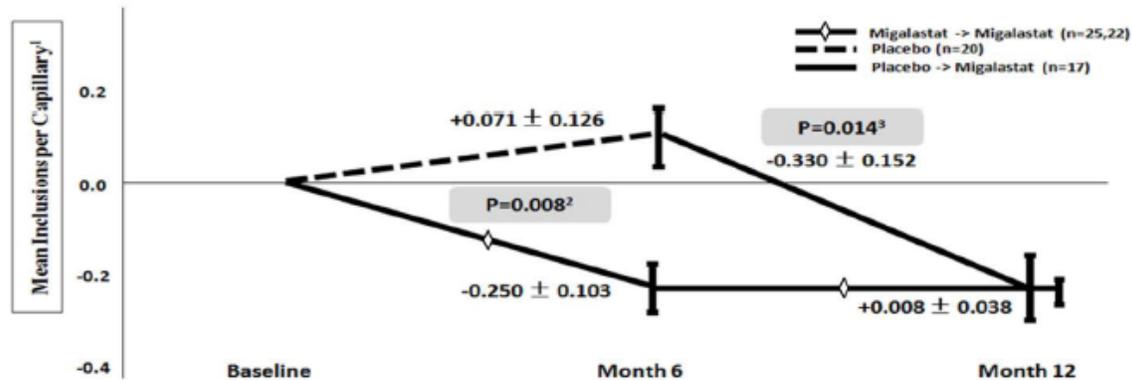
\*Extension phase is ongoing; \*\* Agalsidase-alfa is approved in Europe but not in the United States, in part because of questions regarding efficacy; ERT= enzyme replacement therapy

### Renal-related findings

#### Treatment effects on clearance of GL-3 from renal interstitial capillaries

According to the pre-NDA briefing document, study AT1001-011 failed to meet its prespecified primary endpoint, the proportion of subjects with a  $\geq 50\%$  reduction from baseline to Month 6 in the average number of GL-3 inclusions per renal interstitial capillary. In the ITT population (all randomized subjects), 41% of subjects in the migalastat group as compared to 28% in the control arm had a  $\geq 50\%$  reduction from baseline to Month 6 in the average number of GL-3 inclusions per renal interstitial capillary ( $p=0.3$ ;  $n=64$ ).

Although the trial failed on its primary endpoint, the sponsor notes that in a subpopulation (i.e., those with “amenable mutations” as defined by a GLP HEK assay and not the preliminary assay used to select patients for enrollment) migalastat was effective in reducing GL-3 inclusions in renal interstitial capillaries. Specifically, in a post-hoc analysis of Stage 1 data from patients with “amenable mutations”, there was a “statistically significant” greater reduction in the average number of GL-3 inclusions per interstitial capillary in the migalastat as compared to placebo arm (nominal  $p=0.008$ ). According to the sponsor, in Stage 2 of the trial, the mean number of inclusions decreased in patients with “amenable mutations” who were previously treated with placebo in Stage 1 and remained stable in those previously treated with migalastat in Stage 1 (see figure below). Of note, according to the briefing document there were 50 patients with “amenable mutations” in Stage 1. Based on the numbers presented in the sponsor’s figure below, it appears that 45 of these patients contributed to the analysis in Stage 1 and 39 of these subjects contributed to the analysis for Stage 2.



**Figure 1: Change from Baseline in the Mean Number of GL-3 Inclusions per Renal Interstitial Capillary in Trial AT1001-11 (Figure 5 of sponsor’s pre-NDA briefing document)**

The pre-NDA briefing document also contains a cross-trial comparison of the treatment effect of migalastat and Fabrazyme on GL-3 deposition. In general, we believe that cross-trial comparisons are difficult to interpret and hence, did not review the analyses for the purpose of this consult.

#### Treatment effects on renal function

The briefing document highlights the following analyses related to migalastat’s effect on renal function: (1) a comparison of the annualized rate of change in GFR during treatment with migalastat in Study AT1001-011 with published data for untreated patients; (2) a comparison of the annualized rate of change in eGFR and mGFR in patients treated with migalastat vs ERT in Study AT1001-012; and (3) the findings in patients who continued from AT1001-011 into extension study AT1001-041. The results of these analyses are summarized below.

*Comparison of annualized change in GFR in study AT1001-011 with published data for untreated patients:* The Statistical Analysis Plan for study AT1001-011, submitted in February 2014, describes a number of analyses related to changes in renal function (and proteinuria); none of these analyses were to be conducted within a plan that controlled the overall type 1 error rate.

In the pre-NDA briefing document, the sponsor provides a table with information on the annualized change at Month 18/24 in eGFR<sub>MDRD</sub>, eGFR<sub>CKD-EPI</sub>, and mGFR<sub>iohexol</sub>. The footnote to the table indicates that the analyses were performed in “patients with amenable mutations in the modified intent to treat population with data”; the document does not provide other information about the analyses or clearly indicate the number of patients that were excluded or the reasons for their exclusion (e.g., missing data, non-amenable mutation, etc.). The different methods give somewhat different point estimates for the annualized change at Month 18/24, but the 95% confidence intervals are wide (particular for mGFR<sub>iohexol</sub>) and overlapping. The sponsor interprets these data as showing that “renal function remained stable over 18 to 24 months of migalastat treatment...”

**Table 2: Table 11 of Sponsor’s preNDA briefing document, titled “Study AT1001-011: Annualized GFR Change at Month 18/24**

GFR Method	N	Mean GFR <sup>a</sup> (±SEM) [95% CI]
eGFR <sub>CKD-EPI</sub>	41	-0.30 (0.66) [-1.65, 1.04]
eGFR <sub>MDRD</sub>	41	+0.79 (1.03) [-1.28, 2.87]
mGFR <sub>iohexol</sub>	37	-1.51 (1.33) [-4.20, 1.18]

<sup>a</sup> Glomerular filtration rate (GFR) measured in mL/min/1.73m<sup>2</sup>/year.  
eGFR<sub>CKD-EPI</sub>= estimated glomerular filtration rate based on the Chronic Kidney Disease Epidemiology Collaboration equation; eGFR<sub>MDRD</sub>= estimated glomerular filtration rate based on the Modification of Diet in Renal Disease equation; mGFR<sub>iohexol</sub>=measured glomerular filtration rate; N=number of patients with amenable mutations in the modified intent to treat population with data; SEM=standard error of the mean  
Source: AT1001-011 Table 14.2.7.15-3, Table 14.2.7.16-3, Table 14.2.7.17-3

Since this portion of the trial was uncontrolled, the briefing document compares annualized changes in GFR seen in this trial with rates of disease progression reported in healthy adults (2009 paper by Levey et al) and rates reported in untreated patients with Fabry disease. According to the sponsor, the Levey et al paper reports changes in eGFR in healthy adults of -1 mL/min/1.73m<sup>2</sup> per year. With regard to rates reported in the published literature for untreated patients with Fabry Disease, the briefing document states that “A comprehensive survey of published reports of renal function in untreated patients with Fabry disease revealed annual changes in eGFR (mL/min/1.73m<sup>2</sup>) between -2.2 and -12.2...” and cites 9 papers published between 2001 and 2010 as the source of this information.

The pre-NDA briefing document also includes a comparison of the findings in study AT1001-011 with rates reported in three of the published natural history studies. The sponsor states that these studies were chosen because they most closely matched the AT1001-011 design and patient populations. According to the sponsor:

*“Statistical comparisons of annualized changes in eGFR<sub>MDRD</sub>, stratified by gender and 24-hr urine protein in migalastat-treated patients in AT1001-011 with those reported in untreated patients by Schiffmann 2009 and Schwarting 2006, demonstrated statistically significant differences in annualized rate of change in eGFR, favoring migalastat [2.97 mL/min/m<sup>2</sup> (95% CI: 0.134 to 5.806); p= 0.040 versus Schiffmann et al. 2009; 3.90 mL/min/m<sup>2</sup> (95% CI: 1.142 to 6.666); p=0.006 versus Schwarting et al. 2006]. A similar statistical analysis, stratified by gender and comparing the annualized changes in eGFR<sub>CKD-EPI</sub> in AT1001-011 with untreated patients in Wanner et al 2010, showed a statistically significant difference in annualized rate of change in eGFR, favoring migalastat [1.49 mL/min/m<sup>2</sup> (95% CI: 0.167 to 2.816; p= 0.027)].”*

Finally, the pre-NDA briefing document contains the following table comparing mean annualized GFR in study AT1001-011, stratified by baseline 24-hour urine protein and gender, with those reported in the Schiffmann paper.

**Table 12: Annualized GFR Following 18 or 24 Months Treatment with Migalastat in Study AT1001-011, compared to Published Data on Untreated Patients**

Annualized GFR <sup>a</sup>							
		Male			Female		
	Baseline 24-hr Urine Protein (mg)	N	Mean	(SEM)	N	Mean	(SEM)
<b>Migalastat</b>							
<b>eGFR<sub>CKD-EPI</sub></b>	<100	0	-	-	7	0.22	(1.4)
	100-1000	12	-0.03	(0.90)	18	0.16	(1.2)
	>1000	2	-6.5	(2.0)	2	-1.8	(2.4)
	<b>ALL</b>	<b>14</b>	<b>-0.96</b>	<b>(1.0)</b>	<b>27</b>	<b>0.03</b>	<b>(0.87)</b>
<b>eGFR<sub>MDRD</sub></b>	<100	0	-	-	7	0.26	(1.4)
	100-1000	12	1.0	(1.4)	18	1.8	(2.0)
	>1000	2	-5.9	(1.8)	2	-1.3	(2.8)
	<b>ALL</b>	<b>14</b>	<b>0.01</b>	<b>(1.4)</b>	<b>27</b>	<b>1.2</b>	<b>(1.4)</b>
<b>mGFR<sub>iohexol</sub></b>	<100	0	-	-	7	3.5	(2.9)
	100-1000	11	-3.3	(1.7)	17	-2.8	(2.3)
	>1000	1	0.07	(-)	1	3.5	(-)
	<b>ALL</b>	<b>12</b>	<b>-3.0</b>	<b>(1.6)</b>	<b>25</b>	<b>-0.81</b>	<b>(1.8)</b>
<b>Untreated, as per published natural history data<sup>b</sup></b>							
<b>eGFR<sub>MDRD</sub></b>	<100 <sup>c</sup>	18	-1.6	(1.5)	7	-0.6	(2.6)
	100-1000	21	-3.3	(1.8)	17	-2.2	(2.2)
	>1000	22	-6.9	(1.5)	5	-4.6	(2.3)
	<b>All<sup>d</sup></b>	<b>128</b>	<b>-2.9</b>		<b>51</b>	<b>-1.0</b>	

<sup>a</sup> Annualized GFR in ml/min/1.73m<sup>2</sup>/yr from baseline (for patients randomized to migalastat) or Month 6 (for patients randomized to placebo) to Month 18/24 or last visit; patients with amenable mutations

<sup>b</sup> Schiffmann, Warnock et al. 2009, a retrospective analysis of 447 patients with a median follow-up of 5.6 years

<sup>c</sup> Includes number of patients with baseline 24-hour determinations

<sup>d</sup> Includes patients with ≥3 serum creatinine determinations and not with end-stage renal disease (ESRD).

Sources: AT1001-011 Tables 14.2.7.15-3, 14.2.7.16-3, 14.2.7.17-3; AT1001-011 Dataset Tables 14.1.9.1-3, 14.1.9.2-3.

**Reviewer's comment:** The Schiffmann paper describes the results of a retrospective chart review of 279 men and 168 women diagnosed with Fabry disease during their life or at the time of death at 27 sites (USA, Canada and Europe). For the following reasons, it is unclear whether the cited population of untreated patients is sufficiently similar to the population enrolled in trial AT1001-011 to serve as a control.

- There was a substantial amount of missing follow-up data in patients in the Schiffmann study (see table below). The missing data raise concern that the estimates of disease progression reported in the paper may be biased. Of note, to be included in the eGFR analyses, patients needed to have three follow-up creatinine measurements. It is possible that this requirement resulted in the exclusion of subjects with milder forms of the disease who were not felt to need frequent monitoring, particularly at a time before treatments were available.

**Table 3: Schiffmann Manuscript: Number of patients identified via chart review and number included in various analyses**

	Men	Women
Number of patients identified via chart review	279	168
Number of patients with baseline serum creatinine values	243	152
Number of patients included in eGFR analysis*	145	55
Number of patients included in the eGFR slope analysis stratified by baseline proteinuria and gender (per sponsor's figure)	128	51

\*To be included in the analysis, subjects needed to have 3 follow-up creatinine measurements. Patients who progressed to ESRD were included in the analysis until the start of dialysis or transplant or a serum creatinine > 6mg.

- Data on the natural history of the disease were collected from an earlier time period (1944 to 2002) and it's uncertain whether patients with Fabry disease in this time period are reflective of patients diagnosed with the disease today. Again, one could speculate that patients who were diagnosed with Fabry disease in the past, at a time before treatment was available, may have been more severely affected. Indeed, the manuscript states that the majority of women in the study were "symptomatic with moderate to severe manifestations of Fabry disease."
- The sponsor's analyses focus on a subset of patients with Fabry disease (i.e., those with "amenable" HEK mutations). It is unclear whether rates of disease progression in this subset differ from patients without "amenable" mutations, a population that would have been included in Schiffman's retrospective chart review.
- Finally, the proposed matching based on gender and broad categories of proteinuria levels may not adequately adjust for important covariates that affect disease progression. While both gender and proteinuria levels are important predictors of disease progression, other factors, identified and unidentified, may play a role in disease progression. Of note, the paper by Schiffmann et al reports that progression rates were 2-fold greater for males and females who had eGFRs < 60 as compared to those with eGFRs > 60, suggesting that it may be important to also match patients by baseline renal function status.

#### *Comparison of migalastat and ERT in Study AT1001-012*

According to the briefing document, the study met the prespecified criteria for "comparability" of migalastat and ERT (the differences between the means for annualized change in GFR for migalastat and ERT within 2.2 mL/min/1.73 m<sup>2</sup>/year and a >50% overlap of the 95% CI between migalastat and ERT). The annualized rate of change in GFR from Baseline to Month 18 is shown in the table below for the mITT population (i.e., randomized subjects with amenable mutations who received at least one dose of study medication, have both the baseline and a post-baseline efficacy measure of mGFR<sub>iohexol</sub>, and have a post-baseline measure of eGFR<sub>CKD-EPI</sub>). In both treatment arms, the mean annualized rate of change in GFR is larger when assessed using measured as opposed to estimated GFR. There are no obvious differences between the arms in the rate of change, but the sample size is small and the confidence intervals around the point estimate are relatively wide.

**Table 4: Annualized Rate of Change in GFR from baseline to Month 18 in the mITT population\* in Study AT1001-012**

(b) (4)

Method	Migalastat (mean <sup>1</sup> ± SEM); [95% CI] N = 34	ERT (mean ± SEM) [95% CI] N = 18	Differences in means	Overlap of 95% CIs
eGFR <sub>CKD-EPI</sub>	-0.40 (0.93) [-2.27, 1.48]	-1.03 (1.29) [-3.64, 1.58]	+0.63	100%
mGFR <sub>iohexol</sub>	-4.35 (1.64) [-7.65, -1.06]	-3.24 (2.27) [-7.81, 1.33]	-1.11	100%

<sup>1</sup>Least square means in ml/min/1.73m<sup>2</sup>/year; \*The Background document indicates that 60 subjects were randomized into the trial; of these, 56 had amenable mutations based on the GLP- HEK assay.

*Findings in patients who continued from AT1001-011 into extension study AT1001-041:*

The submission states that “in patients who continued from AT1001-011 into extension study AT1001-041,” the annualized rate of change in eGFR<sub>CKD-EPI</sub> over an average of 36 months (minimum 18 months, maximum 54 months) was -0.81 (95% CI: -2.00, 0.37) mL/min/1.73m<sup>2</sup>. The sponsor comments that the rate of change seen in the study compares favorably to the rates reported in the published literature for untreated patients and to those reported in healthy adults.

(b) (4)

(b) (4)

**Response to Consult Question**

*DCRP Response:* The sponsor believes that the efficacy of migalastat has been demonstrated in trials AT1001-011, AT1001-012 and AT1001-041 and, specifically, that these trials establish that:

- migalastat causes significant reductions of GL-3 in renal interstitial capillaries and reductions in other renal cell types
- migalastat stabilizes renal function for up to 3 years and
- the effects of migalastat on renal function are similar to those observed with ERT and significantly better than those reported in untreated patients with Fabry Disease.

We do not agree with the sponsor’s interpretation of the data. With regard to migalastat’s effect on GL-3 deposition in renal interstitial capillaries, as DGEIP has noted, trial AT1001-011 failed on its prespecified primary endpoint, the proportion of subjects with a ≥ 50% reduction from baseline to Month 6 in the average number of GL-3 inclusions per renal interstitial capillary in the migalastat vs. placebo arm. The sponsor believes that post-hoc analyses in patients with “amenable” mutations (as defined by a GLP HEK assay) provide evidence of migalastat’s effect on GL-3 deposition in renal interstitial capillaries. However, as DGEIP has communicated to the sponsor, the cited analyses are post-hoc, thus raising concern that the findings may not be real. Even if the findings are real, we question whether there is sufficient data to support the use of these findings (i.e., treatment effects on GL-3 inclusions in renal interstitial capillaries of this magnitude and at such an early time point following initiation of therapy) as a “reasonably likely surrogate” and basis for accelerated approval.

*It is also uncertain, based on the data amassed thus far, whether migalastat slows the loss of renal function in patients with Fabry disease. The sponsor believes that the annualized changes in GFR seen in migalastat treated patients in trials AT1001-011 and AT1001-041 compare favorably to rates reported in untreated patients with Fabry disease. However, it is not clear that the cited external controls are sufficiently similar to the patients treated with migalastat in trials AT1001-011 and AT1001-041 to serve as controls for the trial populations (for further discussion of this issue, see the Reviewer's Comment on pages 6-7 of this review). Finally, Amicus believes that the data from trial AT1001-012 demonstrate that the effect of migalastat on renal function is similar to that observed with ERT. To our knowledge, it has not been established that ERT slows the decline in renal function in patients with Fabry disease; hence, we believe these data are difficult to interpret.*

**Additional Comment**

(b) (4)

(b) (4)

**Date:** June 2, 2017

**From:** Kimberly Smith, Medical Officer  
Division of Cardiovascular and Renal Products

**Through:** Aliza Thompson, Team Leader  
Norman Stockbridge, Director  
Division of Cardiovascular and Renal Products

**To:** Hong Vu, Division of Gastroenterology and Inborn Errors Products

**Subject:** Review of new renal function analyses reported in migalastat development program

### **Background**

Migalastat, a low molecular weight iminosugar that is an analogue of the terminal galactose of globotriaosylceramide (GL-3), is being developed by Amicus for the treatment of Fabry disease in patients who have an amenable mutation in the  $\alpha$ -galactosidase A gene (IND 68456).

Fabry Disease is a rare lysosomal storage disease that results from an X-linked mutation in the *GLA* gene causing a deficiency of the enzyme  $\alpha$ -galactosidase A, a lysosomal enzyme that catalyzes the hydrolysis of the terminal glycolipid globotriaosylceramide (Gb3 or GL-3) to galactose and lactosylceramide. In Fabry disease, GL-3 accumulates in tissues leading to systemic manifestations including progressive renal impairment, hypertrophic cardiomyopathy, cardiac conduction system abnormalities, myocardial infarction, stroke, painful neuropathy, and gastrointestinal disturbances. The only therapy currently approved in the U.S. is agalsidase beta (Fabrazyme), an enzyme replacement therapy (ERT) approved in 2003 via accelerated approval “for use in patients with Fabry disease. Fabrazyme reduces globotriaosylceramide (GL-3) deposition in capillary endothelium of the kidney and certain other cell types.” Two post-marketing confirmatory trials have failed to confirm a clinical benefit on renal function.

Amicus has completed two phase 3 trials for migalastat in patients with Fabry disease (AT1001-011 and -012) and, on September 9, 2015, met with the Division of Gastroenterology and Inborn Errors Products (DGIEP) to discuss the content and format of an NDA (minutes dated September 17, 2015). The sponsor proposed to use reduction in GL-3 deposition in renal interstitial capillaries as a surrogate endpoint and basis for Subpart H approval and to conduct a phase 4 CV registry-based trial and a GI trial to verify the clinical benefit. The Division did not agree with their proposed plan, noting that Study 011 did not reach statistical significance for the pre-specified study endpoints and that Study 012 was not powered to test a specific statistical hypothesis. Given the Fabrazyme experience, the Division also noted that the sponsor had not provided sufficient evidence to support treatment effects on GL-3 deposition in renal interstitial capillaries as a surrogate endpoint to support traditional or accelerated approval. The Division of Cardiovascular and Renal Products (DCRP) provided input on these discussions (see consult by Dr. Aliza Thompson dated December 1, 2015.)

On October 26, 2016, Amicus again met with DGIEP to discuss new analyses of the existing trial data and their intention to submit an NDA for Subpart H approval (minutes dated November 24, 2016). DGIEP noted that the additional analyses were considered exploratory and should be used for hypothesis generation only. The meeting focused on the design of a new trial that could be used to establish efficacy based on gastrointestinal symptoms including diarrhea. This was followed by additional discussions on November 21, 2016. On March 21, 2017, Amicus submitted a feasibility report via email that concluded that the trial discussed would take 5-7 years to conduct and therefore is not feasible.

In the current submission, the sponsor states that there were interactions with CDER leadership in March 2017. As a result, on April 24, 2017, Amicus requested that, based on “the collective body of evidence,”

DGIEP issue a written response confirming that no additional Phase 3 studies are required for submission and that the FDA will agree to review an NDA for migalastat. The submission includes additional analyses of eGFR data from the migalastat development program, and DGIEP has requested that DCRP review these data and opine on whether they are adequate to support an NDA submission. Of note, the sponsor's request will be discussed with the Medical Policy Council before DGIEP issues a response.

### Materials Reviewed

1. Sponsor's briefing document dated April 19, 2017.
2. Meeting minutes dated September 17, 2015 and November 24, 2016.
3. Consult by Dr. Aliza Thompson dated December 1, 2015.

### Overview of Phase 3 Trials

As previously noted, Amicus has completed two phase 3 trials in patients with Fabry disease, AT1001-011 and -012 (Table 1). In brief, Study 011 provides placebo-controlled data on the effect of migalastat on GL-3 inclusions in the renal interstitial capillary over a 6-month period. Data on effects on renal function are provided by the two 3 phase trials and an extension study, AT1001-041.

**Table 1: Summary of Phase 3 Trials**

Design	Study population	Primary efficacy endpoint
<b>AT1001-011</b>		
Placebo-controlled, randomized (1:1), international trial. Study consisted of two 6-month stages (stage 1 was double-blind and placebo-controlled; in stage 2, all patients were treated with migalastat) and a 12-month open-label extension phase	67 ERT-naïve patients with Fabry disease who had migalastat-responsive GLA mutations based on a preliminary HEK assay	Stage 1: proportion of subjects with a $\geq 50\%$ reduction from baseline to Month 6 in the average number of GL-3 inclusions per renal interstitial capillary (IC GL-3)
<b>AT1001-012</b>		
Active-controlled, randomized, open-label international trial comparing migalastat to ERT (agalsidase beta or agalsidase alfa**). Study consisted of an 18-month randomized period followed by an optional 12-month open-label extension phase*	60 patients with Fabry disease who were receiving ERT prior to study entry and who had migalastat-responsive GLA mutations based on a preliminary HEK assay	"Primary efficacy parameters": annualized change in measured glomerular filtration rate AND annualized change in estimated GFR (as assessed by plasma clearance of iohexol and the CKD-EPI equation, respectively) from Baseline through Month 18

\*Extension phase is ongoing; \*\* Agalsidase-alfa is approved in Europe but not in the United States, in part because of questions regarding efficacy; ERT= enzyme replacement therapy

### Previously Reviewed Renal Findings

DCRP has previously reviewed the renal findings from the completed studies (see Dr. Thompson's review dated December 1, 2015). In brief, Study AT1001-011 failed to meet its prespecified primary endpoint, the proportion of subjects with a  $\geq 50\%$  reduction from baseline to Month 6 in the average number of GL-3 inclusions per renal interstitial capillary. In the ITT population consisting of all randomized subjects, 41% of subjects in the migalastat group and 28% in the control group reached the endpoint ( $p=0.3$ ;  $n=64$ ). The sponsor then conducted post-hoc analyses of data from patients with "amenable mutations," defined by a GLP HEK assay rather than the preliminary assay used for enrollment. For Stage 1, there was a "statistically significant" greater reduction in the average number of GL-3 inclusions per interstitial capillary in the migalastat group compared with placebo (nominal  $p=0.008$ ). For Stage 2, the mean number of inclusions in patients treated with placebo for Stage 1 decreased after switching to migalastat while the number of inclusions stabilized in patients who

continued on migalastat. Of 50 patients with “amenable mutations,” only 45 and 39 subjects contributed to Stage 1 and 2 analyses, respectively.

Regarding effects on renal function, the sponsor previously provided analyses of the annualized rate of change in eGFR in patients with amenable mutations 1) treated with migalastat for 6-12 months in Study 011 and 2) who continued from Study 011 into Study 041 and were treated with migalastat for an average of 36 months. The sponsor compared these rates with the annualized rate of change in eGFR in published natural history studies. In addition, the sponsor provided a comparison of the annualized rate of change in eGFR and mGFR over an 18-month period in patients treated with migalastat vs. ERT in Study 012.

For Study 011, the sponsor asserted that renal function remained stable over 18-24 months of with an annualized rate of change in eGFR of  $-0.30$  ( $0.66$ ) mL/min/1.73m<sup>2</sup>. Similarly, for ERT-naïve subjects continuing migalastat in Study 041, the annualized rate of change in eGFR over 36 months was  $-0.81$  ( $1.18$ ) mL/min/1.73m<sup>2</sup>. The sponsor believes these findings compared favorably to rates of renal progression reported in three published natural history studies. In her review, Dr. Thompson noted several reasons why the cited external controls may not be sufficiently similar to the patients treated with migalastat in Studies 011 and 041 to serve as appropriate controls for the trial populations. She also noted that the reported analyses were amongst several related to changes in renal function and proteinuria that were not conducted within a plan to control type 1 error and that the analysis populations had been re-defined post hoc.

For Study 012, the sponsor reported the annualized rate of change in GFR from baseline to 18 months in patients treated with migalastat vs. ERT (agalsidase beta and agalsidase alfa) with amenable mutations who received at least one dose of study drug and had both baseline and post-baseline values. In the migalastat arm, the mean annualized change in eGFR was  $-0.40$  ( $0.93$ ) mL/min/1.73m<sup>2</sup> compared with  $-1.03$  ( $1.29$ ) with ERT. Although, there were no obvious differences between the treatment arms, sample sizes were small and the confidence intervals were wide. Of note, ERT has not been shown to slow the decline in renal function.

### **New Analyses of Renal Function Data**

The current submission contains two new analyses of data from the migalastat development program: an analysis of renal function from 12 subjects enrolled in phase 2 studies who continued in extension Study 041 and a post-hoc analysis of subjects enrolled in Studies 011 and 012 who met specified criteria for “classic” Fabry disease.

#### Longer-term Phase 2 Data

The sponsor has provided a one-paragraph summary of analyses of renal function from 12 subjects enrolled in phase 2 studies of migalastat who continued treatment with various doses and regimens for an average of 8.2 years in extension Study 041.<sup>1</sup> According to the sponsor, the annualized rate of change in eGFR in this population was  $-0.67$  ( $0.65$ ) mL/min/1.73 m<sup>2</sup>/year. The sponsor asserts that this rate compares favorably to rates of progression reported in the published literature. The sponsor did not provide information regarding how this subset of 12 subjects was identified, their baseline characteristics, or data regarding the natural history of the disease in patients with similar baseline characteristics.

#### Analyses in Males with Classic Fabry Disease

At the request of the Dutch rapporteur, the sponsor conducted post-hoc subgroup analyses of renal function in all male patients with “classic” Fabry disease defined by multi-organ system involvement and

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<sup>1</sup> For these analyses, the sponsor’s briefing document cites Table 2.2.4, ISE Table 4.4.1.8, and ISE Table 4.4.1.6 but we were unable to locate these tables in the meeting material or previous submissions.

an  $\alpha$ -Gal A activity <3% for Study 011 (n=14) and as multi-organ system involvement in Study 012 (n=12). They provided a brief summary of the findings with the current submission. As shown in Table 2, the mean (95% CI) annualized change from baseline to month 18 or 24 in eGFR in this population was -0.3 (-2.8, 2.3) mL/min/1.73m<sup>2</sup> in Study 011 and -2.4 (-4.5, -0.2) mL/min/1.73m<sup>2</sup> in Study 012. The sponsor concludes that these subjects had a positive response to migalastat based stabilization in GFR. They do not cite a comparator population.

**Table 2: Effect of Migalastat on Renal Function in Males with Classic Fabry disease**

Endpoint	Parameter	Study AT1001-011 Migalastat		Study AT1001-012 Migalastat		Study AT1001-012 ERT	
		"Classic": Male, Multi- organ, <3% $\alpha$ -Gal A (n=14)	Other (n=36)	"Classic": Male, Multi- organ (n=12)	Other (n=22)	"Classic": Male, Multi- organ (n=8)	Other (n=11)
		Mean (SD or 95% CI)					
eGFR <sub>CKD-EPI</sub>	Baseline	87.8 (33.6)	95.3 (19.6)	87.1 (23.3)	89.7 (19.2)	95.7 (17.1)	93.3 (22.3)
	Annualized CFB to Month 18 or 24	-0.3 (-2.8, 2.3)	-0.3 (-2.0, 1.4)	-2.4 (-4.5, -0.2)	-0.6 (-2.6, 1.5)	-1.5 (-7.8, 4.9)	-5.6 (-13.7, 2.5)
	Percent Annualized CFB to Month 18/24	-0.8 (-5.5, 4.0)	-0.3 (-2.0, 1.5)	-3.1 (-6.1, 0.0)	-0.1 (-2.8, 2.7)	-1.6 (-8.7, 5.6)	-5.8 (-15.1, 3.5)

Source: Sponsor, Briefing Document, Table 17.

### Consult Questions

1. Do the submitted new analyses and data on eGFR stabilization over the stated time period of follow up in some Fabry patients treated with migalastat appear adequate for a NDA submission for migalastat?

*DCRP Response: The sponsor has previously asserted that the efficacy of migalastat has been demonstrated in Studies 011, 012, and 041 and, specifically, that these trials have established that:*

1. *migalastat causes significant reductions of GL-3 in renal interstitial capillaries*
2. *migalastat stabilizes renal function for up to 3 years*
3. *the effects of migalastat on renal function are similar to those observed with ERT and significantly better than those reported in untreated patients with Fabry Disease*

*With the current meeting materials, the sponsor has provided additional analyses of renal function from 12 subjects enrolled in phase 2 studies who continued migalastat in Study 041 and from subjects enrolled in Studies 011 and 012 who met specified criteria for "classic" Fabry disease. The sponsor asserts that these subjects had a positive response to migalastat based on stabilization of renal function.*

*As Dr. Thompson noted in her December 2015 review, Study 011 failed on its prespecified primary endpoint related to GL-3 inclusions. The sponsor believes post-hoc analyses in patients with "amenable mutations" provide evidence of migalastat's effect on GL-3 deposition; however, DGIEP has previously communicated to the sponsor that the cited analyses are post-hoc and raise concerns that the findings may not be real. Even if they are real, given the Fabrazyme experience, we question whether there are sufficient data to support the use of treatment effects on GL-3 inclusions as a validated or reasonably likely surrogate and basis for traditional or accelerated approval.*

*It is also uncertain, based on the data amassed thus far, whether migalastat slows the loss of renal function in patients with Fabry disease. The sponsor believes that the annualized changes in GFR seen in migalastat treated patients in Studies 011 and 041 compare favorably to rates reported in untreated patients with Fabry disease. However, as Dr. Thompson noted, it is not clear that the cited*

*external controls are sufficiently similar to the patients treated with migalastat in Studies 011 and 041 to serve as controls for the trial populations. Amicus also believes that the data from Study 012 demonstrate that the effect of migalastat on renal function is similar to that observed with ERT. To our knowledge, it has not been established that ERT slows the decline in renal function in patients with Fabry disease; hence, we believe these data are difficult to interpret.*

2. Does the finding of eGFR stabilization in some patients with Fabry disease treated with migalastat, as presented by the sponsor, appear adequate to serve as a surrogate endpoint, which is reasonably likely to predict clinical benefit in this patient population?

*DCRP Response: See our response to Question 1 regarding our concerns with the available data on “eGFR stabilization” from the migalastat development program.*

*As noted in the minutes from the October 26, 2016 meeting with the sponsor, we believe an endpoint based on annualized change in eGFR is a reasonable basis for full approval for an uncommon, slowly progressive renal disease that has a high likelihood of progressing to end-stage kidney disease, assuming that the magnitude of the treatment effect on eGFR is clinically meaningful and the treatment effect continues to accrue across the various stages of the disease and over time.*

*We further noted that, according to the Guidance for Industry: E10 Choice of Control Group and Related Issues in Clinical Trials, inability to control bias is the major and well-recognized limitation of externally controlled trials and generally restricts use of the external control design to situations in which the effect of treatment is dramatic and the usual course of the disease is highly predictable. In addition, the use of external controls should be limited to cases in which the endpoints are objective and the impact of baseline and treatment variables on the endpoint is well characterized. Given variability in the severity of Fabry disease and, specifically, in the progression of renal disease, it is not clear to us that all of these criteria can be satisfied in a trial of patients with Fabry disease such that it would be acceptable to use a historical control; hence, we believe comparison with a concurrent control group is necessary* (b) (4)

3. If accelerated approval may be reasonable based on eGFR stabilization, what trial features/considerations would a confirmatory trial need to have to confirm clinical benefit on renal disease in Fabry patients? Which clinical endpoints should such a trial evaluate?

*DCRP Response: As noted in our response to Question 1, we believe the available data on change in renal function with migalastat have significant limitations and are not likely to provide sufficient basis for traditional or accelerated approval. As noted in our response to Question 2, we believe that demonstration of a treatment effect on the annualized change in eGFR in the context of an adequate and well-controlled trial would be sufficient for full approval and would therefore not require a confirmatory post-marketing trial.*

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**This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.**  
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/s/  
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KIMBERLY A SMITH  
05/02/2018

ALIZA M THOMPSON  
05/03/2018

NORMAN L STOCKBRIDGE  
05/03/2018



# Memorandum

DEPARTMENT OF HEALTH AND HUMAN SERVICES  
PUBLIC HEALTH SERVICE  
FOOD AND DRUG ADMINISTRATION  
CENTER FOR DRUG EVALUATION AND RESEARCH  
DIVISION OF CARDIOVASCULAR AND RENAL PRODUCTS

Date: April 19, 2018

From: Stephen M. Grant, M.D.  
Deputy Director  
Division of Cardiovascular and Renal Products

Through: Norman Stockbridge, M.D., Ph.D.  
Director  
Division of Cardiovascular and Renal Products

To: Hong H. Vu, PharmD, MS  
Regulatory Project Manager  
Division of Gastroenterology and Inborn Errors Products

Subject: DCaRP consult to comment about labeling of migalastat (NDA 208623).

On 13 December 2017 Amicus Therapeutics submitted NDA 208623 seeking approval under subpart H to market migalastat “for the treatment of patients with Fabry disease with amenable mutations” based on effects on renal endpoints. As part of your review of this NDA, you asked us the following questions:

“1. Please review and provide input [REDACTED] (b) (4)

[REDACTED] (b) (4)

2. Are there any PMCs or PMRs that should be considered?”

We received and reviewed the following materials:

- Your consult dated 17 Jan 2018
- The proposed PI submitted in NDA 208623

[REDACTED] (b) (4)

## Background

Fabry disease is a rare hereditary, X-linked recessive, lysosomal storage disorder caused by the partial or complete deficiency of the lysosomal enzyme alpha galactosidase A, resulting in excess cellular glycosphingolipid deposition. Clinical cardiovascular disease typically begins in the third to fifth decades of life and manifestations include hypertension, anginal chest pain, myocardial infarction, conduction defects, valvular insufficiency, and heart failure. In Fabry patients with left ventricular hypertrophy (LVH), the underlying mechanism leading to the increase in left ventricular mass (LVM) is unknown, as glycosphingolipid deposition represents only a fraction of the increase in LV mass. Life expectancy is approximately 60 years and death is caused by renal failure, cardiovascular, or cerebrovascular disease.

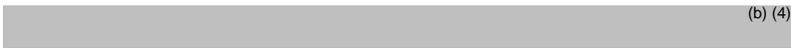
Under IND 68456 Amicus Therapeutics has developed migalastat (tradename: GALFOLD) for treatment of Fabry Disease. Amicus conducted two clinical studies intended to demonstrate that migalastat reduces kidney interstitial cell GL-3 (KIC GL-3) substrate accumulation. One study (AT1011-011) compared migalastat to placebo for 6 months in treatment naïve patients and then allowed subjects on placebo to roll over to migalastat and continued follow-up for up to an additional 18 months. The second study (AT1011-013) compared migalastat to marketed enzyme replacement therapy with agalsidase alfa or agalsidase beta for 18 months with an optional 12-month open label extension. Both studies collected echocardiographic data.

(b) (4)



**DGIEP QUESTIONS**

1. Please review and provide input





Hence [redacted] (b) (4), we do not believe the applicant has established [redacted] (b) (4).

As described in 21 CFR 201.56, the intent of a label is to provide prescribers with the information needed to safely and effectively use the drug. [redacted] (b) (4).

2. Are there any PMCs or PMRs that should be considered?

We do not suggest any PMCs or PMRs.

Thank you for requesting our input into the labeling proposed in this NDA. We welcome more discussion with you now and in the future.

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