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A Novel Recombinant Human Acid Alpha-glucosidase, ATB200, Co-administered with a Pharmacological Chaperone, Leads to Greater Substrate Reduction and Improvement in Pompe Disease-relevant Markers Compared to Alglucosidase Alfa in *Gaa* KO Mice

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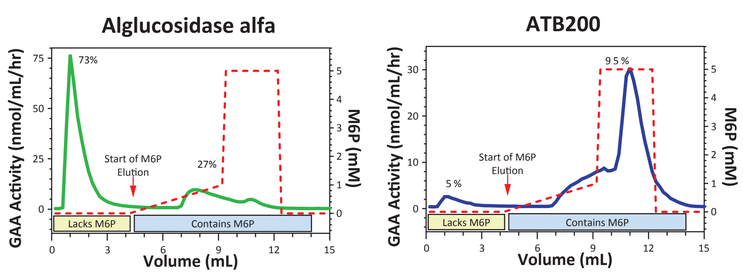
INTRODUCTION

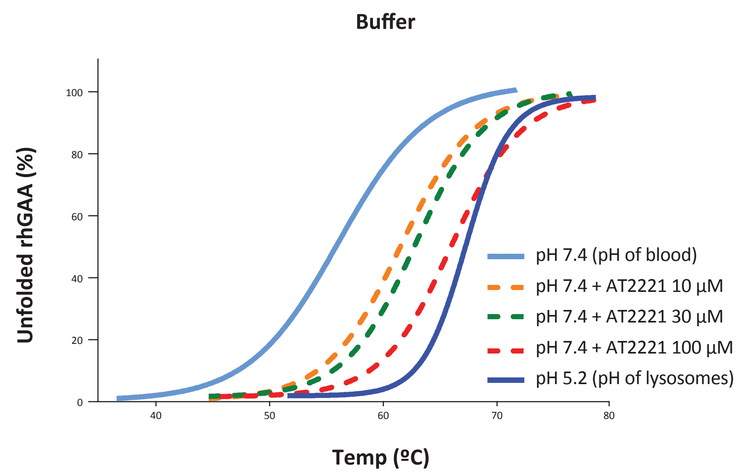
Pompe disease is an inherited lysosomal storage disorder caused by a deficiency in acid alpha-glucosidase (GAA) activity and is characterized by progressive accumulation of lysosomal glycogen in cardiac and skeletal muscles. Enzyme replacement therapy using alglucosidase alfa, a recombinant human GAA (rhGAA), is currently the only approved treatment for Pompe disease. Although alglucosidase alfa has demonstrated some clinical benefits, there may be limitations in its delivery to skeletal muscles due to sub-optimal levels of mannose-6-phosphate (M6P), a carbohydrate that binds the cation-independent M6P receptor (CI-MPR) at the cell surface to mediate enzyme internalization and lysosomal delivery. We have developed a novel next-generation therapy (designated Advanced and Targeted Acid α-glucosidase [AT-GAA]), which utilizes a novel rhGAA (ATB200) co-administered with AT2221 (miglustat), a small molecule pharmacological chaperone. ATB200 has a substantially higher M6P content compared with alglucosidase alfa to enhance uptake and is further stabilized by AT2221. In this study, we compared the effects of ATB200 co-administered with AT2221 (ATB200/AT2221) with those of alglucosidase alfa in *Gaa* knock-out (KO) mice.

1. ATB200 HAS A HIGHER M6P CONTENT COMPARED WITH ALGLUCOSIDASE ALFA



The M6P content was measured by affinity chromatography, whereby alglucosidase alfa or ATB200 was loaded onto a CI-MPR column and only enzyme that contained M6P was retained and then eluted from the column using free M6P of increasing concentration (dotted red line). Both M6P-lacking and M6P-containing rhGAA fractions were collected and assayed for GAA activity. The majority of ATB200 (95%) was bound, compared with 27% of alglucosidase alfa, suggesting that ATB200 has a higher M6P content, which is key to the efficient endocytosis and lysosomal targeting of rhGAA.

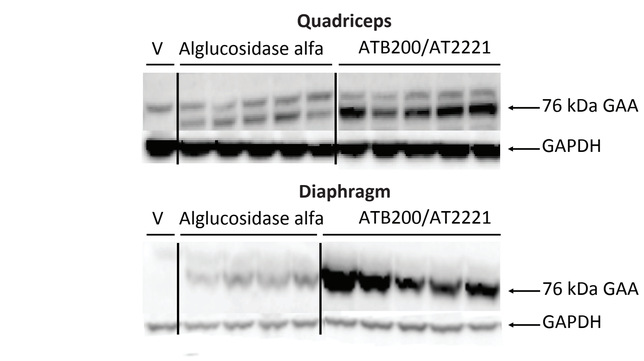
2. THE PHARMACOLOGICAL CHAPERONE AT2221 STABILIZES ATB200 IN VITRO AND EX VIVO

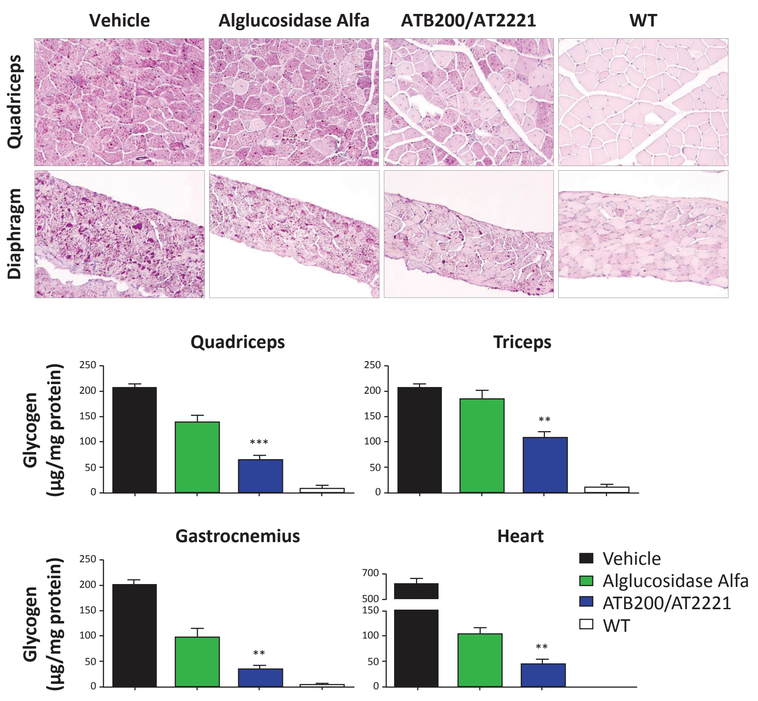


(Upper panel) The stability of ATB200 in acidic or neutral pH buffers was evaluated using SYPRO Orange, a dye that increases fluorescence when binding denatured proteins. AT2221 stabilizes ATB200 at pH 7.4 in a concentration-dependent manner; the increased stability of ATB200 at pH 7.4 approached that seen at pH 5.2, a condition that mimics the acidic environment of the lysosome.

(Lower panel) The activity of ATB200 was evaluated following incubation at 37ºC in human blood. AT2221 prevented the inactivation of ATB200 over 4 hours. Note that 17 µM approximates the peak plasma concentration observed in Pompe patients administered 260 mg AT2221 in the clinical trial (NCT02675465, ATB200-02—Amicus' ongoing clinical trial).

3. ATB200/AT2221 IMPROVES LYSOSOMAL TARGETING OF rhGAA AND LEADS TO GREATER GLYCOGEN REDUCTION IN THE MUSCLES OF *Gaa* KO MICE



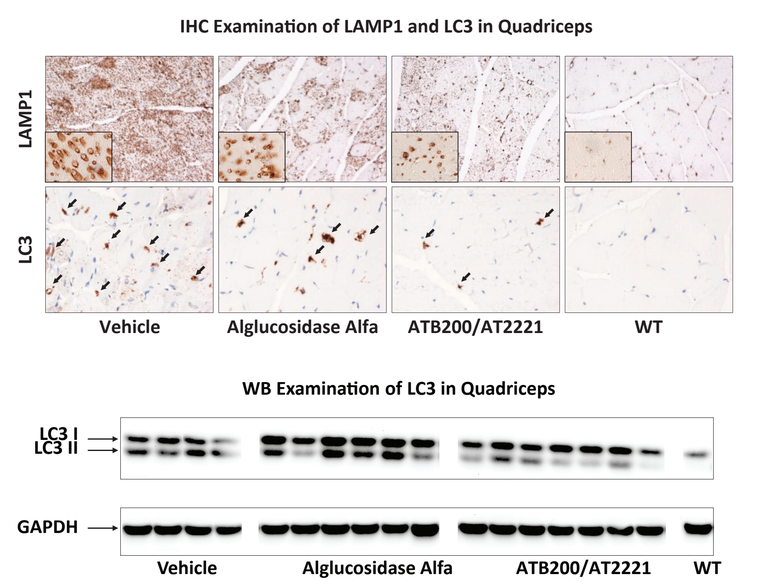


(Upper) Male *Gaa* KO mice received a single administration of intravenous (IV) vehicle (V), IV 20 mg/kg alglucosidase alfa, or IV 20 mg/kg ATB200 with oral co-administration of 10 mg/kg AT2221 30 minutes prior to IV (ATB200/AT2221). Western blot (WB) was performed using an anti-human GAA antibody on quadriceps and diaphragm collected 72 hours post-dose. ATB200/AT2221 led to higher level of the mature, lysosomal form of GAA (76 kDa) compared with alglucosidase alpha. GAPDH was blotted as the loading control.

(Middle) Male *Gaa* KO mice received two biweekly administrations of vehicle, alglucosidase alfa, or ATB200/AT2221. Tissues were collected 14 days after the last administration. ATB200/AT2221 led to greater glycogen reduction in quadriceps and diaphragm as demonstrated by Periodic Acid-Schiff (PAS) staining. Age-matched wild-type (WT) animals were included as a control. Each image is representative of 5-6 mice/group. Magnification = 200x.

(Lower) In a similar study with 6 biweekly administrations, glycogen levels were determined in various muscles using amyloglucosidase digestion. ATB200/AT2221 led to significantly greater glycogen reduction in the quadriceps, triceps, gastrocnemius, and heart. Bars represent mean ± standard error of the mean (SEM) of 7 mice/group. $^{**}P<0.005$ and $^{***}P<0.0005$ vs alglucosidase alfa in a two-tailed t-test.

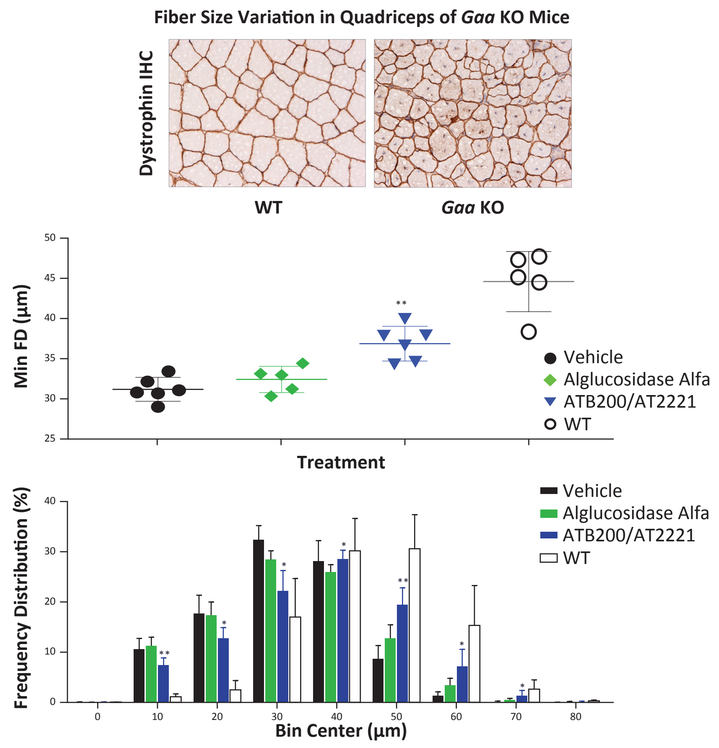
4. ATB200/AT2221 CORRECTS THE LYSOSOMAL-AUTOPHAGIC DEFECTS IN MUSCLES OF *Gaa* KO MICE



(Upper panel) Male *Gaa* KO mice received 6 biweekly administrations of vehicle, alglucosidase alfa (20 mg/kg) or ATB200/AT2221 (20/10 mg/kg). Quadriceps were collected 14 days after the sixth administration for immunochemistry. Lysosomal proliferation (as shown in the enlargement of LAMP1-positive vesicles) and autophagic buildup (as indicated by LC3-positive puncta) in the quadriceps were preferentially and drastically reduced following ATB200/AT2221 administration compared with alglucosidase alfa. Images are representative of 7 mice per group. Magnification = 200x (1,000x in insets) for LAMP1 staining and 400x for LC3 staining.

(Lower panel) LC3 levels in quadriceps were evaluated after 2 biweekly administrations of vehicle, alglucosidase alfa (20 mg/kg), or ATB200/AT2221 (20/10 mg/kg). Most animals treated with ATB200/AT2221 showed a significant decrease in levels of LC3 II, the lipidated form of LC3 that is associated with autophagosomes. In comparison, the effect of alglucosidase alfa was modest.

5. ATB200/AT2221 IMPROVES MUSCLE FIBER SIZE IN QUADRICEPS OF *Gaa* KO MICE

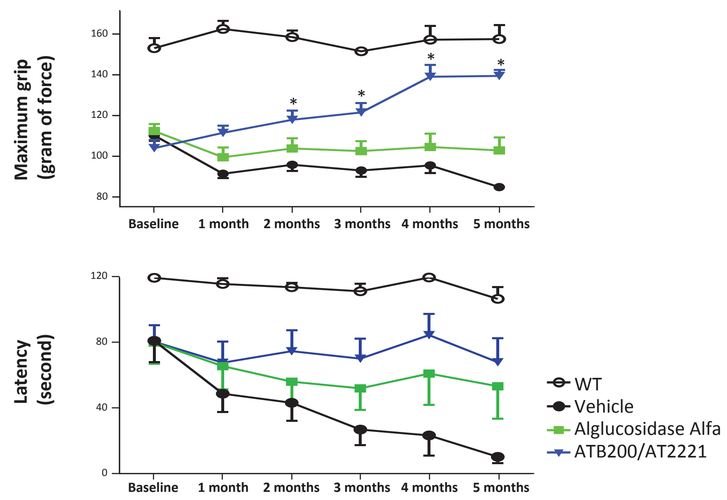


(Upper) The presence of smaller muscle fibers in *Gaa* KO mice was demonstrated by dystrophin IHC, which led to investigation of the effect of ATB200/AT2221 on fiber size in *Gaa* KO mice.

(Middle) Twelve biweekly administrations of ATB200/AT2221 led to significant improvement in fiber size compared with alglucosidase alfa, as determined by minimal Feret's diameter (Min FD). Each data point represents the average Min FD from 2000–5000 fibers per animal. $^{**}P<0.005$ versus alglucosidase alfa in a two-tailed t-test.

(Lower) The histogram shows a consistent shift toward larger fiber size in quadriceps of *Gaa* KO mice following 12 biweekly administrations of ATB200/AT2221. $^{*}P<0.05$ and $^{**}P<0.005$ vs alglucosidase alfa in a two-tailed *t* test.

6. ATB200/AT2221 IMPROVES FUNCTIONAL MUSCLE STRENGTH IN *Gaa* KO MICE



In a long-term study, *Gaa* KO mice received biweekly administration of alglucosidase alfa or ATB200/AT2221 for 5 months. Muscle function was measured using grip strength and wire hang tests. Although the measurements in mice treated with alglucosidase alfa declined over the course of study, they were stabilized in the wire hang test and improved progressively in the grip strength test following ATB200/AT2221 administration. Each data point represents the mean ± SEM of 7-15 mice/group. $^{*}P<0.05$ vs alglucosidase alfa in a two-sided t-test.

SUMMARY AND CONCLUSIONS

- Co-administration of ATB200/AT2221 is more effective than alglucosidase alfa in tissue uptake and lysosomal targeting. Consequently, ATB200/AT2221 demonstrated a greater capability in correcting some of the disease-relevant pathologies in *Gaa* KO mice, such as glycogen accumulation, lysosomal proliferation, and impairment of autophagic pathway. These positive changes correlated with the increase in muscle fiber size and ultimately led to improvement in functional muscle strength.
- Taken together, the combination of ATB200 and AT2221 offers potential advantages over the standard of care and warrants further clinical investigation as a next-generation treatment for Pompe disease.

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DISCLOSURE

Conflict of Interest

All authors are employees of and hold stock in Amicus Therapeutics, Inc.

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