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Duvoglustat HCl Increases Systemic and Tissue Exposure of Active Acid α-Glucosidase in Pompe Patients Co-administered with Alglucosidase α

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Duvoglustat HCl (AT2220, 1-deoxynojirimycin) is an investigational pharmacological chaperone for the treatment of acid α-glucosidase (GAA) deficiency, which leads to the lysosomal storage disorder Pompe disease, which is characterized by progressive accumulation of lysosomal glycogen primarily in heart and skeletal muscles. The current standard of care is enzyme replacement therapy with recombinant human GAA (alglucosidase alfa [AA], Genzyme). Based on preclinical data, oral co-administration of duvoglustat HCl with AA increases exposure of active levels in plasma and skeletal muscles, leading to greater substrate reduction in muscle. This phase 2a study consisted of an open-label, fixed-treatment sequence that evaluated the effect of single oral doses of 50 mg, 100 mg, 250 mg, or 600 mg duvoglustat HCl on the pharmacokinetics and tissue levels of intravenously infused AA (20 mg/kg) in Pompe patients. AA alone resulted in increases in total GAA activity and protein in plasma compared to baseline. Following co-administration with duvoglustat HCl, total GAA activity and protein in plasma were further increased 1.2- to 2.8-fold compared to AA alone in all 25 Pompe patients; importantly, muscle GAA activity was increased for all co-administration treatments from day 3 biopsy specimens. No duvoglustat-related adverse events or drug-related tolerability issues were identified.

INTRODUCTION

Pompe disease, also referred to as glycogen storage disorder type II or acid maltase deficiency, is a lysosomal storage disorder (LSD) caused by mutations in the *GAA* gene that encodes the lysosomal hydrolase acid α-glucosidase (GAA).[1,2] Deficiency of GAA activity results in progressive accumulation and deposition of glycogen in the lysosomes of heart, skeletal muscles, and other tissues. The disease encompasses a broad spectrum of phenotypes that range from severe classic infantile Pompe disease to the more slowly progressing late-onset form.[2–5] Late-onset Pompe disease (LOPD) can present as early as the first year of life to adulthood, has a slower rate of progression than the infantile-onset form, and is typically characterized by musculoskeletal and pulmonary involvement that leads to progressive weakness and respiratory insufficiency.[1,3–5] Cardiac involvement can occur in LOPD as well.[4]

Enzyme replacement therapy (ERT) is currently the primary treatment for Pompe disease.[6] ERT is based on the intravenous administration of recombinant human GAA (rhGAA), of which Myozyme and Lumizyme (alglucosidase alfa [AA]; Genzyme) are the only two approved products. Although infantile and late-onset Pompe patients have shown some improvements and stabilization in motor and respiratory functions following therapy with ERT, residual disease persists, suggesting that ERT is not completely effective in clearing glycogen and correcting all of the associated underlying pathologies.[7,8] Despite the clinical benefits of ERT, correction of the skeletal muscle phenotype is particularly challenging, and not all patients

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Table 1. Patient Disposition and Demographic Characteristics by Dose Cohort and Overall

	Duvoglustat HCl Dose Cohort Co-administered with 20 mg/kg AA				
Characteristic	50 mg (n = 6)	100 mg (n = 6)	250 mg (n = 6)	600 mg (n = 7)	Total (n = 25)
Sex (no. of patients)	3 males, 3 females	3 males, 3 females	2 males, 4 females	5 males, 2 females	13 males, 12 females
Race (no. of patients)	6 white	6 white	6 white	7 white	25 white
Mean (SD) age (years)	47.7 (5.35)	52.8 (5.71)	50.0 (12.9)	40.0 (6.61)	47.3 (9.13)
Mean (SD) BMI (kg/m^2)	27.5 (5.41)	29.7 (5.40)	24.8 (5.44)	28.3 (7.58)	27.6 (5.99)

respond equally well to treatment.[2,7,9,10] These limitations are at least partially due to insufficient targeting/uptake into disease-relevant tissues, as well as poor tolerability due to severe ERT-mediated anaphylactic and immunologic reactions in a small subset of patients.[5,8,11–15]

An approach that has recently attracted much interest for the treatment of LSDs uses small-molecule pharmacological chaperones (PCs). Results from in vitro and in vivo studies suggest that a substantial fraction of AA provided as ERT denatures in the blood and is rapidly cleared by the liver and other organs, resulting in poor targeting to muscle cells and thus lack of effectiveness.[13,16] It was shown that interaction of different ERTs with respective PCs can enhance, at least in part, enzyme stability.[13,16] To this end, studies in rats and *Gaa* knockout mice indicate that oral pre-administration of duvoglustat HCl increases the circulating half-life of AA and leads to significant increases in total GAA activity in disease-relevant tissues.[17] Duvoglustat-mediated increases in total GAA tissue levels translated to greater glycogen reduction than administration of AA alone, thus indicating an increase in the net lysosomal activity from the exogenous recombinant enzyme. Taken together, these data indicate that duvoglustat HCl can increase the stability and improve the pharmacokinetic (PK) properties of AA, thereby leading to increased tissue enzyme activity and greater substrate reduction.[17]

Significant increases in plasma rhGAA activity area under the plasma drug conentration-time curve (AUC) following co-administration with miglustat were observed in 13 Pompe patients. These results suggested improved stability of rhGAA in blood in the presence of a pharmacological chaperone.[18]

The PK of duvoglustat HCl has been well characterized in four phase 1 studies conducted in healthy volunteers. Duvoglustat was well absorbed in 2–3 hr; however, the exposures were less than dose proportional over a dose range of 50–2,500 mg. Terminal half-life varied between studies (range: 4.5–14 hr between 50 mg and 600 mg) and was dependent on the duration of the terminal phase blood sampling (range: 24–168 hr).

The rationale for this study was to provide drug-drug interaction information after co-administration of a single oral dose of duvoglustat HCl administered 1 hr prior to initiation of infusion of AA in adults with LOPD. In addition, information on the effect of duvoglustat HCl on AA was obtained for proof of concept that duvoglustat HCl has the potential to improve the PK properties of AA in Pompe patients. Four dose levels of duvoglustat HCl were co-administered with 20 mg/kg AA in this study: 50 mg, 100 mg, 250 mg, and 600 mg. These doses were selected based upon the estimated clearance rates of duvoglustat from plasma and muscle tissue to concentrations that are below the limit of quantification prior to the end of 14-day dosing interval of AA from a phase 1 clinical study conducted in healthy volunteers and from maximal glycogen clearance at the translated human dose of 600 mg estimated in the mouse model.[17,19] Increased GAA activity in blood and tissue as a result of co-administration with the pharmacological chaperone, duvoglustat HCl, may provide improved clinical benefit compared to ERT alone. However, since this was a single-dose study, patients participating in this trial were not expected to gain any additional therapeutic benefit from co-administration of duvoglustat HCl with ERT.

RESULTS

Demographics

A summary of the patient disposition and demographic characteristics is presented in Table 1. A total of 25 Pompe disease patients (13 males and 12 females) were enrolled into the study. At least six patients were assigned to each of the four duvoglustat HCl dose cohorts in a single-ascending dose fashion. All 25 patients completed the study. There were no clinically meaningful differences between dose cohorts for age, race, sex, or BMI. Generally, clinical characteristics of the Pompe population in this study were similar between duvoglustat HCl dose groups.

Total GAA Activity and Protein in Plasma

Generally, greater increases in total plasma GAA activity following co-administration of AA with duvoglustat HCl relative to AA administered alone started to occur during the infusion phase, with greater peaks at the end of infusion. During the terminal elimination phase, the rate of decline was longer following co-administration with duvoglustat HCl than following administration of AA alone (Figure 1).

When comparing administration of AA alone to co-administration with duvoglustat HCl, plasma $AUC_{0-\infty}$ of total GAA enzyme activity increased for all 25 Pompe disease patients (100%) (Figure 2). Increases in total GAA activity exposure were duvoglustat-HCl-dose related, ranging from 1.5- to 2.1-fold relative to AA alone, based upon mean $AUC_{0-\infty}$ ratios (Table 2).

The observed maximum drug concentration (C_{max}) for total plasma GAA activity following co-administration with duvoglustat HCl

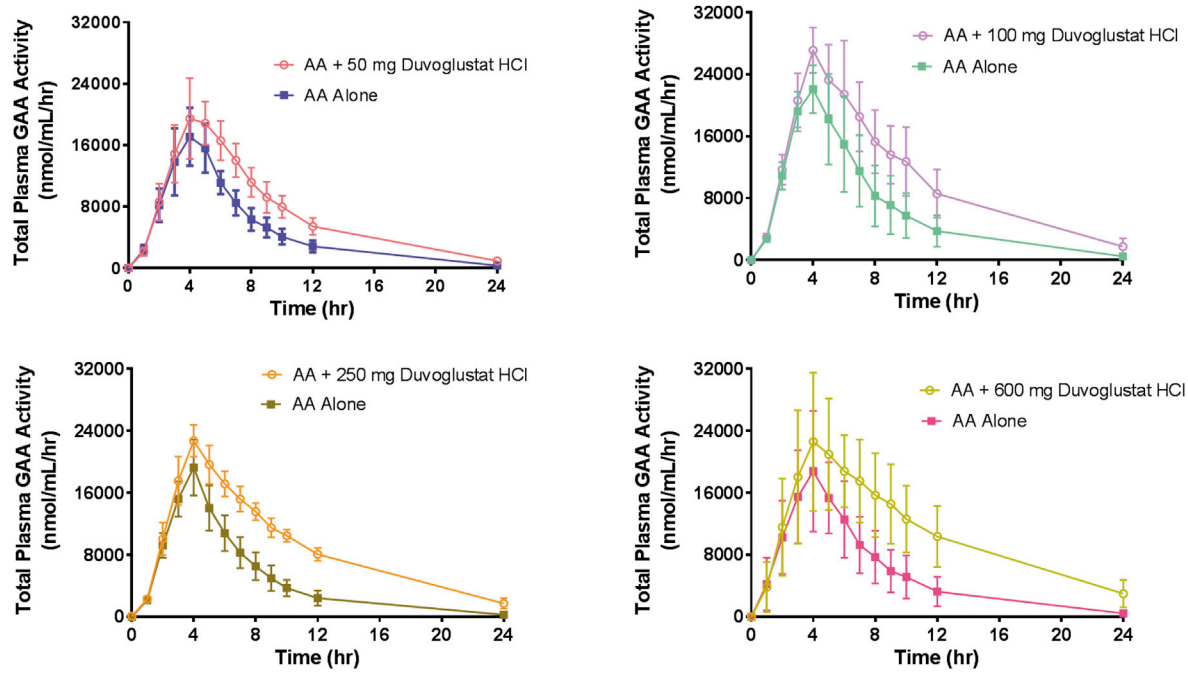

Figure 1. Mean and SD Total Plasma GAA Activity-Time Profiles for All Treatments

increased for 24 of 25 Pompe patients (96%) relative to when AA was administered alone. Mean duvoglustat-mediated relative increases in C_{max} of ~19%–26% were observed across all doses of duvoglustat HCl. However, these increases were not dose related. Median observed time of maximum drug concentration (t_{max}) values for total plasma GAA activity were generally consistent with the duration of the infusion of AA (4 hr) (Table 2). Mean terminal half-life values were similar (~3.8 hr) for AA alone (Table 2); however, terminal half-life increased in a dose-related manner from 4.4 hr to 6.3 hr following co-administration with duvoglustat HCl.

Because of the small sample sizes, C_{max} and AUC statistical comparisons between each duvoglustat HCl co-administration dose group and AA alone were not performed.

In addition to total GAA activity, total GAA protein was measured in plasma by western blot analysis. Generally, increases in total plasma GAA protein followed a similar pattern to total GAA activity. Following co-administration, quantifiable total plasma GAA protein concentrations were observed during the infusion phase and peaked at the end of infusion. During the terminal elimination phase, the rate of decline was longer for co-administration with duvoglustat HCl than for administration of AA alone (Figure S1).

When evaluating exposures (AUCs) of total GAA protein, the extrapolated areas of $AUC_{0-\infty}$ were greater than 50% of the areas to the last measurable time point (AUC_{0-t}). Therefore, AUC_{0-t} was the selected exposure parameter for evaluation of total plasma GAA protein exposures. When comparing administration of AA alone to co-administration with duvoglustat HCl, plasma AUC_{0-t} of total GAA protein increased for all 25 Pompe patients (100%) (Figure S2).

Increases in total GAA protein exposures also were duvoglustat-HCl-dose related, ranging from 1.5- to 2.3-fold relative to administration of AA alone, based upon mean AUC_{0-t} ratios (Table S1). The C_{max} for total plasma GAA protein following co-administration of duvoglustat HCl increased in 23 of 25 Pompe disease patients (92%) relative to when AA was administered alone. Mean duvoglustat-mediated relative increases in C_{max} values of ~17%–26% were observed across all doses of duvoglustat HCl. However, these increases were not dose related. Variability in C_{max} and AUC, as measured by coefficient of variation (CV)%, ranged from 7.0% to 68.2% (Table S1). Median t_{max} values for total plasma GAA protein were generally consistent with the duration of the infusion of AA (4 hr) (Table S1). Mean terminal terminal half-life values were similar (approximately 2.5 hr) for AA alone (Table S1); however, mean terminal half-life increased for all doses from 4.4 hr to 5.8 hr following co-administration with duvoglustat HCl.

PK of Plasma Duvoglustat

Dose-related increases in mean (SD) plasma duvoglustat concentration-time profiles were observed following co-administration of AA with 50 mg, 100 mg, 250 mg, and 600 mg duvoglustat HCl (Figure 3).

Median t_{max}, when examined across the four treatment cohorts, occurred 1–3 hr after dosing for most subjects. Mean terminal half-life of plasma duvoglustat was ~3.5 hr for all treatment cohorts (Table 3). Plasma duvoglustat exposure ($AUC_{0-\infty}$ and AUC_{0-t}) and

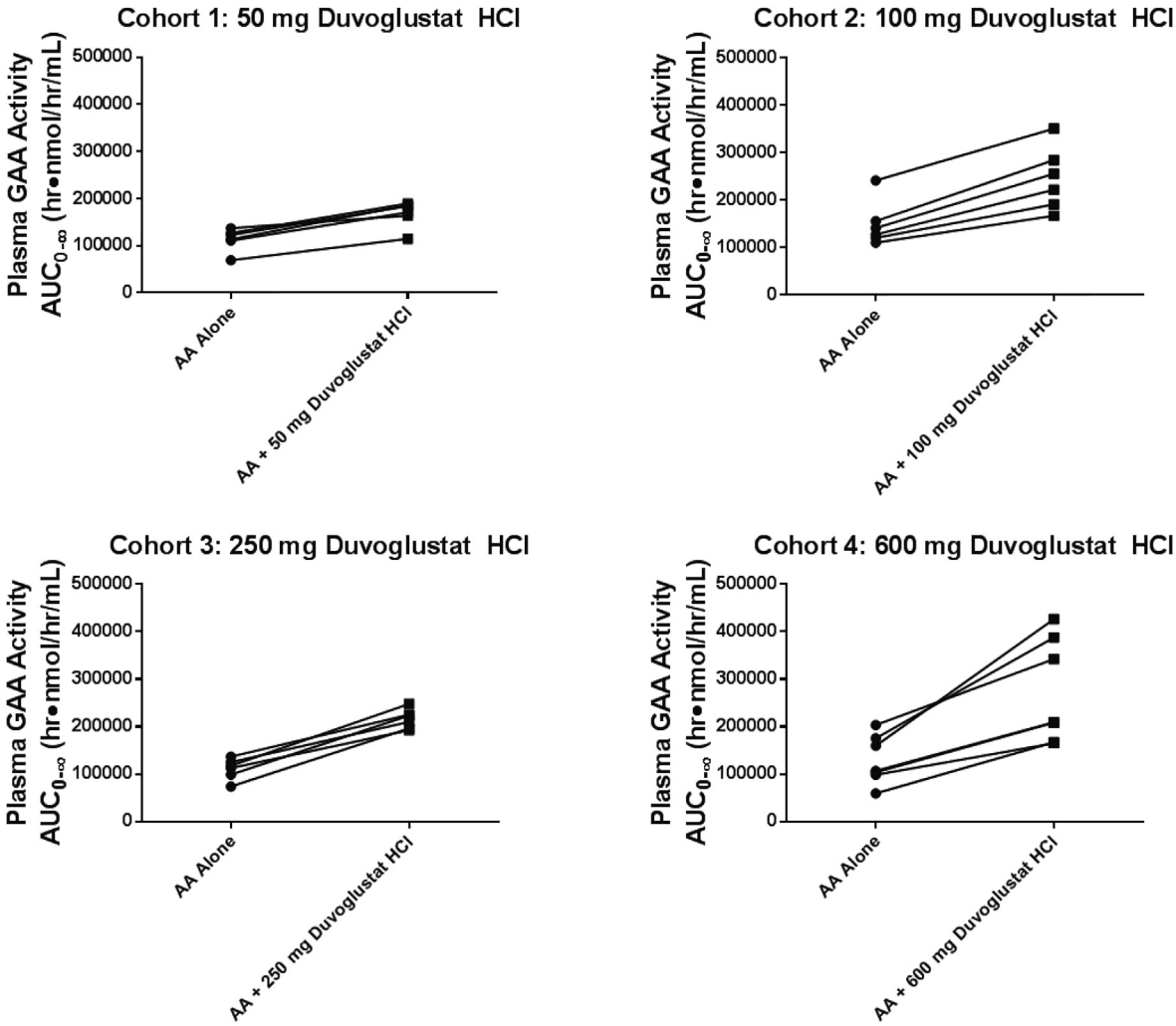

Figure 2. Two-by-Two Panel of Total Plasma GAA Activity AUC Stick Plots for AA Alone and Co-administered with Duvoglustat HCl

peak exposure (C_{max}) increased less than proportionally with dose (Table 3). The upper limit of the 90% confidence intervals (CIs) for each tested parameter was less than 1, which indicates that increases in total and peak exposure of plasma duvoglustat were less than dose proportional over the dose range of 50–600 mg.

Muscle Biopsies: Total GAA Activity and Duvoglustat Concentrations

23 out of 25 patients had evaluable treatment-matched (AA alone and AA co-administered with duvoglustat HCl) muscle biopsy samples on either day 3 or day 7. The reasons for unevaluable biopsy specimens included insufficient sample quantity to measure total GAA activity or the sample was mislabeled at the clinical site.

Although co-administration of AA with 50, 100, 250, or 600 mg duvoglustat HCl resulted in increased AA activity for day 3 muscle biopsies and 100, 250, and 600 mg duvoglustat HCl for day 7 muscle biopsies, there were no clear dose-related trends for either study day (Table 4). For example, following day 3 muscle biopsies, increases in total GAA activity relative to AA alone were 38.5%, 20.5%, 4.7%, and 43.6% for AA co-administered with 50, 100, 250, and 600 mg, respectively. As a result of small sample sizes (n = 2–4), variability in total muscle GAA activity, as expressed by CV%, was high (CV% >50%) for some treatments. Again, the increases were not duvoglustat-dose related. The range of individual patient muscle GAA activity ratios (rhGAA + duvoglustat HCl vs. rhGAA alone) were 0.3 to >7 (one patient had a muscle GAA activity below the limit of quantification [BLQ] after receiving rhGAA alone and 2,091 pmol/mg/hr after receiving rhGAA + 50 mg duvoglustat HCl).

Nine patients had a third optional muscle biopsy taken on days 28–42 post-administration of period 2. Eight patients had evaluable total GAA activity levels. One patient's biopsy sample had low protein levels and was unevaluable. The mean (CV%) total GAA activity level

Table 2. Summary of Total Plasma GAA Activity Pharmacokinetic Parameters by Treatment

Treatment	Cohort	Period	C_{max}[a] (nmol/hr/mL)	t_{max}[b] (hr)	AUC_{0-t}[a] (hr × nmol/hr/mL)	$AUC_{0-\infty}$[a] (hr × nmol/hr/mL)	AUC Ratio[a]	Terminal Half-Life[c] (hr)
AA alone (n = 6)	1	1	17,269 (25.6)	4.7 (4–5)	108,578 (24.1)	110,388 (24.5)	—	3.8 (12.7)
AA + 50 mg duvoglustat HCl (n = 6)	1	2	20,539 (21.2)	4.5 (4–6)	159,994 (18.1)	165,983 (19.1)	1.5 (12.0)	4.4 (16.9)
AA alone (n = 6)	2	1	22,785 (18.1)	4.0 (3–5)	141,515 (27.9)	144,056 (28.6)	—	3.8 (17.7)
AA + 100 mg duvoglustat HCl (n = 6)	2	2	28,607 (14.1)	4.0 (3–6)	226,198 (25.4)	237,613 (27.6)	1.7 (9.8)	4.8 (14.2)
AA alone (n = 6)	3	1	18,986 (19.5)	4.0 (4–4)	107,489 (21.7)	108,862 (22.1)	—	3.6 (13.7)
AA + 250 mg duvoglustat HCl (n = 6)	3	2	22,651 (9.0)	4.0 (4–4)	201,044 (8.3)	215,276 (9.5)	2.0 (19.7)	5.4 (26.0)
AA alone (n = 7)	4	1	18,628 (34.6)	4.0 (3–6)	118,483 (43.1)	120,604 (44.3)	—	3.7 (19.9)
AA + 600 mg duvoglustat HCl (n = 7)	4	2	22,505 (36.4)	4.0 (3–6)	228,413 (38.4)	254,074 (42.0)	2.1 (20.8)	6.3 (22.8)

[a]Geometric mean (CV%).
[b]Median (range).
[c]Arithmetic mean (CV%).

for the optional muscle biopsy specimens was 612 (44.5) pmol/mg/hr. Since these samples were taken at trough or some recent time before initiation of the next AA infusion, the values were low relative to the day 3 and day 7 muscle biopsy total GAA activity levels. Consideration was given to utilizing the optional biopsy value as a baseline; however, the time of biopsy relative to the next infusion could not be confirmed for all samples.

The concentration of duvoglustat was determined in skeletal muscle tissue homogenate following co-administration of AA with duvoglustat HCl in treatment period 2. Bioanalysis for muscle duvoglustat concentration was performed on 26 samples from 20 patients (6 patients had a second, optional biopsy on days 28–42). Of these, 12 had quantifiable muscle duvoglustat concentrations ranging from 10.0 to 83.6 ng/g and 14 had concentrations BLQ (<8 ng/g). Muscle duvoglustat concentrations that were BLQ were set to zero for the statistical calculations presented in Table S2. Based on the observed mean concentrations for each duvoglustat HCl dose level, dose-related increases in concentrations were generally observed on day 3 and day 7. Muscle duvoglustat concentrations generally decreased from day 3 to day 7; all day 28 to day 42 samples were BLQ.

Safety Data

Of the 25 Pompe patients enrolled in the study that received AA alone or co-administered with duvoglustat HCl, 70 treatment-emergent adverse events (TEAEs) were reported in 16 patients (64%) during the study. For patients who had at least one AE during the study, TEAEs were evenly distributed across the four treatment groups in both periods of the study. No TEAE resulted in discontinuation from study treatment. All TEAEs were either unrelated or unlikely to be related to study medication. Most of the TEAEs (53) were mild in severity, 13 were considered moderate, and 4 were considered severe (constipation, back pain, neck pain, and urinary incontinence). No deaths were reported during the study; however, one patient had a TEAE that was serious. The serious TEAE was an incident of QT prolongation of 20 ms (473–493 ms) that was considered unlikely related to study drug. The patient had a normal electrocardiogram (ECG) result at screening and at baseline; however, all subsequent ECG results, throughout periods 1 and 2 were also abnormal, but not clinically significant. Relevant medical history included heart valve incompetence and hypertension. The patient was receiving benazepril HCl 40 mg for hypertension. No treatment for this event was reported, and no action was taken with the study drug. The event resolved without sequelae.

Clinical laboratory evaluations, ECGs, vital signs, and physical examinations were unremarkable during the study.

Results for exploratory evaluations for anti-GAA antibodies, urine Hex4 concentrations, and manual muscle strength tests were not clinically different from period 1 to period 2. Differences between AA alone and AA with single doses of duvoglustat HCl were not anticipated from a single-dose study.

DISCUSSION

In the current phase 2 study, the ability to extend our preclinical proof-of-concept findings for co-administration of AA with duvoglustat HCl was explored for the first time in Pompe disease patients. The primary objective was to determine whether a single dose of duvoglustat HCl co-administered with ERT is safe and whether it can increase the exposure ($AUC_{0-\infty}$) of AA (as measured by total active GAA, total GAA protein levels in plasma, and total active GAA in muscle), an indication of a positive drug-drug interaction.

Co-administration of duvoglustat HCl at doses of 50 mg, 100 mg, 250 mg, or 600 mg with AA was generally well tolerated. No deaths or discontinuations of study treatment due to TEAEs were reported. Overall, all treatment-related adverse events were either unlikely related or unrelated to study drug. When compared to phase 1 studies conducted in healthy volunteers at similar doses in the current study, there was no trend toward the incidence of any particular adverse event.

As primary objectives of the study, the effect of plasma duvoglustat on AA was evaluated by comparing 24-hr exposures (AUC) of total GAA activity and protein following co-administration with AA and

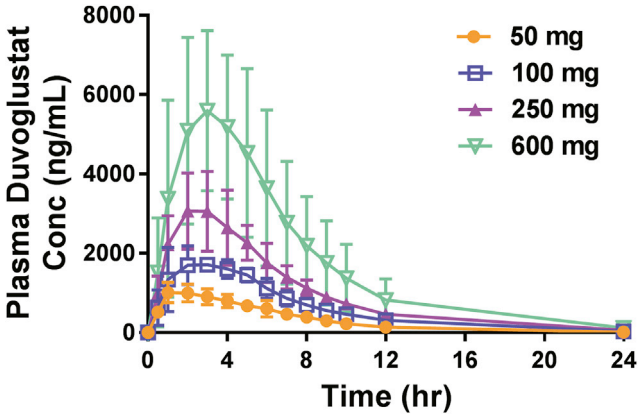

Figure 3. Mean and SD Plasma Duvoglustat Concentration-Time Profiles following Co-administration of AA with 50 mg, 100 mg, 250 mg, or 600 mg Duvoglustat HCl

duvoglustat HCl relative to AA administered alone. All dose levels of duvoglustat HCl resulted in ~2-fold increases in total plasma GAA activity and protein exposures following co-administration relative to AA administered alone. Incremental increases in mean total GAA activity and protein exposures with increasing duvoglustat HCl doses were small but clearly dose related. This is borne out when observing individual total GAA activity and protein AUCs; the higher doses of 250 mg and 600 mg duvoglustat HCl co-administered with AA resulted in the greatest individual increases in total GAA activity and protein exposures relative to AA alone. Likewise, co-administration of duvoglustat HCl with AA increased total plasma GAA activity and protein C_{max} values for over 90% of patients. Increases in mean C_{max} values for total plasma GAA activity and protein were similar (~20%) for each duvoglustat HCl co-administration dose level relative to AA alone. However, mean total plasma GAA activity terminal half-life demonstrated duvoglustat dose-related increases of 15.8%, 26.3%, 50.0%, and 70.3% for 50 mg, 100 mg, 250 mg, and 600 mg, respectively, relative to the total GAA activity alone terminal half-life. Mean plasma total GAA protein terminal half-life demonstrated duvoglustat dose-related increases of 33.3%, 152%, 121%, and 149% for 50 mg, 100 mg, 250 mg, and 600 mg, respectively, relative to AA alone terminal half-life. The dose-related increases in terminal half-life suggest that increases in overall total GAA activity and protein exposures were driven mainly by longer half-life than peak total GAA activity and protein levels. The results of a longer half-life with co-administration can be observed in Figure 1 as increased AUC during the terminal elimination phase (i.e., post-C_{max}) relative to AA alone. These data indicate that duvoglustat HCl co-administration can increase the exposure of both active total GAA and total GAA protein in the circulation. These increases suggest greater stabilization of GAA in plasma that may result in less immunogenicity and provide more active GAA for increased uptake into tissues.

The secondary objectives of this study were to characterize the effects of co-administration of duvoglustat HCl on the distribution (uptake) of active GAA to skeletal muscle tissue and to characterize the plasma and muscle pharmacokinetics of duvoglustat. The majority (16 out of 24 [66.7%]) of patients demonstrated greater levels of active GAA in muscle on day 3 or day 7 following co-administration with duvoglustat HCl relative to AA administration alone, suggesting greater uptake of duvoglustat-mediated rhGAA into muscle tissue. Due to the small sample sizes, statistically significant differences between cohorts could not be achieved; however, the overall duvoglustat-mediated increases of active GAA in muscle are quite consistent given the variability introduced by differing tissue sample weights, protein content, and dilution factors for homogenate preparation. An average relative increase of up to 2.0-fold ([duvoglustat + AA] / AA alone) in active GAA muscle levels was seen in patients who had a muscle biopsy on day 3 following administration of any dose of duvoglustat HCl in combination with AA. By day 7, co-administration with duvoglustat HCl maintained muscle GAA activity levels 1.2-fold greater on average (10 of 13 remained elevated) than when AA was administered alone.

Although dose-related increases in plasma duvoglustat exposures were observed, they were less than dose proportional over the evaluated dose range of 50 mg to 600 mg. The dose ratios relative to the lowest dose were 2× (100 mg/50 mg), 5× (250 mg/50 mg), and 12× (600 mg/50 mg). The exposure ratios relative to the 50 mg dose were 1.9-fold (100 mg/50 mg), 3.1-fold (250 mg/50 mg), and 5.5-fold (600 mg/50 mg) for AUC and, similarly, 1.8-fold (100 mg/50 mg), 3.0-fold (250 mg/50 mg), and 5.7-fold (600 mg/50 mg) for C_{max}. It is readily apparent that exposures steadily decrease relative to proportional increase in dose to approximately half of a dose-proportional exposure at the highest dose (600 mg). The relative decreases in exposure with proportional increase in dose observed in these Pompe disease patients is consistent with characterization of exposures in healthy volunteers from phase 1 studies up to a dose of 2,500 mg, a 50-fold increase from the 50-mg dose. This resulted in only a 13-fold increase in AUC, ~25% of a dose-proportional exposure. The exposure data suggest that a saturation mechanism may be limiting absorption. Duvoglustat HCl is highly soluble (700 mg/mL across a pH range of 1.2 to 9.0). Two possible routes of absorption are passive diffusion and, being a highly polar iminosugar, active transport via sodium-glucose transporters. Duvoglustat HCl is known to inhibit the gastrointestinal (GI) disaccharidases isomaltase and sucrase in solubilized Caco-2 cell membranes. Previous studies with duvoglustat HCl and other potent inhibitors of intestinal α-glucosidases have been shown to suppress post-prandial elevations in blood glucose levels.[23] Whether the cause of limited absorption with increasing dose is due to poor permeability and/or competitive binding with digestive enzymes and sodium-glucose transporters in the gut is not known.

Sustained elevated concentrations of duvoglustat in muscle tissue have the potential to inhibit GAA activity if not cleared before the following co-administration with AA. Due to the standard-of-care regimen of biweekly administrations of AA, it is necessary to clear duvoglustat from muscle tissue before day 14 post-dose. The

Table 3. Plasma Duvoglustat PK Summary and Dose Proportionality Analysis

Duvoglustat HCl Dose (n)	C_{max}[a] (ng/mL)	t_{max}[b] (hr)	AUC_{0-t}[a] (hr × ng/mL)	$AUC_{0-\infty}$[a] (hr × ng/mL)	Terminal Half-Life[c] (hr)
50 mg (6)	1060 (20.7)	1.08 (0.98, 2.05)	7,073 (24.0)	7,122 (24.1)	3.26 (6.0)
100 mg (6)	1870 (14.1)	2.49 (2.00, 4.00)	1 3,136 (20.8)	1 3,271 (21.0)	3.40 (9.9)
250 mg (6)	3150 (34.0)	2.02 (1.97, 3.00)	2 1,693 (18.1)	2 2,010 (17.5)	3.67 (14.4)
600 mg (7)	6050 (38.8)	3.00 (2.00, 5.00)	3 8,490 (40.9)	3 9,068 (41.1)	3.73 (9.9)
Beta (90% CI)	0.69 (0.59, 0.78)	—	0.66 (0.57, 0.76)	0.67 (0.57, 0.76)	—

[a]Geometric mean (CV%).
[b]Median (minimum, maximum).
[c]Arithmetic mean (SD).

observed dose-related increases in plasma GAA activity AUC are incremental and flatten at the 250-mg dose of duvoglustat, and increases are not dose proportional. This flattening effect reflects the attainment of the most effective molar concentration ratio of duvoglustat to GAA. Being a small molecule, even the lowest dose of duvoglustat administered in the study (50 mg) has a high molar ratio to 20 mg/kg GAA. Once a maximal ratio is attained, which provides optimal binding to GAA with potentiated stabilization from plasma to muscle, further increases in GAA activity levels can no longer be observed. Lack of an inhibitory effect may be suggested in muscle PK data. Muscle GAA activity levels attained were highest at the 600-mg dose of duvoglustat on day 3 (Table 4). Based on muscle PK, 600 mg duvoglustat appears to be the optimal dose to ensure stabilization in plasma with maximum uptake to muscle tissue. Co-administration with comparable doses in the knockout (KO) mouse model achieved the greatest glycogen-lowering effect in muscle.[24] The highest muscle duvoglustat concentrations were recorded following administration of 600 mg duvoglustat HCl from a day 3 sample, 83.6 ng/g, and a day 7 sample of 64.8 ng/g. Both of the Pompe disease patients with these muscle duvoglustat concentrations had follow-up biopsy results (~28 days after duvoglustat HCl administration) that were BLQ. The limit of quantification in muscle tissue was 8 ng/g, which is similar to the Ki value. Modeling of the day 3 and day 7 concentrations suggests that both would be BLQ by day 14; therefore, accumulation of muscle duvoglustat would be negligible in a multiple dosing scenario. The data from this study appear sufficient to warrant further investigation for multiple dose administrations at or below 600 mg duvoglustat HCl.

The observed increases in total GAA activity levels in plasma of this study provided additional proof of concept for co-administration of duvoglustat HCl with AA in Pompe disease patients, consistent with observed increases in plasma rhGAA activity from co-administration with miglustat,[18] and confirmed preclinical findings.[17] Further, muscle biopsy specimens with observed increases in muscle total GAA activity provided novel aspects that support proof of concept for co-administration. Overall, it can be concluded that the pharmacological consequences for the duvoglustat HCl doses evaluated in this study were improved exposure of active total GAA in plasma and greater levels of active enzyme in skeletal muscle.

MATERIALS AND METHODS

Prior to patient enrollment, each investigator provided written institutional review board or ethics committee approval to conduct the study at each study center, and at the time of enrollment, all study patients provided written informed consent before partaking in any study procedure. The following institutional review boards and ethics committees, including trial registration number with site-associated investigator's initials, provided approval prior to conduct of this clinical trial: Western Institutional Review Board 1129606 (P.K.), 1128173 (K.S.), 1128363 (O.G.A.), 1129130 (K.A.G.), 1127077 (T.L.), and 1129080 (B.B.); Hamilton Health Sciences/McMaster Health Sciences 11-488 (M.T.); North West Liverpool Central Centre of Research Ethics Committee 11/NW/0745 (M.R. and R.L.); University of Kansas Medical Center Human Subjects Review Board 12978 (M.D.); Oregon Health & Sciences University Institutional Review Board 00008174 (E.F.); University of California, Irvine, Office of Research Institutional Review Board 2011-8560 (T.M.); Emory Institutional Review Board 00054549 (M.A.P.); and CPP Ile-de-France VI 106-11 (P.L.).

The primary objectives of the study were to characterize the effects of 50 mg, 100 mg, 250 mg, and 600 mg duvoglustat HCl orally administered 1 hr prior to initiation of AA intravenous infusion on the safety and PK of total GAA activity and protein in patients with Pompe disease. Secondary objectives were to evaluate the effect of duvoglustat HCl on skeletal muscle tissue levels of total GAA activity, as well as to assess plasma and muscle duvoglustat concentrations. Exploratory objectives assessed anti-rhGAA antibody titers, urine hexose tetrasaccharide levels, and muscle strength.

This was a single-ascending dose, open-label study composed of two fixed-sequence periods per dose group. Patients underwent screening to determine eligibility prior to dosing with duvoglustat HCl. During period 1, patients received their regularly scheduled bi-weekly infusion of 20 mg/kg AA alone. Following a minimum 2-week dosing interval, the same patients returned for period 2 to receive a single oral dose of 50 mg, 100 mg, 250 mg, or 600 mg duvoglustat HCl 1 hr prior to initiation of infusion with 20 mg/kg AA. Each dose group was performed in a new cohort of patients. To characterize the PK of total plasma GAA activity and protein, the dose and duration of AA infusion were kept as close to identical as possible for periods 1 and 2.

Table 4. Summary of Total GAA Activity Levels in Muscle

Duvoglustat HCl Co-administered with AA (mg)	Total GAA Activity in Muscle (pmol/mg/hr) Mean (CV%)							
	Day 3 Biopsy				Day 7 Biopsy			
	n	Period 1 (AA Alone)	Period 2 (Co-administration)	Change from Period 1	n	Period 1 (AA Alone)	Period 2 (Co-administration)	Change from Period 1
50	2	1,409 (130)	1,952 (8.0)	543	4	1216 (24.0)	1,197 (18.0)	−19
100	3	1,926 (67.0)	2,320 (77.0)	394	3	861 (16.0)	1,064 (39.0)	203
250	3	2,622 (14.0)	2,764 (35.0)	142	2[a]	1,144 (25.5)	1,534 (19.6)	390
600	3[b]	1,887 (47.0)	2,710 (37.0)	823	3	916 (25.0)	1,085 (14.0)	169

[a]One of three subjects had a total GAA activity value below the limit of quantification for period 1 and was excluded.
[b]In one of four subjects, period 1 and period 2 biopsy samples were mislabeled at the site, could not be confirmed, and were subsequently not analyzed.

A total of two muscle biopsies were taken, one from the left and one from the right *vastus lateralis* muscle of each patient. One muscle biopsy was taken each treatment period.

Since there are no human safety data from co-administration of duvoglustat HCl and AA, increasing the dose to the next dose level of duvoglustat HCl was not made until thorough evaluation of all available safety and tolerability information from each dose cohort was completed by an independent Drug Safety Monitoring Board (DSMB). The DSMB 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, and identifying any clinically relevant signals and/or trends in each cohort and assessing whether it is safe to continue and enroll the next sequential dose level/cohort. Enrollment for the next cohort only began upon the DSMB's recommendation to proceed.

A schematic of the study design is provided in Figure 4.

All study patients were males or females between 18 and 65 years of age in whom Pompe disease had been diagnosed and (inclusive) were on a stable regimen and dose of AA for at least 3 months before screening (stable regimen defined as receiving AA every 2 weeks and stable dose defined as not varying by more than ±10%); had an estimated creatinine clearance $\geq$50 mL/min (creatinine clearance was estimated using the four-parameter modification of diet in renal disease [MDRD] equation: estimated glomerular filtration rate [eGFR] (mL/min/1.73 m^2) = 186 × $(Scr)^{-1.154}$ × $(Age)^{-0.203}$ × (0.742 if female) × (1.212 if African American)); agreed to use medically accepted methods of contraception during the study and for 30 days after study completion; and provided written informed consent. A patient was not considered for enrollment for any of the following documented clinical conditions: had a documented transient ischemic attack, stroke, unstable angina or myocardial infarction within the 3 months before screening; unstable cardiac disease (e.g., cardiac disease requiring active management, such as symptomatic arrhythmia, unstable angina, or New York Heart Association (NYHA) class III or IV congestive heart failure); required mechanical ventilation or was confined to a wheelchair; had a history of allergy or sensitivity to study drug (including excipients) or other iminosugars (e.g., miglustat or miglitol); required Glyset (miglitol) or Zavesca (miglustat) therapy; was pregnant or breastfeeding; tested positive for hepatitis B surface antigen or hepatitis C antibody; received investigational/experimental drug or device within 30 days prior to screening; and had an intercurrent illness or condition that may have precluded the patient from fulfilling the protocol requirements or suggested to the investigator that the potential patient may have had an unacceptable risk by participating in this study.

Primary outcome measures included total plasma GAA activity and protein PK parameter values (C_{max}, t_{max}, AUC, and terminal half-life) after AA infusion alone and when co-administered with oral duvoglustat HCl; plasma duvoglustat PK parameter values (C_{max}, t_{max}, AUC, and terminal half-life) following single, oral administration alone and when co-administered with AA; safety variables including adverse events, clinical laboratory tests (hematology, urinalysis, serum chemistry including creatine kinase, lactate dehydrogenase [LDH], alkaline phosphatase, alanine aminotransferase [ALT], and aspartate aminotransferase [AST]), 12-lead ECGs, physical examinations, vital signs, and infusion-associated reactions. Tissue (muscle) levels of total GAA activity and duvoglustat measured either on day 3 (48 hr) or on day 7 (144 hr) after administration of AA alone or when co-administered with duvoglustat HCl were evaluated as secondary outcomes. Exploratory measurements included anti-rhGAA antibody titers, urine hexose tetrasaccharide levels, and muscle strength tests.

Plasma and muscle duvoglustat concentrations were analyzed using a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method.[20] The analytical ranges of the plasma and muscle assays were 2.00 to 1,000 ng/mL and 8.00 to 1,600 ng/g, respectively.

Determination of GAA activity in human plasma and muscle homogenates was performed using 4-methylumbelliferyl-α-D-glucopyranoside (4MU-Glc) as the substrate using different dilutions. The product of the reaction was quantified by measuring against a 4-methyl-umbelliferone (4-MU) standard curve. GAA activity from the highest dilution factors within the linear range of the standard curve was

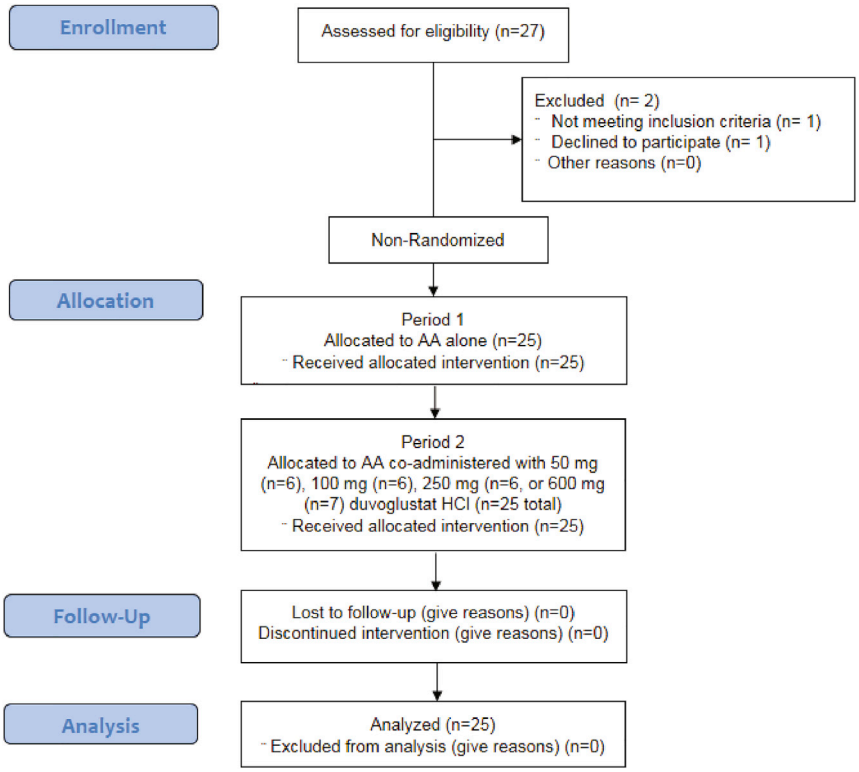

Figure 4. Flow Diagram of Patient Disposition
Period 1 patients received AA alone on day 1. In period 2, the same patients received AA co-administered with 50 mg, 100 mg, 250 mg, or 600 mg duvoglustat HCl on day 1. All 25 patients completed the study, and all 25 had plasma total GAA activity and protein and plasma duvoglustat analyzed.

reported. Plasma GAA activity was reported as nanomoles per milliliter per hour, while muscle GAA activity was reported as picomoles per milligram protein per hour.[21]

Total GAA protein was estimated by western blot. Results were reported as nanograms of GAA protein per milliliter plasma.[17]

Serial blood sampling for total plasma GAA activity and protein following AA infusion was performed for 24 hr on day 1 of periods 1 and 2 at pre-dose (time 0) and at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, and 24 hr after initiation of infusion. Serial blood sampling for plasma duvoglustat concentrations was performed pre-dose (time 0) and at 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, and 24 hr post-dose of period 2 only. Total plasma GAA activity and protein and duvoglustat PK parameters (AUC_{0-t}, $AUC_{0-\infty}$, C_{max}, t_{max}, k_{el}, and terminal half-life) were determined by non-compartmental analysis using WinNonlin software (Pharsight, version 5.2 or higher). PK parameters were summarized by treatment using descriptive statistics. Descriptive statistics were provided for PK data by treatment group. Descriptive statistics included n, arithmetic and geometric means, SD, coefficient of variation, median, minimum, and maximum values. Total plasma GAA activity, total plasma GAA protein, and duvoglustat AUC ratios for co-administration relative to AA alone were calculated for each patient. A dose-proportionality analysis for plasma duvoglustat AUC_{0-t}, $AUC_{0-\infty}$, and C_{max} was performed using the power model, where ln(parameter) = intercept + beta*ln(dose) + error, was used to estimate the slope (beta), corresponding 90% CI, and the p value testing dose proportionality (beta = 1). Assuming the relationship between log(parameter) and log(dose) is linear, a value of 1 for beta would indicate perfect dose proportionality.

The ability of active GAA in the plasma to be taken up into cells and tissues was evaluated in this trial, as was the ability of duvoglustat HCl to affect these cellular and tissue levels. Two muscle biopsy samples were taken from each Pompe disease patient on either day 3 (48 hr) or day 7 (144 hr) post-administration of AA of periods 1 and 2. If a patient was assigned for a muscle biopsy on day 3 of period 1, the second biopsy specimen was taken on the same day of period 2. Likewise, if a patient was assigned a muscle biopsy on day 7 of period 1, the second biopsy specimen was taken on the same day of period 2. An optional muscle biopsy specimen was taken from 23 subjects 28 to 42 days post-dose from period 1, day 1 for determination of trough total GAA activity levels.

Total urinary hexose tetrasaccharide (urine Hex4) was determined as butyl 4-aminobenzoate (BAB) derivatives using stable isotope dilution electrospray ionization tandem mass spectrometry (MS/MS) with selected reaction monitoring (SRM). Urine Hex4 concentration was normalized to urine creatinine concentration.[22] Urine collections for urine Hex 4 determination were taken at screening; on days −1, 2, and 7 of periods 1 and 2; and at study follow-up.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Materials and Methods, two figures, and two tables and can be found with this article online at http://dx.doi.org/10.1016/j.ymthe.2017.02.017.

AUTHOR CONTRIBUTIONS

R.L, S.S., R.K., E.B., J.F., J.B., K.J.V., and F.K.J. contributed to experiments, data analysis, data review, and study design and are currently employees and stockholders of Amicus Therapeutics. M.A., J.J.F., D.J.L., and P.B. contributed to experiments, data review, and study design and are former employees of Amicus Therapeutics. C.B. contributed to data review and analysis and is a former paid consultant with Amicus Therapeutics. P.K., M.T., M.R., K.S., M.D., M.M.D., E.F., O.G.A., K.A.G., T.M., M.A.P., R.L., P.L., T.L., and B.B. were the clinical investigators who recruited and treated the Pompe patients

enrolled in this trial and were not, nor currently are, employees or stockholders of Amicus Therapeutics.

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