Abstract #94

Novel rhGAA with Optimal Glycosylation Is Significantly Better than Alglucosidase Alfa for Glycogen Clearance in Skeletal Muscles of *Gaa* KO Mice

Gotschall R, Xu S, Lun Y, Schilling A, Soska, R, Frascella M, Garcia A, Feng J, Chang K, Brignol N, Khanna R, Valenzano KJ, and Do H

Amicus Therapeutics, 1 Cedar Brook Drive, Cranbury, NJ 08512

Amicus Therapeutics

Abstract

Pompe disease is an inherited lysosomal storage disorder that results from a deficiency in acid alpha-glucosidase (GAA), and is characterized by progressive accumulation of lysosomal glycogen in cardiac and skeletal muscles. Enzyme replacement therapy (ERT) using recombinant human GAA (rhGAA) is the only approved treatment available for Pompe disease. This ERT requires the specialized carbohydrate mannose 6-phosphate (M6P) for cellular uptake and subsequent delivery to lysosomes via cell surface cation-independent M6P receptors (CI-MPRs). However, the current rhGAA ERT (alglucosidase alfa) contains low amounts of M6P that limit drug targeting and efficacy in disease-relevant tissues.

We have developed a proprietary production cell line and manufacturing process that yield a novel rhGAA (designated as ATB200) with optimal glycosylation and higher M6P content, particularly the high-affinity bis-M6P N-glycan structure, for improved drug targeting. ATB200 binds the CI-MPR with high affinity (K_D ~ 2-4 nM) and was efficiently internalized by Pompe fibroblasts and skeletal muscle myoblasts (K_{uptake} ~ 7-14 nM).

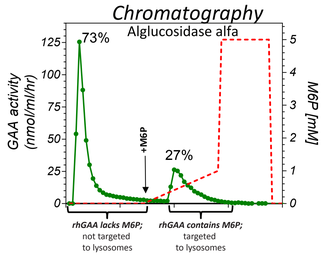
ATB200 was evaluated in vivo and shown to be much more effective for clearing accumulated glycogen in skeletal muscles of *Gaa* KO mice than alglucosidase alfa. Our results indicate that bi-weekly administrations of 5 mg/kg ATB200 were equivalent to 20 mg/kg rhGAA for reducing glycogen, while substantially greater glycogen clearance was observed with 20 mg/kg ATB200 compared to 20 mg/kg rhGAA in key skeletal muscles. These results were confirmed by histological examination. The addition of a pharmacological chaperone (PC) enhanced glycogen reduction by ATB200.

Taken together, these data demonstrate that the higher M6P content of ATB200 results in better lysosomal targeting and substrate reduction, which can be further improved by combination with a PC, thus warranting further investigation of this next-generation treatment for Pompe disease.

Existing rhGAA ERTs Have Low M6P and Are Poorly Targeted

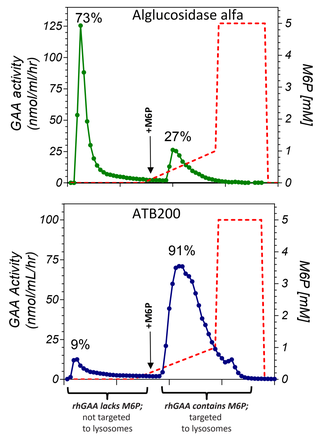
- Recombinant human acid α-glucosidase enzyme replacement therapies (Myozyme® and Lumizyme® rhGAA ERT; Sanofi-Genzyme) are the only approved treatments for Pompe disease
- Existing rhGAA ERTs contain mixture of mannose 6-phosphate (M6P) and non-M6P carbohydrates
- Only the minor fraction containing M6P can bind M6P receptor (CIMPR) for uptake and delivery to lysosomes
- Existing ERTs therefore require significantly higher drug doses for clinical efficacy
- Higher drug doses have significant drawbacks:
  - Substantially longer infusion times
  - Higher probability for developing immune responses
  - Require more protein manufacturing

M6P Receptor (CIMPR) Chromatography



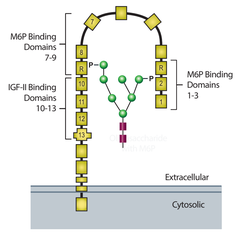
ATB200: A Pompe ERT Optimized for Lysosomal Targeting *via* the CIMPR

- We developed proprietary cell line and manufacturing/purification processes that yield rhGAA (ATB200) with significantly higher M6P content than alglucosidase alfa
- Higher amounts of M6P enable ATB200 to bind the CIMPR substantially better than alglucosidase alfa
- Optimized carbohydrate structures are maintained during process scale up to current 250 L bioreactor

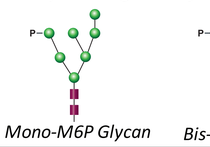


Bis-Phosphorylated Oligosaccharides Required for High-Affinity Binding to CIMPR Receptor

Structure of CIMPR



Glycan Ligands for CIMPR



Ligand	Binding affinity (Apparent K_D; nM)
bis-M6P N-glycan	2
beta-galactosidase	20
pentamannose-M6P	6,000
free M6P	7,000

Data from Tong et al., 1989

- Type of M6P N-glycan mediates binding to CIMPR
- Bis-phosphorylated N-glycans have ~3,000-fold greater affinity for the CIMPR receptor than mono-phosphorylated N-glycans

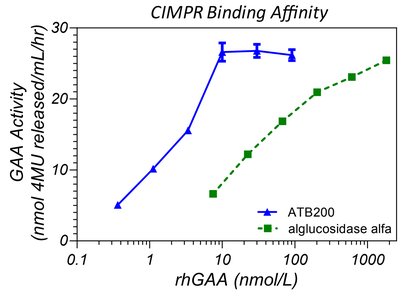
Goal: develop an rhGAA ERT with natural bis-M6P N-glycans for efficient drug targeting to lysosomes

ATB200 Contains Bis-M6P N-glycans and Has Very High-Affinity for CIMPR

Bis-Phosphorylated Glycan Analysis

Glycan	Alglucosidase alfa (*mol bis-glycan/ mol protein*)	ATB200 (*mol bis-glycan/ mol protein*)
Bis-M6P	0.1	1.3

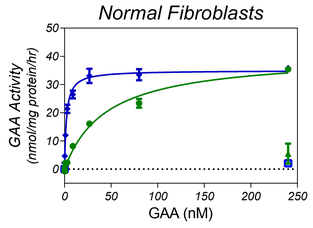
CIMPR Binding Affinity



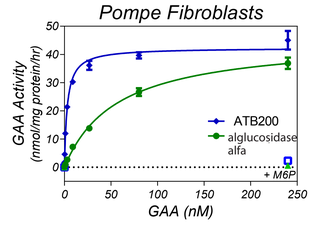
- N-glycan analyses via MALDI-TOF mass spectrometry confirmed that on average, each ATB200 molecule contains the natural bis-M6P N-glycan structure
- Higher bis-M6P N-glycan content on ATB200 directly correlated with high-affinity binding to CIMPR in M6P receptor plate binding assays (K_D~2-4 nM)

ATB200 is Internalized Efficiently into Cells

Normal Fibroblasts



Pompe Fibroblasts



Cell Line	K_{uptake} (nM)	
	ATB200	alglucosidase alfa
normal	2	56
Pompe	3	57

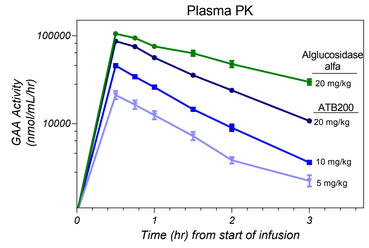
- ATB200 is internalized substantially better than alglucosidase alfa into normal and Pompe fibroblasts
- Receptor saturation observed with ~25 nM ATB200 while ~250 nM Lumizyme is needed
- Extrapolated uptake efficiency constant (K_{uptake}) is 2-3 nM for ATB200 and ~56 nM for alglucosidase alfa
- Cellular uptake is dependent on M6P; free excess M6P blocks rhGAA cellular uptake

These results suggest that ATB200 should be a well-targeted ERT for Pompe disease

ATB200 Has a Favorable Pharmacokinetic Profile

- Compared PK of ATB200 and alglucosidase alfa
  - Single dose
  - 30 min infusion in rats
  - Plasma GAA levels determined by enzyme activity
- ATB200 is cleared faster than alglucosidase alfa
  - Likely due to faster uptake via M6P pathway
  - ATB200 had similar half-lives across different test doses
- Dose proportional C_{max} and AUC

Plasma PK

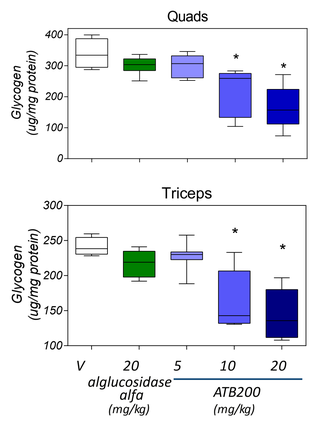


	Dose (*mg/kg*)	C_{max} (*nmol/mL/hr*)	AUC (*nmol/mL*)	Half-life (*hr*)
alglucosidase alfa	20	105,543	480,552	1.3
ATB200	20	86,805	215,238	0.8
	10	45,721	74,880	0.6
	5	21,165	34,145	0.6

ATB200 has a favorable PK profile and exposure for developing an effective ERT for Pompe Disease

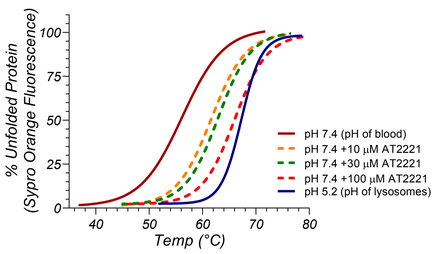
ATB200 Clears Glycogen Significantly Better than Alglucosidase Alfa in Skeletal Muscles of *Gaa* KO Mice

- Evaluated alglucosidase alfa and ATB200 for glycogen clearance in *Gaa* KO mice
  - Two IV bolus administrations (every other week)
  - Tissues harvested 2 weeks after last dose
  - Tissues analyzed for GAA activity and glycogen content
- ATB200 at 5 mg/kg was equivalent to alglucosidase alfa at 20 mg/kg for reducing glycogen in skeletal muscles
- ATB200 dosed at 10 and 20 mg/kg was significantly better than alglucosidase alfa for clearing glycogen in skeletal muscles
- ATB200 and alglucosidase alfa were equally effective for clearing glycogen in heart



Stability of rhGAA ERT Vastly Improved with CHART Technology

ATB200 rhGAA ± AT2221



Buffer	T_m (°C) of ATB200
pH 7.4	56.2
pH 7.4 + 10 mM AT2221	61.6
pH 7.4 + 10 mM AT2221	62.9
pH 7.4 + 10 mM AT2221	66.0
pH 5.2	67.3

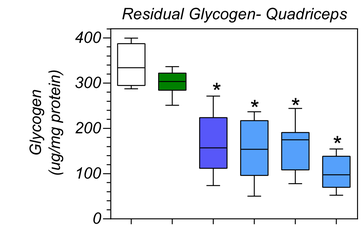
- Chaperone binds to and stabilizes ATB200
- Chaperone increases uptake of active enzyme into tissues
- Chaperone improves tolerability

Protein stability of ERT at unfavorable conditions substantially improved with CHART™

Combination of Pharmacological Chaperone and ATB200 Enhances Glycogen Clearance in *Gaa* KO Mice

- Evaluated alglucosidase alfa and ATB200 for glycogen clearance in *Gaa* KO mice
  - Two IV bolus administrations of ERT (every other week)
  - Pharmacological chaperone AT2221 administered orally 30 min prior to ERT
  - Tissues harvested 2 weeks after last dose
  - Tissues analyzed for GAA activity and glycogen content
- ATB200 (20 mg/kg) alone was significantly better than Lumizyme for clearing glycogen in skeletal muscles
- Addition of AT2221 improved glycogen clearance in muscles
- Addition of 10 mg/kg AT2221 with 20 mg/kg ATB200 reduced skeletal muscle glycogen to near normal levels

Residual Glycogen- Quadriceps



AT2221 (mg/kg)	–	–	–	1	3	10
rhGAA ERT (20 mg/kg)	–	Lumi	ATB200			

Summary

- A proprietary cell line and manufacturing processes have been developed to produce a novel rhGAA (ATB200) with optimal glycosylation for efficient lysosomal targeting
  - Significant amounts of M6P (particularly bis-M6P N-glycans) for efficient lysosomal targeting
  - Well-processed complex-type N-glycans for minimizing non-productive drug clearance
- ATB200 has favorable PK and good drug targeting to key muscle tissues in vivo
- ATB200 is significantly better than standard of care for reducing glycogen in skeletal muscles of *Gaa* KO mice
- Optimized carbohydrate structures maintained throughout scale up of manufacturing process (currently at 250 L scale)
- ATB200 + Pharmacological Chaperone further improves glycogen reduction in skeletal muscle of *Gaa* KO mice

  Additional development of ATB200 + pharmacological chaperone is warranted.