Mechanism of action, pharmacokinetic profiles, and pharmacokinetic/pharmacodynamic relationships differ between cipaglucosidase alfa/miglustat and alglucosidase alfa in late-onset Pompe disease

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INTRODUCTION

- Pompe disease is an inherited metabolic disease of impaired lysosomal glycogen clearance due to acid α-glucosidase (GAA) deficiency, which leads to accumulation of the substrate most prominently in the heart, skeletal muscle, and smooth muscle.[1,2]
- Treatment with recombinant human GAA (rhGAA; enzyme replacement therapy [ERT]) is available; however, delivery to skeletal muscles is challenging due in part to inherently poor phosphorylation of GAA and instability of rhGAAs in the bloodstream.[3]
- Once in the skeletal muscle, rhGAA requires both proteolytic processing and N-glycan trimming into the mature form of the enzyme, with maximal activity toward substrate (7–10-fold higher activity than precursor protein).[4]
- Cipaglucosidase alfa plus miglustat is a novel two-component candidate therapy in development for late-onset Pompe disease (LOPD) designed to address these key mechanistic challenges.

MECHANISM OF ACTION (MOA)

Challenge #1: Cation-independent mannose 6-phosphate receptor (CI-MPR)-mediated uptake into muscle

- Highly efficient cellular uptake mechanisms from properly glycosylated rhGAA are needed when treating Pompe disease (**Figure S1**; available in the Supplement, which is accessible via quick response [QR] code)[3]
  - Relatively large amounts of rhGAA proteins are injected into the blood, but only about 1% reach the intended tissues[3]
  - Resultant rhGAA protein concentrations in the interstitial space are typically in the low nanomolar range
  - At such low rhGAA protein concentrations, highly efficient CI-MPR-mediated uptake is needed for internalization in muscle.[3]

Challenge #2: rhGAA processing (both proteolytic and N-glycan trimming) is required to achieve maximal enzyme activity toward glycogen

- Mammalian GAA is synthesized as a precursor of ~110 kDa that is N-glycosylated (**Figure S2**).[4]
- After endolysosomal delivery, the single-chain, 110 kDa GAA precursor undergoes proteolytic processing and N-glycan trimming to the mature 76/70 kDa forms.[4]
- Mature GAA has been reported to exhibit 7–10-fold higher affinity for glycogen than the precursor.[4]

Challenge #3: rhGAA is rapidly inactivated in the blood following infusion

- rhGAA is most stable and active at acidic pH, such as that of lysosomes.[3]
- At neutral pH, like that of blood, rhGAA is considerably less stable, which can lead to irreversible enzyme inactivation (**Figure S3**)[3]
  - >70% of rhGAA enzyme activity is lost over 4 hours (typical infusion time).

Cipaglucosidase alfa: addressing challenges #1 and #2

- Cipaglucosidase alfa is a next-generation rhGAA ERT with more mono- (M6P) and bis-mannose 6-phosphate (bis-M6P) than alglucosidase alfa (**Figure 1**).
- The bis-M6P N-glycans on cipaglucosidase alfa enable the enzyme to bind CI-MPR with higher affinity than alglucosidase alfa and at the low nanomolar concentrations expected in the interstitium following dosing.
- Cipaglucosidase alfa reaches peak (saturable) enzyme activity for CI-MPR at approximately 1000-fold lower concentrations than alglucosidase alfa (**Figure 2A**).
- Uptake of cipaglucosidase alfa into Pompe patient fibroblasts resulted in greater internalized GAA activity than with alglucosidase alfa at concentrations that can be achieved in the interstitium (**Figure 2B**).
- Cipaglucosidase alfa is internalized substantially better than alglucosidase alfa and is fully processed via proteolysis and N-glycan trimming identical to endogenous GAA (**Figure 3**).

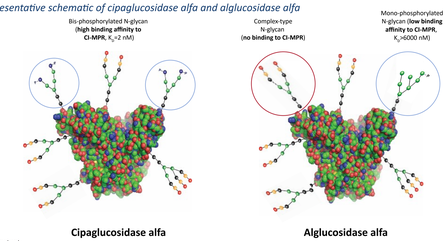
Miglustat: addressing challenge #3

- Miglustat functions as an enzyme stabilizer that competitively binds to the active site, stabilizing cipaglucosidase alfa while in circulation (**Figure 4**).
- In the acidic pH of the lysosome, miglustat dissociates from cipaglucosidase alfa to deliver active ERT.[5,7]

Cipaglucosidase alfa/miglustat: substrate reduction

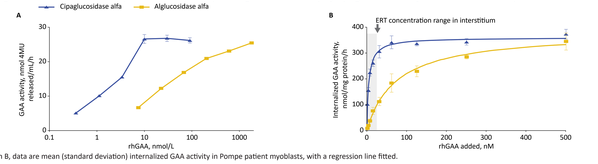
- A pharmacokinetic (PK)/pharmacodynamic (PD) translational model from *Gaa* knockout mice predicted that cipaglucosidase alfa 20 mg/kg administered with miglustat 10 mg/kg (comparable to 260 mg in humans) provides much improved glycogen reduction compared with alglucosidase alfa 20 mg/kg (**Figure 5**).[8]

Figure 1. Representative schematic of cipaglucosidase alfa and alglucosidase alfa



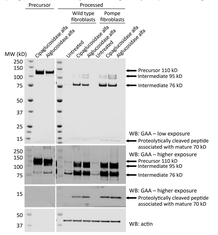
K_d, dissociation constant.

Figure 2. Enhanced cellular uptake as indicated by the higher GAA activity of cipaglucosidase alfa is demonstrated in preclinical models



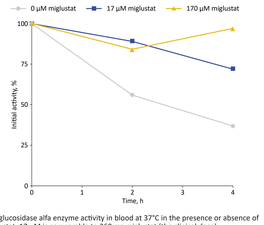
In B, data are mean (standard deviation) internalized GAA activity in Pompe patient myoblasts, with a regression line fitted.

Figure 3. Cipaglucosidase alfa undergoes full processing in cellulo



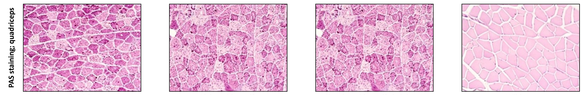
MW, molecular weight; WB, western blot.

Figure 4. Stabilization of cipaglucosidase alfa by miglustat[6]



Cipaglucosidase alfa enzyme activity in blood at 37°C in the presence or absence of miglustat. 17 μM is comparable to 260 mg miglustat (the clinical dose).

Figure 5. Enhanced cellular uptake of cipaglucosidase alfa leads to improved glycogen reduction in Gaa knockout mice



20x magnification; PAS, Periodic acid–Schiff.

PK AND PK/PD OBJECTIVES

- To characterize the disposition of cipaglucosidase alfa/miglustat and alglucosidase alfa exposures in adults with Pompe disease.
- To describe the effect of miglustat on cipaglucosidase alfa disposition.
- To quantify demographic covariate effects including ERT history on cipaglucosidase alfa/miglustat.
- To explore cipaglucosidase alfa/miglustat PK/PD in comparison with alglucosidase alfa.

METHODS

- Total GAA protein and miglustat was measured via signature peptides unique to human GAA using a validated liquid chromatography with tandem mass spectrometry (LC-MS/MS) assay.
- Blood sampling for plasma total GAA protein and plasma miglustat were taken at day 1 and week 52, just prior to start of cipaglucosidase alfa infusion (time 0), and at 1, 4, 6, 12, and 24 hours after the start of infusion.
- A population PK model was prepared from pooled data in ERT-experienced and ERT-naïve adults with LOPD enrolled in the PROPEL (ATB200-03; NCT03729362) and ATB200-02 (NCT02675465) clinical trials.
- Separate population PK analyses (cipaglucosidase alfa/miglustat and alglucosidase alfa/placebo) were performed on the PROPEL study to assess differences between treatments.
- Additional methodology is available in the Supplement, which is accessible via QR code.

RESULTS

- A summary of demographic characteristics and patient disposition in PROPEL is presented in **Table 1**.

PK of plasma total GAA protein

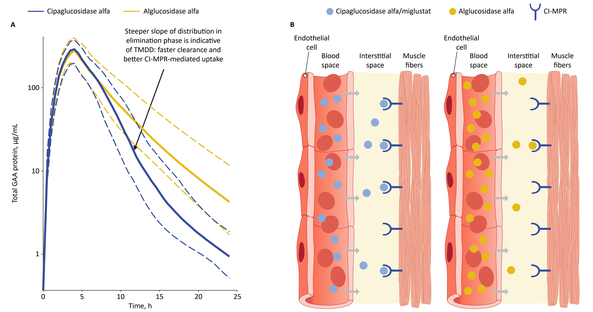
- The model-predicted median (5th and 95th quantile) plasma total GAA protein concentrations at week 52 for cipaglucosidase alfa/miglustat and alglucosidase alfa are presented in **Figure 6A**.
- At week 52, both drugs reached peak levels by approximately the end of infusion (4 hours) and declined to negligible levels 24 hours after the start of infusion.
- The terminal elimination phase of both drugs is observed on the log scale (**Figure 6A**)
  - Predicted median total GAA protein concentrations declined at a faster rate for cipaglucosidase alfa during the distribution phase of elimination than for alglucosidase alfa because of better CI-MPR-mediated uptake (as represented by the schematic in **Figure 6B**). A nonlinear clearance pathway was needed to describe the higher elimination rate of cipaglucosidase alfa, which is indicative of target-mediated drug disposition (TMDD)
    - The faster clearance rate is likely to be due to the presence of high amounts of bis-M6P N-glycan ligands in cipaglucosidase alfa, which provides the highest affinity for CI-MPRs and greatly improves muscle uptake and, therefore, clinical response.
  - These observations are consistently demonstrated without observable accumulation at the end of study (week 52; **Figure 6, Table 2**)
    - Cipaglucosidase alfa is rapidly eliminated from systemic circulation because of better CI-MPR-mediated uptake
    - The sole function of miglustat as part of this two-component therapy is to stabilize cipaglucosidase alfa while in circulation, and is then rapidly eliminated and excreted.

Table 1. Summary of demographic characteristics and patient disposition in PROPEL

	Cipaglucosidase alfa/miglustat N=79	Alglucosidase alfa/placebo N=35
Age, years, mean (min., max.)	47.4 (19, 74)	44.7 (22, 66)
Sex, male:female	35:44	18:17
Weight, kg, mean (min., max.)	72.8 (40.1, 109.0)	78.3 (40.5, 134.0)
ERT history, ERT experienced:ERT naïve	60:19	27:8
Number of PK observations, rhGAA:miglustat or placebo	536:661	242:0

PK analysis set: all randomized patients who received at least one dose of study drug and had at least one PK assessment.

Figure 6. Plasma total GAA protein profile at week 52 of PROPEL for cipaglucosidase alfa and alglucosidase alfa on a log scale (A), with a schematic of a plausible mechanism for differences between treatments in GAA protein profile (B)



Data in A are median (5th and 95th quantile) model-predicted plasma total GAA protein at week 52 of PROPEL. B is a schematic showing how the optimized M6P glycosylation structure of cipaglucosidase alfa enables the enzyme to bind CI-MPR with higher affinity than alglucosidase alfa, which is a plausible mechanism underlying the differences between treatments in GAA protein profile. Figure 6B adapted from Do H *et al. Ann Transl Med* 2019;7:291, with permission from AME Publishing Company.

Impact of cipaglucosidase alfa MOA on differences in PK compared with alglucosidase alfa

- Cipaglucosidase alfa area under the plasma drug concentration–time curve (AUC) was lower than alglucosidase alfa at day 1 and week 52 in both ERT-experienced and ERT-naïve patients (**Table 2**).
- Lower cipaglucosidase alfa exposures may be attributed to the additional nonlinear clearance pathway that may be the result of better CI-MPR-mediated uptake into target tissues because of more bis-M6P.
- The maximum observed concentration (C_{max}) was similar between treatment groups for ERT-experienced patients, but lower than alglucosidase alfa for ERT-naïve patients.
- Cipaglucosidase alfa mean plasma total GAA protein AUC and C_{max} were similar between ERT-experienced and ERT-naïve patients at day 1 and week 52.

Table 2. PK summary for cipaglucosidase alfa and alglucosidase alfa

Treatment	ERT history	Visit	AUC, μg·h/mL	C_{max}, μg/mL
Cipaglucosidase alfa/miglustat	Experienced (n=56)	Day 1	1395 (21.5)	280 (18.5)
Alglucosidase alfa/placebo	Experienced (n=26)	Day 1	1700 (17.6)	289 (13.2)
Cipaglucosidase alfa/miglustat	Experienced (n=44)	Week 52	1476 (21.8)	296 (19.9)
Alglucosidase alfa/placebo	Experienced (n=21)	Week 52	1688 (23.9)	283 (17.6)
Cipaglucosidase alfa/miglustat	Naïve (n=18)	Day 1	1343 (25.7)	273 (18.1)
Alglucosidase alfa/placebo	Naïve (n=7)	Day 1	1859 (22.4)	342 (31.0)*
Cipaglucosidase alfa/miglustat	Naïve (n=16)	Week 52	1457 (19.2)	290 (17.4)
Alglucosidase alfa/placebo	Naïve (n=7)	Week 52	1964 (26.8)	359 (28.1)*

Data are mean (CV%). *Small sample size; some patients had a shorter infusion time, which resulted in higher C_{max} and increased variability.
CV, coefficient of variation.

- Alglucosidase alfa mean plasma total GAA protein AUC and C_{max} were similar between day 1 and week 52; however, ERT-naïve patients had notably higher C_{max} levels than ERT-experienced patients because of a shorter infusion duration in some patients.
- Cipaglucosidase alfa mean plasma total GAA protein AUC was somewhat lower than alglucosidase alfa at both day 1 and week 52, and between ERT-experienced and ERT-naïve patients; however, C_{max} was similar between treatments
  - The lower cipaglucosidase alfa AUC may be a function of better CI-MPR-mediated uptake.

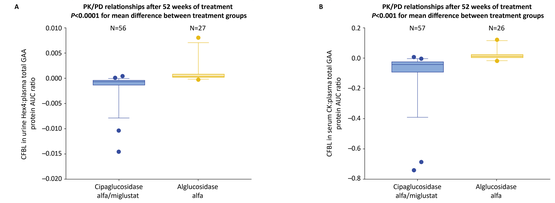
Covariate effects on cipaglucosidase alfa AUC

- A covariate analysis was performed to assess the effect of miglustat, sex, ERT history, race, age, and body weight on cipaglucosidase alfa AUC (**Figure S4**).
- Administration with miglustat had a clinically meaningful impact on cipaglucosidase alfa PK
  - Miglustat increased 20 mg/kg cipaglucosidase alfa AUC by approximately 35% (95% confidence interval 29–41) for the 260 mg dose relative to 20 mg/kg cipaglucosidase alfa alone.
- Additional results of the covariate analysis are available in the Supplement, which is accessible via QR code.

PK/PD analyses

- PK/PD analyses of change from baseline (CFBL) in urine glucose tetrasaccharide (Hex4) or serum creatine kinase (CK) to plasma total GAA protein AUC ratios were compared between cipaglucosidase alfa/miglustat and alglucosidase alfa ERT-experienced treatment groups
  - ERT-experienced patients were selected as a more homogenous group for clinical response evaluations.
- Differences between PROPEL treatment groups were highly significant, with cipaglucosidase alfa demonstrating greater decreases for PD assessments (Hex4 and CK) than alglucosidase alfa after 52 weeks of treatment in ERT-experienced patients with LOPD (**Figure 7**).

Figure 7. PK/PD analyses for CFBL in urine Hex4 or serum CK to plasma total GAA protein AUC ratios in PROPEL



Box and whisker plots: horizontal line within box = median; box = 25th and 75th quartiles; vertical error bars = 5th and 95th percentiles; closed circles = outliers.

IMPACT OF CIPAGLUCOSIDASE ALFA/MIGLUSTAT MOA ON PK, PK/PD, AND CLINICAL RESPONSE

MOA

- The addition of miglustat 260 mg provides an optimal molar ratio to total GAA protein to balance stabilization of cipaglucosidase alfa in systemic circulation and maintenance of catalytic activity, while minimizing any potential inhibition in the lysosome
  - Miglustat rapidly dissociates within 24 hours for delivery of intact cipaglucosidase alfa to lysosomes.
- Improved CI-MPR-binding affinity via bis-M6P-enhanced cellular uptake and delivery to lysosomes and an enzyme that can be fully processed into the mature/most active form provides:
  - Enhanced biodistribution to muscle
  - Improved intracellular catalytic activity leading to greater glycogen reduction in a knockout mouse model approaching wild type.

Effect of MOA on cipaglucosidase alfa PK, PK/PD, and clinical outcomes

- Key improvements conferred by this two-component therapy, such as stabilization by miglustat, enhanced glycosylation, higher CI-MPR-binding affinity, and improved targeting to muscle, were demonstrated by TMDD in PK characterization.
- PK/PD analyses of the ratio of CFBL in Hex4 or CK to AUC further confirms improved CI-MPR-mediated uptake.
- Greater glycogen-lowering effect of cipaglucosidase alfa plus miglustat observed in animal models.
- The sum-total of the MOA of this two-component therapy—including stabilization by miglustat; improvements in rhGAA structure, delivery, and processing into the most active form of the enzyme; and glycogen-lowering effects—have led to significant improvements in clinical response in adults with LOPD.[9]

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This presentation shares information about Amicus Therapeutics' investigational therapy, cipaglucosidase alfa/miglustat, which is in development for the treatment of Pompe disease. This investigational therapy is not approved by any regulatory agency at this time.

The presenter, Franklin K. Johnson, is an employee of, and holds stock in, Amicus Therapeutics, Inc.

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