A Novel Phase 2a Study Design to Investigate the Effect of AT2220 (Duvoglustat HCl) on the Pharmacokinetics of Acid α-Glucosidase in Subjects with Pompe Disease

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Introduction

Pompe disease is caused by mutations in the gene that encodes the lysosomal enzyme acid α-glucosidase (*GAA*), which hydrolyzes glycogen. AT2220 (1-deoxynojirimycin [DNJ] hydrochloride [HCl]) is an iminosugar that is a potent, competitive inhibitor of GAA. We have shown that AT2220 can increase GAA enzyme activity and protein processing in cell lines derived from Pompe patients and in COS-7 cells that transiently express various mutant forms of GAA. AT2220 stabilizes human recombinant GAA (rhGAA) *in vitro.* Co-administration of AT2220 and alglucosidase alfa results in dose-related increases in muscle rhGAA levels with corresponding decreases in glycogen levels in a mouse model of Pompe disease.

The current study is designed in a single ascending dose fashion with safety reviews between doses as typically performed in healthy volunteer first in human studies. The key difference however is that this study is being conducted in patients with Pompe Disease which presents unique challenges. As a healthy volunteer study can be performed in a single Phase 1 clinic with trained staff, proper equipment, housing facilities for overnight stays, *etc.*, for obtaining , processing, and shipping serial blood, urine, and tissue samples. In contrast, this study is being conducted by specialized clinicians in Pompe Disease who may require training, extra staff, and equipment to perform this type of study. Each period patients are required to have serial blood samples taken for up to 24 hours and up to 3 muscle biopsies for rhGAA activity and AT2220 concentrations. Not only are sites trained in these specialized pharmacokinetic techniques, but also for an innovative muscle biopsy technique that requires specialized investigator training. However, this unique fixed-sequence design allows each patient to serve as their own control and reduces variability compared to parallel designs that require larger sample sizes. The design is especially useful for visual comparisons of PK and safety data between treatments.

Objectives

Primary Objectives

- To evaluate the safety of single ascending oral doses of AT2220 administered 1 hour before administration of alglucosidase alfa in patients with Pompe disease
- To evaluate the effect of single ascending oral doses of AT2220 on the plasma pharmacokinetics of rhGAA

Secondary Objectives

- To assess GAA enzyme activity and protein levels in skeletal muscle at Day 3 or Day 7 following a single intravenous infusion with alglucosidase alfa alone and after pre-administration of single ascending oral doses of AT2220
- To evaluate the concentration of AT2220 in skeletal muscle on Day 3 or Day 7

Study Design and Selection Criteria

AT2220-010 is an ongoing, open-label, non-randomized, fixed-sequence, single-ascending dose study comprised of 2 periods per dose level in patients with Pompe disease.

- Period 1: IV infusion of rhGAA alone
- Period 2: AT2220 orally administered 1 hour prior to IV infusion of rhGAA (at the same dose and infusion duration as in Period 1)

Each period is separated by a minimum 14-day rhGAA dosing interval. AT2220 dose cohorts 1 – 4 (N of 4 to 6 patients per cohort) evaluated: 50 mg, 100 mg, 250 mg, and 600 mg administered as an oral solution.

Preliminary results are available for Cohorts 1 and 2 (50 mg and 100 mg) for Periods 1 and 2:

- Subjects receive their current dose and regimen of rhGAA alone as an IV infusion (approximately 20 mg/kg for 3 - 6 hrs) followed by oral AT2220 administered one hour prior to the next rhGAA infusion.
- IV infusions of rhGAA are balanced each period for dose and duration of infusion

A schematic of the study design is presented in Figure 1.

Patient Selection Criteria

- Male or female, diagnosed with Pompe disease and between 18 and 65 years of age, inclusive

Key inclusion criteria

- Subject has been on a stable regimen and dose of alglucosidase alfa for at least 3 months before screening (stable regimen defined as currently receiving alglucosidase alfa every 2 weeks and stable dose defined as not varying by more than ± 10%)
- Estimated glomerular filtration rate (eGFR) ≥ 50 mL/min at screening.

Key exclusion criteria

- A documented transient ischemic attack, ischemic stroke, unstable angina, or myocardial infarction within 3 months before screening;
- Clinically significant unstable cardiac disease;
- Subject requiring mechanical ventilation or is confined to a wheelchair
- Sensitivity to or concomitant therapy with iminosugars (*e.g.*, miglustat, miglitol)

All subjects gave written informed consent.

Bioanalytical Methods and PK Analysis

rhGAA Activity in Plasma and Muscle

rhGAA activity in plasma and muscle is measured by a fluorescence enzyme assay using 4-methylumbelliferyl-α-D-glucopyranoside (4-MUG). GAA activity using 4-MUG is measured *in vitro* following serial dilutions to minimize reassociation of AT2220. An aliquot of plasma and muscle lysate was run along with 4 methylumbelliferone (4-MU) as a reference for conversion of fluorescence counts to absolute α-Gal A activity of rhGAA protein in each sample. The analytical range of the assay is 50 - 30000 nM. The activity values obtained in plasma are reported as nmol/hr/mL. An aliquot of muscle lysate was also used to measure total protein in lysate using a colorimetric kit from Pierce with BSA standard curve. The signal (absorbance) was read on a spectrophotometer. The analytical range of the protein assay is 125 to 1800 µg/mL. In muscle lysates the activity is reported as as nanomoles of 4-MU released per milligram of protein per hour (nmol//hr/mg protein).

AT2220 in Plasma

Plasma and muscle AT2220 concentrations were determined by a validated LC/MS/MS assay. An aliquot of plasma containing the analyte and internal standard (AT-2220-$^{13}C_6$) was extracted using a solid phase extraction procedure. The extracted samples were analyzed by an HPLC equipped with an AB/MDS Sciex API 4000 mass spectrometer in APCI Positive Mode. A Tosoh Bioscience, 50x2mm, 5um analytical column was used with two mobile phases. Inter-assay variability ranged between 5.82 - 12.0% CV and 100.2 - 105.8% theoretical. Intra-assay variability ranged between 2.42 - 10.7% CV and 96.2 - 118.4% theoretical. The analytical range of the assay is 2.00 to 1000 ng/mL.

AT2220 in Muscle

An aliquot of muscle lysate containing the analyte and internal standard (AT-2220-$^{13}C_6$) was extracted using a solid phase extraction procedure. The extracted samples were analyzed by an HPLC equipped with an AB/MDS Sciex API 5000 mass spectrometer in APCI Positive Mode. A Thermo Betasil Silica-100, 50x3mm, 5um analytical column with a Metaguard Pursuit C18, 2mm, 5um guard column was used with two mobile phases. Inter-assay variability ranged between 5.71 - 10.3% CV and 98.3 - 107.9% theoretical. Intra-assay variability ranged between 2.30 - 10.7% CV and 92.4 -103.3% theoretical. The analytical range of the assay is 8.00 to 1600 ng/g.

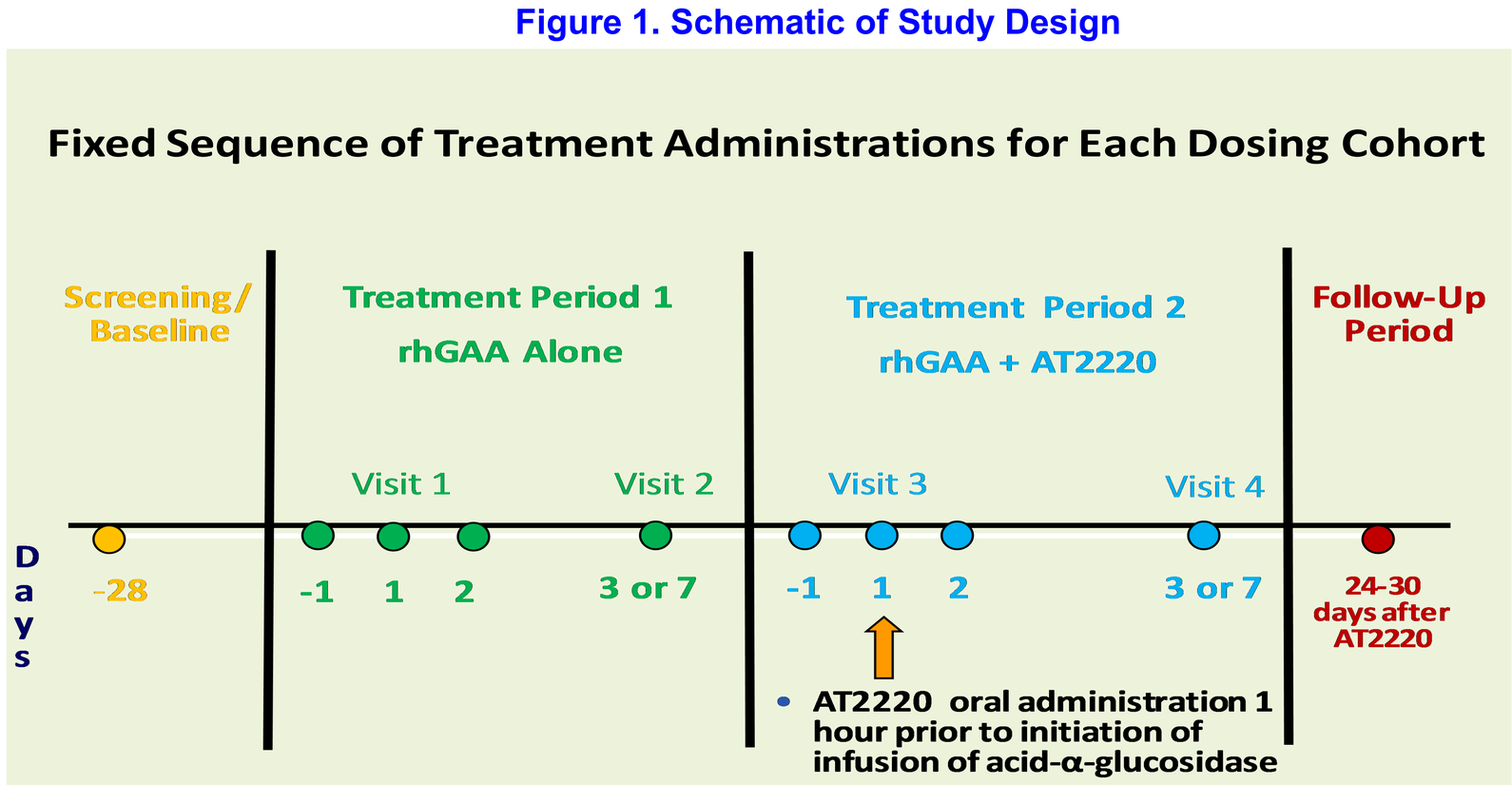
Total GAA Protein by Western Blot

Western blot analysis of GAA protein were performed on plasma samples. Plasma samples from each subject were diluted (1:2500) in Pompe Lysis Buffer (1% Triton X-100, 150 mM NaCl, 25 mM Bis-Tris, pH 6.5). Twelve µL of diluted plasma was subjected to SDS-PAGE on 12% polyacrylamide gels, transferred to PVDF membranes (Bio-Rad), and immunoblotted with rabbit anti-human GAA primary antibody (1:1000 dilution). Precursor GAA protein bands (~110 kDa) were detected using peroxidase-conjugated goat anti-rabbit secondary antibody (1:1000) in combination with enhanced chemiluminescence (Pierce, Rockford, IL). Blots were scanned on an Kodak Image Station 4000R to determine the intensity of GAA protein. An rhGAA standard curve ranging from 0.125 to 2 ng/lane was run in parallel with plasma samples on each gel to calculate the approximate concentration of GAA protein in each plasma sample. The GAA protein data in each sample was reported as nanograms of protein per mL (ng/mL) of plasma used.

Pharmacokinetic Analysis

Serial blood samples were taken each period at predose (Time 0), and at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, and 24 hours post-dose for plasma rhGAA activity and total rhGAA protein levels. During Period 2 only, serial blood samples for plasma AT2220 concentrations were taken at predose (Time 0), and at 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, and 24 hours post-dose. Plasma rhGAA activity and total rhGAA protein, and plasma AT2220 PK parameters included C_{max}, T_{max}, AUC_{0-t}, $AUC_{0-\infty}$, and T½. Pharmacokinetic parameters were calculated using standard non-compartmental procedures (WINNONLIN version 5.0 or higher). Muscle biopsies for rhGAA activity and muscle AT2220 levels were taken on Day 7 for Cohort 1 during Periods 1 and 2. Following Cohort 1, the protocol was amended to increase the sample size from 4 to 6 patients so that muscle biopsies could be taken for 3 patients each on Days 3 and 7 for Cohort 2 during Periods 1 and 2, and all remaining cohorts. The amendment also allowed for a third optional muscle biopsy per patient to be taken on Day 28 of the study for baseline assessment.

Figure 1. Schematic of Study Design



Safety Measurements and Drug Safety Monitoring Board

The safety parameters include adverse events (AEs), vital signs, clinical laboratory tests (hematology, serum chemistry, and urinalysis), electrocardiograms (ECGs), physical examinations, and use of concomitant medications. A Drug Safety Monitoring Board (DSMB) comprised of external experts in the rare disease field and the Amicus Clinical Team was chartered to monitor and evaluate the safety of all subjects in this trial by periodically reviewing summaries of safety data, evaluating risk/benefit where possible, identifying any clinically relevant signals and/or trends in each cohort, and assessing whether it was safe to continue and enroll the next sequential dose level/cohort. The DSMB convened for each cohort when data from visits 1 to 4 were available for at least 4 subjects in the cohort. Enrollment for the next cohort may only begin upon the DSMB's recommendation to proceed. Safety data reviewed included adverse events (including infusion-associated reactions), clinical laboratory tests (hematology, urinalysis, serum chemistry including creatine kinase, LDH, alkaline phosphatase, ALT, and AST), urine Hex4, 12-lead ECGs, physical examinations, vital signs, and muscle strength tests.

Based upon review of safety data by the DSMB, both cohorts were approved for advancement to the next dose level.

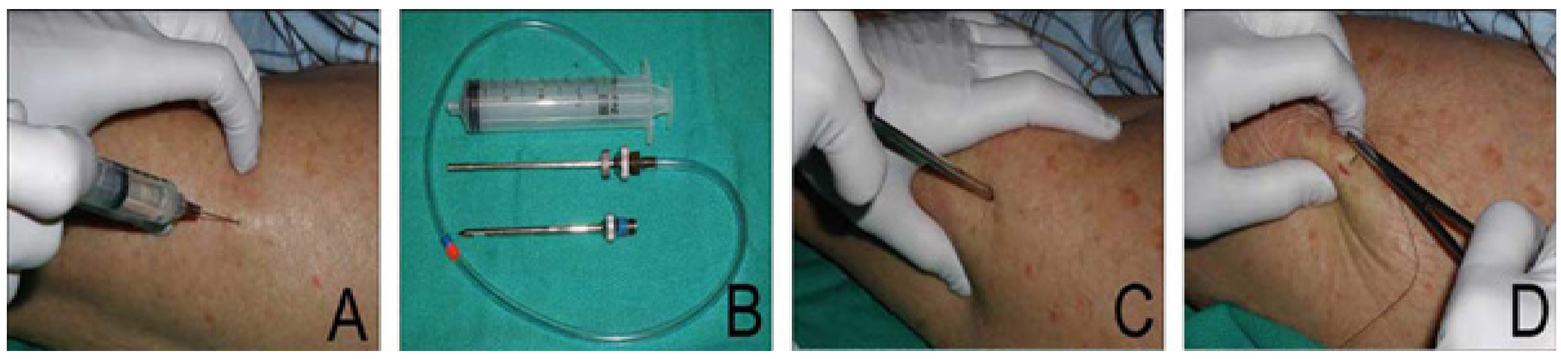
Innovative Technique for Muscle Biopsy

All participating investigators have undergone training for an innovative muscle biopsy technique. The technique was developed by Dr. Mark Tarnopolsky, who also provided on-site training.

Procedure Overview

Skeletal muscle samples from the quadriceps muscle (specifically, the vastus lateralis) by core needle biopsy were performed on Cohort 1 and 2 patients. Open muscle biopsies were not permitted. The needle biopsies were performed at Period 1, Visit 2 (**Day 3 or 7**) and at Period 2, Visit 4 (**Day 3 or 7**). The preferred location for the second biopsy to be performed is the opposite leg. If the physician feels that this location is not suitable then the second biopsy may be performed on the same muscle at both visits. Each of the muscle biopsies were performed on the same number of days post-rhGAA infusion. If the patient could not return on Day 3 or 7 in Period 1 the sample was collected on the same day in Period 2, however, all patients have had their biopsies taken on the predefined study day. At both visits the muscle biopsies were performed after all assessments have been completed. At each study center, a qualified physician was responsible for performing the core needle muscle biopsies. For consistency, all patients at a particular site had the procedure performed by the same experienced physician. Ideally, a minimum of 150 mg of muscle tissue should have been obtained to provide sufficient material for rhGAA enzyme activity, rhGAA protein level, and AT2220 tissue levels. In the literature, several modifications to the standard muscle biopsy needle procedure aimed at increasing sample size and quality have been described *(Dietrichson et al, 1987; Bourgeois and Tarnopolsky, 2004; Melendez et al, 2007; Tarnopolsky, 2011).* The recommended instruments for obtaining muscle samples in this study are the modified (*Tarnopolsky, 2011*) Bergstrom muscle biopsy needle (5 mm) or the U.C.H. skeletal muscle biopsy needle (5.0 mm OD), both of which consist of a hollow outer cannula with a lateral window near the tip, and a hollow, sharpened inner trocar that serves to cut muscle that protrudes through the window. NOTE – suction must be applied (Fig 2).

Figure 2. Core Needle Muscle Biopsy Procedure



(A) Anesthesia with 2% lidocaine (or equivalent), do not infiltrate the muscle; (B) Modified Bergstrom needle with airtight custom-made machined slip fit tap and die screw-on apparatus (including a disposable Teflon O-ring) and 60 mL syringe as a suction apparatus; (C) Introduction of needle. Once inside the muscle, the trocar is opened and suction is applied and then the trocar is closed; (D) Suture or sterile tape post-muscle biopsy. (Bourgeois and Tarnopolsky, 2004)

Discussion of Study Design and Methods

Single dose, Phase 2a, proof of concept clinical studies typically employ a randomized, placebo-controlled design. However, in rare genetic disorders, as in oncology, randomization and blinding becomes impractical due to the severity of the disease. An open-label, fixed sequence design allows for immediate evaluation of the data as well as each patient serving as their own control, in this case evaluation of rhGAA activity after enzyme replacement by itself and after co-administration with the pharmacological chaperone, AT2220. Additionally, parallel study designs require power calculations in order to detect a statistically significant difference between treatments. But in rare diseases, sample sizes are extremely limited, as patients are difficult to find, and in such a study as this, may be reluctant to participate. As such, the fixed-sequence, 2-period crossover provides direct, within-patient comparison of the data with minimal statistical analysis.

Of primary importance in this study is the evaluation of AT2220 dose-response in terms of increases of active rhGAA and subsequent distribution to muscle tissue. Bioanalysis of rhGAA activity is performed in two steps. First, reassociation of the chaperone to the active site of the enzyme is minimized by serial dilutions. Next, the rate of turnover of the artificial fluorescent substrate, 4-MUG to MU, is measured. The dilution that provides the highest value within the analytical linear range is selected for each time point for non-compartmental PK analysis. With an open-label design, sample analysis can proceed as each cohort completes allowing for immediate evaluation of the data. The Western Blot determination of total rhGAA protein levels along with rhGAA activity at each time point allow for estimation of the effect of a particular dose of AT2220 for stabilizing rhGAA and therefore, increasing the level of active enzyme for tissue distribution. Additionally, an evaluation of clearance of rhGAA by rhGAA-antibodies and subsequent increase in of active enzyme due to stabilization of rhGAA by AT2220 is under investigation.

Paired muscle biopsies on either Day 3 or 7 of each period also allows for within-patient comparison of rhGAA activity in muscle tissue with and without AT2220. An exposure-response comparison (along with measurement of muscle AT2220 levels), allows for selection of the appropriate dose(s) for evaluation in a multiple-dose, dose-ranging follow-up study.

Although this single dose study offers no immediate benefit to patients with Pompe Disease, the study design provides for a fast turn-around of data to allow optimization of dose selection and planning for follow up multiple dose, dose-ranging, and follow-on Phase 3 studies. As shown in the adjacent poster on preliminary data for Cohorts 1 and 2, the results are encouraging for an improved ERT. A large part of the potential success of this clinical development program depends on the willingness and enthusiasm of the patients who choose to participate in these clinical trials. We at Amicus offer our sincere gratitude to them, their caretakers, and the physicians in their care.

Disclosure Statement: Authors footnoted #1 – 11 above are currently participating investigators in the ongoing clinical trial, AT2220-010. Authors footnoted #12 are full-time employees of Amicus Therapeutics and are stockholders.