

(19) **DANMARK**



Patent- og
Varemærkestyrelsen

(10) **DK/EP 3624831 T5**

(12) **Rettet oversættelse af
europæisk patentskrift**

-
- (51) Int.Cl.: **A 61 K 38/43 (2006.01)** **A 61 K 38/47 (2006.01)** **A 61 P 21/00 (2006.01)**
A 61 P 25/00 (2006.01) **A 61 P 43/00 (2006.01)**
- (45) Oversættelsen bekendtgjort den: **2024-09-02**
- (80) Dato for Den Europæiske Patentmyndigheds bekendtgørelse om meddelelse af patentet: **2023-03-29**
- (86) Europæisk ansøgning nr.: **18802722.1**
- (86) Europæisk indleveringsdag: **2018-05-15**
- (87) Den europæiske ansøgnings publiceringsdag: **2020-03-25**
- (86) International ansøgning nr.: **US2018032815**
- (87) Internationalt publikationsnr.: **WO2018213340**
- (30) Prioritet: **2017-05-15 US 201762506561 P** **2017-05-15 US 201762506574 P**
2017-05-15 US 201762506569 P **2017-07-06 US 201762529300 P**
2017-09-27 US 201762564083 P **2017-10-03 US 201762567334 P**
2018-01-16 US 201862618021 P **2018-01-31 US 201862624638 P**
2018-04-20 US 201862660758 P
- (84) Designerede stater: **AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO RS SE SI SK SM TR**
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- (54) Benævnelse: **REKOMBINANT HUMAN SUR ALFA-GLUCOSIDASE**
- (56) Fremdragne publikationer:
WO-A1-2016/054231
WO-A1-2017/049161
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DESCRIPTION

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application Serial No. 62/506,561 filed May 15, 2017, U.S. Provisional Application Serial No. 62/506,569 filed May 15, 2017, U.S. Provisional Application Serial No. 62/506,574 filed May 15, 2017, U.S. Provisional Application Serial No. 62/564,083 filed September 27, 2017, U.S. Provisional Application Serial No. 62/567,334 filed October 3, 2017, U.S. Provisional Application Serial No. 62/618,021 filed January 16, 2018, U.S. Provisional Application Serial No. 62/624,638 filed January 31, 2018, and U.S. Provisional Application Serial No. 62/660,758 filed April 20, 2018, to each of which priority is claimed.

FIELD OF INVENTION

[0002] The present invention involves the fields of medicine, genetics and recombinant glycoprotein biochemistry, and, specifically, relates to recombinant human α -glucosidase (rhGAA) compositions that have a higher total content of mannose-6-phosphate-bearing N-glycans that efficiently target CIMPR on cells and subsequently deliver rhGAA to the lysosomes where it can break down abnormally high levels of accumulated glycogen. The rhGAA of the invention exhibits superior uptake into muscle cells and subsequent delivery to lysosomes compared to conventional rhGAA products and exhibits other pharmacokinetic properties that make it particularly effective for enzyme replacement therapy of subjects having Pompe disease.

[0003] The present disclosure also provides a method for treating Pompe disease comprising administering to an individual a combination of an rhGAA and a pharmacological chaperone. For example, in some examples, the present disclosure provides a method for treating Pompe disease comprising administering to an individual a combination of rhGAA and miglustat. The rhGAA of the invention exhibits surprising efficacy in treating and reversing disease progression in subjects suffering from Pompe disease.

BACKGROUND

[0004] Pompe disease is an inherited lysosomal storage disease that results from a deficiency in acid α -glucosidase (GAA) activity. A person having Pompe Disease lacks or has reduced levels of acid α -glucosidase (GAA), the enzyme which breaks down glycogen to glucose, a main energy source for muscles. This enzyme deficiency causes excess glycogen accumulation in the lysosomes, which are intra-cellular organelles containing enzymes that ordinarily break down glycogen and other cellular debris or waste products. Glycogen accumulation in certain tissues of a subject having Pompe Disease, especially muscles, impairs the ability of cells to function normally. In Pompe Disease, glycogen is not properly metabolized and progressively accumulates in the lysosomes, especially in skeletal muscle cells and, in the infant onset form of the disease, in cardiac muscle cells. The accumulation of glycogen damages the muscle and nerve cells as well as

those in other affected tissues.

[0005] Traditionally, depending on the age of onset, Pompe disease is clinically recognized as either an early infantile form or as a late onset form. The age of onset tends to parallel the severity of the genetic mutation causing Pompe Disease. The most severe genetic mutations cause complete loss of GAA activity and manifest as early onset disease during infancy. Genetic mutations that diminish GAA activity but do not completely eliminate it are associated with forms of Pompe disease having delayed onset and progression. Infantile onset Pompe disease manifests shortly after birth and is characterized by muscular weakness, respiratory insufficiency and cardiac failure. Untreated, it is usually fatal within two years. Juvenile and adult onset Pompe disease manifest later in life and usually progress more slowly than infantile onset disease. This form of the disease, while it generally does not affect the heart, may also result in death, due to weakening of skeletal muscles and those involved in respiration.

[0006] Current non-palliative treatment of Pompe disease involves enzyme replacement therapy (ERT) using recombinant human GAA (rhGAA) known as Lumizyme[®], Myozyme[®], or alglucosidase alfa. This conventional enzyme replacement therapy seeks to treat Pompe Disease by replacing the missing GAA in lysosomes by administering rhGAA thus restoring the ability of cells to break down lysosomal glycogen. "Lumizyme[®]" and "Myozyme[®]" are conventional forms of rhGAA produced or marketed as biologics by Genzyme and approved by the U.S. Food and Drug Administration, and are described by reference to the Physician's Desk Reference (2014). Alglucosidase alfa is identified as chemical name [199-arginine, 223-histidine]prepro- α -glucosidase (human); molecular formula, C₄₇₅₈H₇₂₆₂N₁₂₇₄O₁₃₆₉S₃₅; CAS number 420794-05-0. These products are administered to subjects with Pompe Disease, also known as glycogen storage disease type II (GSD-II) or acid maltase deficiency disease.

[0007] The cellular uptake of a rhGAA molecule is facilitated by the specialized carbohydrate, mannose-6-phosphate (M6P), which binds to the cation-independent mannose-6-phosphate receptor (CIMPR) present on target cells such as muscle cells. Upon binding, rhGAA molecule is taken up by target cells and subsequently transported into the lysosomes within the cells. Most of the conventional rhGAA products, however, lack a high total content of mono-M6P- and bis-M6P-bearing N-glycans (i.e., N-glycans bearing one M6P residue or N-glycans bearing two M6P residues, respectively), which limits their cellular uptake via CIMPR and lysosomal delivery, thus making conventional enzyme replacement therapy insufficiently effective. For example, while conventional rhGAA products at 20 mg/kg or higher doses do ameliorate some aspects of Pompe disease, they are not able to adequately, among other things, (i) treat the underlying cellular dysfunction, (ii) restore muscle structure, or (iii) reduce accumulated glycogen in many target tissues, such as skeletal muscles, to reverse disease progression. Further, higher doses may impose additional burdens on the subject as well as medical professionals treating the subject, such as lengthening the infusion time needed to administer rhGAA intravenously. There remains a need for further improvements to enzyme replacement therapy for treatment of Pompe disease, such as rhGAA with improved tissue uptake, improved enzymatic activity, improved stability, or reduced immunogenicity.

[0008] The glycosylation of GAA or rhGAA can be enzymatically modified in vitro by the

phosphotransferase and uncovering enzymes described by Canfield, et al., U.S. Patent No. 6,534,300, to generate M6P groups. Enzymatic glycosylation cannot be adequately controlled and can produce rhGAA having undesirable immunological and pharmacological properties. Enzymatically modified rhGAA may contain only high-mannose oligosaccharide which all could be potentially enzymatically phosphorylated in vitro with a phosphotransferase or uncovering enzyme and may contain on average 5-6 M6P groups per GAA. The glycosylation patterns produced by in vitro enzymatic treatment of GAA are problematic because the additional terminal mannose residues, particularly non-phosphorylated terminal mannose residues, negatively affect the pharmacokinetics of the modified rhGAA. When such an enzymatically modified product is administered in vivo, these mannose groups increase non-productive clearance of the GAA, increase the uptake of the enzymatically-modified GAA by immune cells, and reduce rhGAA therapeutic efficacy due to less of the GAA reaching targeted tissues, such as skeletal muscle myocytes. For example, terminal non-phosphorylated mannose residues are known ligands for mannose receptors in the liver and spleen which leads to rapid clearance of the enzymatically-modified rhGAA and reduced targeting of rhGAA to target tissue. Moreover, the glycosylation pattern of enzymatically-modified GAA having high mannose N-glycans with terminal non-phosphorylated mannose residues resembles that on glycoproteins produced in yeasts and molds, and increases the risk of triggering immune or allergic responses, such as life-threatening severe allergic (anaphylactic) or hypersensitivity reactions, to the enzymatically modified rhGAA.

[0009] In view of these deficiencies of conventional rhGAA products and in vitro methods to phosphorylate rhGAA, the inventors diligently sought and identified ways to produce rhGAA with an optimized N-glycan profile for enhanced biodistribution and lysosomal uptake and thereby to minimize non-productive clearance of rhGAA once administered. The present invention provides stable or declining Pompe patients an effective therapy that reverse disease progression at the cellular level. The inventors also report that the rhGAA of the present invention reverses the disease progression - including clearing lysosomal glycogen more efficiently than the current standard of care - and that patients treated with the rhGAA of the present invention exhibit surprising and significant health improvements, including improvements in muscle strength, motor function, and/or pulmonary function, and/or including a reversal in disease progression, as demonstrated in various efficacy results (e.g., Examples 10 and 11) from the clinical studies.

[0010] WO 2016/054231 discloses recombinant human alpha glucosidase (rhGAA) composition derived from CHO cells that contains a more optimized glycan composition consisting of a higher amount of rhGAA containing N-glycans carrying mannose-6-phosphate (M6P) or bis-M6P than conventional rhGAAs, along with low amount of non-phosphorylated high mannose glycans, and a low amount of terminal galactose on complex oligosaccharides. Compositions containing the rhGAA, and methods of use are described.

SUMMARY

[0011] The present invention is set out in the appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0012]

Fig. 1A shows non-phosphorylated high mannose N-glycan, a mono-M6P N-glycan, and a bis-M6P N-glycan. Fig. 1B shows the chemical structure of the M6P group. Each square represents N-acetylglucosamine (GlcNAc), each circle represents mannose, and each P represents phosphate.

Fig. 2A describes productive targeting of rhGAA via N-glycans bearing M6P to target tissues (e.g. muscle tissues of subject with Pompe Disease). Fig. 2B describes non-productive drug clearance to non-target tissues (e.g. liver and spleen) or by binding of non-M6P N-glycans to non-target tissues.

Fig. 3 is a schematic diagram of an exemplary process for the manufacturing, capturing and purification of a recombinant lysosomal protein.

Fig. 4 shows a DNA construct for transforming CHO cells with DNA encoding rhGAA.

Fig. 5 is a graph showing the results of CIMPR affinity chromatography of ATB200 rhGAA with (Embodiment 2) and without (Embodiment 1) capture on an anion exchange (AEX) column.

Figs. 6A-6H show the results of a site-specific N-glycosylation analysis of ATB200 rhGAA, using two different LC-MS/MS analytical techniques. Fig. 6A shows the site occupancy of the seven potential N-glycosylation sites for ATB200. Fig. 6B shows two analyses of the N-glycosylation profile of the first potential N-glycosylation site for ATB200. Fig. 6C shows two analyses of the N-glycosylation profile of the second potential N-glycosylation site for ATB200. Fig. 6D shows two analyses of the N-glycosylation profile of the third potential N-glycosylation site for ATB200. Fig. 6E shows two analyses of the N-glycosylation profile of the fourth potential N-glycosylation site for ATB200. Fig. 6F shows two analyses of the N-glycosylation profile of the fifth potential N-glycosylation site for ATB200. Fig. 6G shows two analyses of the N-glycosylation profile of the sixth potential N-glycosylation site for ATB200. Fig. 6H summarizes the relative percent mono-phosphorylated and bis-phosphorylated species for the first, second, third, fourth, fifth, and sixth potential N-glycosylation sites.

Fig. 7 is a graph showing Polywax elution profiles of Lumizyme[®] (αglucosidase alfa, thinner line, eluting to the left) and ATB200 (thicker line, eluting to the right).

Fig. 8 is a table showing a summary of N-glycan structures of Lumizyme[®] compared to three different preparations of ATB200 rhGAA, identified as BP-rhGAA, ATB200-1 and ATB200-2.

Figs. 9A and 9B are graphs showing the results of CIMPR affinity chromatography of Lumizyme[®] and Myozyme[®], respectively.

Fig. 10A is a graph comparing the CIMPR binding affinity of ATB200 rhGAA (left trace) with that of Lumizyme[®] (right trace). Fig. 10B is a table comparing the bis-M6P content of Lumizyme[®] and ATB200 rhGAA.

Fig. 11A is a graph comparing ATB200 rhGAA activity (left trace) with Lumizyme[®] rhGAA activity (right trace) inside normal fibroblasts at various GAA concentrations. Fig. 11B is a table comparing

ATB200 rhGAA activity (left trace) with Lumizyme® rhGAA activity (right trace) inside fibroblasts from a subject having Pompe Disease at various GAA concentrations. Fig. 11C is a table comparing K_{uptake} of fibroblasts from normal subjects and subjects with Pompe Disease.

Fig. 12 depicts the stability of ATB200 in acidic or neutral pH buffers evaluated in a thermostability assay using SYPRO Orange, as the fluorescence of the dye increases when proteins denature.

Fig. 13 shows tissue glycogen content of WT mice or *Gaa* KO mice treated with a vehicle, alglucosidase alfa, or ATB200/AT2221, determined using amyloglucosidase digestion. Bars represent Mean $\pm$ SEM of 7 mice/group. * $p < 0.05$ compared to alglucosidase alfa in multiple comparison using Dunnett's method under one-way ANOVA analysis.

Fig. 14 depicts LAMP1-positive vesicles in muscle fibers of *Gaa* KO mice treated with a vehicle, alglucosidase alfa, or ATB200/AT2221 or WT mice. Images were taken from *vastus lateralis* and were representative of 7 mice per group. Magnification = 200x (1,000x in insets).

Fig. 15A shows LC3-positive aggregates in muscle fibers of *Gaa* KO mice treated with a vehicle, alglucosidase alfa, or ATB200/AT2221 or WT mice. Images were taken from *vastus lateralis* and were representative of 7 mice per group. Magnification = 400x. Fig. 15B shows a western blot analysis of LC3 II protein. A total of 30 mg protein was loaded in each lane.

Fig. 16 shows Dysferlin expression in muscle fibers of *Gaa* KO mice treated with a vehicle, alglucosidase alfa, or ATB200/AT2221 or WT mice. Images were taken from *vastus lateralis* and were representative of 7 mice per group. Magnification = 200x.

Fig. 17 depicts co-immunofluorescent staining of LAMP1 (green) (see for example, "B") and LC3 (red) (see, for example, "A") in single fibers isolated from the white gastrocnemius of *Gaa* KO mice treated with a vehicle, alglucosidase alfa, or ATB200. "C" depicts clearance of autophagic debris and absence of enlarged lysosome. A minimum of 30 fibers were examined from each animal.

Fig. 18 depicts stabilization of ATB200 by AT2221 at 17 μM , and 170 μM AT2221, respectively, as compared to ATB200 alone.

Figs. 19A and 19B show the ATB200-02 study design. Low dose = 130 mg. High dose = 260 mg. In Fig. 26A, "6MWT" = 6-minute walk test; "FVC" = forced vital capacity; "QOW" = every other week. "a" = safety data from 2 sentinel patients from Cohort 1 were reviewed at each dose level before dosing in Cohorts 2 and 3; "b" = during stages 2 and 3, AT2221 was orally administered prior to the start of ATB200 intravenous infusion. For all doses, ATB200 was intravenously infused for a 4-hour duration, "c" = the first 2 patients in Cohorts 2 and 3 served as sentinel patients for their respective cohorts. Fig. 19C summarizes the baseline characteristics of patients enrolled across Cohorts 1, 2, and 3. "NA" = not applicable. "SD" = standard deviation. "a" = Cohort 1 patients were required to have been on alglucosidase alfa for 2-6 years at baseline. LOPD = late onset Pompe disease.

Fig. 20 depicts pharmacokinetic data for AT2221. "AUC" = area under the curve; "CL/F" = plasma clearance adjusted for AT2221 oral bioavailability; " C_{max} " = maximum drug concentration; "CV" = coefficient of variability; " $t_{1/2}$ " = half-life; " t_{max} " = time to maximum drug concentration; " V_z/F " = apparent terminal phase volume of distribution adjusted for AT2221 oral bioavailability. "a" = geometric mean (CV%); "b" = median (min-max); "c" = arithmetic mean (CV%).

Fig. 21 depicts total GAA protein by signature peptide T09 for Cohorts 1 and 3. "AUC" = area under the curve; "CL_T" = total body clearance; "C_{max}" = maximum drug concentration; "CV" = coefficient of variability; "MD" = multiple doses; "t_{1/2}" = half-life; "t_{max}" = time to maximum drug concentration; "F_{rel}" = AUC Ratio of 20 mg/kg ATB 200 alone and 10 mg/kg ATB200 alone versus 5 mg/kg ATB200 alone, and 20 mg/kg ATB200 + low dose or high dose AT2221 versus 20 mg/kg ATB200 alone. "a" = geometric mean (CV%); "b" = median (min-max); "c" = arithmetic mean (CV%); "d" = n=11; "e" = n=5. Low dose = 130 mg. High dose = 260 mg.

Figs. 22A, 22B, 22C, 22D, 22E, and 22F depict total GAA protein by cohort. Low dose = 130 mg. High dose = 260 mg. Fig. 22A shows the mean total GAA protein concentration-time profiles for Cohort 1 (single dose). Fig. 22B shows the mean total GAA protein concentration-time profiles for Cohort 1 (multiple dose). Fig. 22C shows the mean total GAA protein concentration-time profiles for Cohort 1 vs Cohort 3 (single dose). Fig. 22D shows the mean total GAA protein concentration-time profiles for Cohort 1 vs Cohort 3 (multiple dose). Fig. 22E shows total GAA protein comparisons to 20 mg/kg ATB200 at 12 hours post-dose; * = p<0.05; ** = p <0.01; *** p<0.001. Fig. 22F shows total GAA protein comparisons to 20 mg/kg ATB200 at 24 hours post-dose; * = p<0.05; ** = p <0.01; "ns" = not significant.

Fig. 23 shows an analysis of variance (ANOVA) for total GAA protein by signature peptide T09. The area under the curve (AUC) is provided in µg·h/mL; "CI" = confidence interval.

Fig. 24A depicts a summary of the analyses and available interim data from the 6-Minute Walk Test ("6MWT"), showing the change from baseline ("CFBL") at month 6, month 9, and month 12 for patients in Cohort 1 and Cohort 3.

Fig. 24B depicts 6MWT data for individual Cohort 1 and Cohort 3 patients.

Fig. 24C depicts a summary of the analysis and available interim data from other motor function tests: the Timed up and Go motor function test; the 4-stair climb test; the ten-meter (10M) walk test; gowers; and the gait-stair-gower-chair ("GSGC") motor function test, showing the change from baseline ("CFBL") at month 6, month 9, and month 12 for patients in Cohort 1 and Cohort 3. GSGC is an observer-rated combined score of four motor function assessments: gait (10-meter walk), 4-stair climb, gowers (stand from floor), and rising from chair. Each test is scored from 1 (normal) to 7 (cannot perform; max score of 6 for rising from chair test). Total scores range from 4 to 27. "a" = n=9, missing values not obtained due to patient refusal to perform test; "b" = median change from baseline was -1.5, and 7/9 patients had a decrease; "c" = median change from baseline was -0.8, and 4/5 patients had a decrease.

Fig. 25 depicts a summary of the analysis and available interim data from Muscle Strength Testing (QMT), showing the change from baseline ("CFBL") at month 6 and month 9 for patients in Cohort 2. QMT = quantified muscle test. The values shown represent pounds of force for right and left sides combined. "a" = shoulder adduction not available for one subject; "b" = scoring: (1) visible muscle movement, but no movement at the joint; (2) movement at the joint, but not against gravity; (3) movement against gravity, but not against added resistance; (4) movement against resistance, but less than normal; (5) normal strength.

Fig. 26A depicts of summary of the analysis and available interim data from manual muscle test (MMT) scores in Cohort 1 patients. MMT scores were calculated for upper body (maximum score:

40), lower body (maximum score: 40), and total body (maximum score: 80). Increases in manual muscle strength were observed in Cohort 1 patients at months 6, 9, and 12. "SD" = standard deviation.

Fig. 26B depicts of summary of the analysis and available interim data from manual muscle test (MMT) scores in Cohort 2 patients. MMT scores were calculated for upper body (maximum score: 40). Increases in manual muscle strength were observed in Cohort 2 patients at months 6 and 9. "SD" = standard deviation. MMT results were generally consistent with QMT results (shown in Fig. 28).

Fig. 26C depicts of summary of the analysis and available interim data from manual muscle test (MMT) scores in Cohort 3 patients. MMT scores were calculated for upper body (maximum score: 40), lower body (maximum score: 40), and total body (maximum score: 80). Increases in manual muscle strength were observed in Cohort 3 patients at each of months 6, 9, and 12. "SD" = standard deviation.

Fig. 27 depicts a summary of the analysis and available interim data from sitting forced vital capacity ("FVC"), maximal inspiratory pressure ("MIP"), and maximal expiratory pressure ("MEP"), showing the change from baseline ("CFBL") at month 6, month 9, and month 12 for patients in Cohort 1 and Cohort 3. "a" = FVC not available for one subject. MEP and MIP were measured in cmH₂O.

Fig. 28 depicts a summary of the analysis and available interim data from the Fatigue Severity Scale ("FSS"), a self-assessment questionnaire consisting of nine questions, each scored on a scale of 1 to 7. The total score ranges from 9 to 63, with higher values representing higher level of fatigue due to the disease condition. The normative value in the healthy population is ~21. Fig. 28 shows the change from baseline ("CFBL") at month 6, month 9, and month 12 for patients in Cohort 1, Cohort 2, and Cohort 3.

Figs. 29A-29C depict the mean percentage change from baseline in markers of marker injury (alanine aminotransferase, aspartate aminotransferase, and creatine kinase) in all patient cohorts. Fig. 29A depicts data from Cohort 1 patients over 58 weeks, Fig. 29B depicts data from Cohort 2 patients over 24 weeks, and Fig. 29C depicts data from Cohort 3 patients over 36 weeks. Fig. 29D depicts the mean percentage change from baseline in markers of muscle injury (CK = creatine kinase) and disease substrate (Hex4 = urine hexose tetrasaccharide) for up to 12 months for patients in Cohort 1, Cohort 2, and Cohort 3. "BL" = baseline. "SE" = standard error. "WK" = week. "M" = month.

Fig. 30 summarizes safety data from the ATB200-02 study. "AE" = adverse events. "IAR" = infusion-associated reaction; "a" = Reported through interim data analysis (maximum 20+ months); "b" = Includes upper and lower abdominal pain.

Fig. 31 summarizes available efficacy and safety data from the ATB200-02 study.

Figs. 32A-32H show the results of a site-specific N-glycosylation analysis of ATB200 rhGAA, including an N-glycosylation profile for the seventh potential N-glycosylation site, using LC-MS/MS analysis of protease-digested ATB200. Figs. 32A-32H provide average data for ten lots of ATB200 produced at different scales.

Fig. 32A shows the average site occupancy of the seven potential N-glycosylation sites for ATB200. The N-glycosylation sites are provided according to SEQ ID NO: 1. CV = coefficient of variation.

Figs. 32B-32H show the site-specific N-glycosylation analyses of all seven potential N-glycosylation sites for ATB200, with site numbers provided according to SEQ ID NO: 5. Bars represent the maximum and minimum percentage of N-glycan species identified as a particular N-glycan group for the ten lots of ATB200 analyzed. Fig. 32B shows the N-glycosylation profile of the first potential N-glycosylation site for ATB200. Fig. 32C shows the N-glycosylation profile of the second potential N-glycosylation site for ATB200. Fig. 32D shows the N-glycosylation profile of the third potential N-glycosylation site for ATB200. Fig. 32E shows the N-glycosylation profile of the fourth potential N-glycosylation site for ATB200. Fig. 32F shows the N-glycosylation profile of the fifth potential N-glycosylation site for ATB200. Fig. 32G shows the N-glycosylation profile of the sixth potential N-glycosylation site for ATB200. Fig. 32H shows the N-glycosylation profile of the seventh potential N-glycosylation site for ATB200.

Figs. 33A-33B further characterize and summarize the N-glycosylation profile of ATB200, as also shown in Figs. 32A-32H. Fig. 33A shows 2-Anthranilic acid (2-AA) glycan mapping and LC/MS-MS analysis of ATB200 and summarizes the N-glycan species identified in ATB200 as a percentage of total fluorescence. Data from 2-AA glycan mapping and LC-MS/MS analysis are also depicted in Table 5. Fig. 33B summarizes the average site occupancy and average N-glycan profile, including total phosphorylation, mono-phosphorylation, bis-phosphorylation, and sialylation, for all seven potential N-glycosylation sites for ATB200. ND = not detected.

DETAILED DESCRIPTION

[0013] Before describing several exemplary embodiments of the invention, it is to be understood that the invention is not limited to the details of construction or process steps set forth in the following description. The invention is capable of other embodiments and of being practiced or being carried out in various ways.

I. Definition

[0014] The terms used in this specification generally have their ordinary meanings in the art, within the context of this invention and in the specific context where each term is used. Certain terms are discussed below, or elsewhere in the specification, to provide additional guidance to the practitioner in describing the compositions of the invention and how to make and use them. The articles "a" and "an" refer to one or to more than one (i.e., to at least one) of the grammatical object of the article. The term "or" means, and is used interchangeably with, the term "and/or," unless context clearly indicates otherwise. In this application, the use of the singular includes the plural unless specifically stated otherwise. Furthermore, the use of the term "including," as well as other forms, such as "includes" and "included," are not limiting. Any range described herein will be understood to include the endpoints and all values between the endpoints. In the present

specification, except where the context requires otherwise due to express language or necessary implication, the word "comprises", or variations such as "comprising" is used in an inclusive sense, i.e., to specify the presence of the stated features but not to preclude the presence or addition of further features in various embodiments of the invention.

[0015] The term "GAA" refers to human acid α -glucosidase (GAA) enzyme that catalyzes the hydrolysis of α -1,4- and α -1,6-glycosidic linkages of lysosomal glycogen as well as to insertional, relational, or substitution variants of the GAA amino acid sequence and fragments of a longer GAA sequence that exert enzymatic activity. Human acid α -glucosidase is encoded by the GAA gene (National Centre for Biotechnology Information (NCBI) Gene ID 2548), which has been mapped to the long arm of chromosome 17 (location 17q25.2-q25.3). An exemplary DNA sequence encoding GAA is NP 000143.2. More than 500 mutations have currently been identified in the human GAA gene, many of which are associated with Pompe disease. Mutations resulting in misfolding or misprocessing of the acid α -glucosidase enzyme include T1064C (Leu355Pro) and C2104T (Arg702Cys). In addition, GAA mutations which affect maturation and processing of the enzyme include Leu405Pro and Met519Thr. The conserved hexapeptide WIDMNE at amino acid residues 516-521 is required for activity of the acid α -glucosidase protein. As used herein, the abbreviation "GAA" is intended to refer to human acid α -glucosidase enzyme, while the italicized abbreviation "GAA" is intended to refer to the human gene coding for the human acid α -glucosidase enzyme. The italicized abbreviation "Gaa" is intended to refer to non-human genes coding for non-human acid α -glucosidase enzymes, including but not limited to rat or mouse genes, and the abbreviation "Gaa" is intended to refer to non-human acid α -glucosidase enzymes.

[0016] The term "rhGAA" is intended to refer to the recombinant human acid α -glucosidase enzyme and is used to distinguish endogenous GAA from synthetic or recombinant-produced GAA (e.g., GAA produced from CHO cells transformed with DNA encoding GAA). The term "rhGAA" encompasses a population of individual rhGAA molecules. Characteristics of the population of rhGAA molecules are provided herein. The term "conventional rhGAA product" is intended to refer to products containing α -glucosidase, such as Lumizyme[®] or Myozyme[®].

[0017] The term "genetically modified" or "recombinant" refers to cells, such as CHO cells, that express a particular gene product, such as rhGAA, following introduction of a nucleic acid comprising a coding sequence which encodes the gene product, along with regulatory elements that control expression of the coding sequence. Introduction of the nucleic acid may be accomplished by any method known in the art including gene targeting and homologous recombination. As used herein, the term also includes cells that have been engineered to express or overexpress an endogenous gene or gene product not normally expressed by such cell, e.g., by gene activation technology.

[0018] The term "purified" as used herein refers to material that has been isolated under conditions that reduce or eliminate the presence of unrelated materials, i.e., contaminants, including native materials from which the material is obtained. For example, a purified protein is preferably substantially free of other proteins or nucleic acids with which it is associated in a cell; a purified nucleic acid molecule is preferably substantially free of proteins or other unrelated nucleic acid molecules with which it can be found within a cell. As used herein, the term "substantially free" is used operationally, in the context of analytical testing of the material. Preferably, purified material

substantially free of contaminants is at least 95% pure; more preferably, at least 97% pure, and more preferably still at least 99% pure. Purity can be evaluated by chromatography, gel electrophoresis, immunoassay, composition analysis, biological assay, enzymatic assay and other methods known in the art. In a specific embodiment, purified means that the level of contaminants is below a level acceptable to regulatory authorities for safe administration to a human or non-human animal. Recombinant proteins, such as rhGAA may be isolated or purified from CHO cells using methods known in the art including by chromatographic size separation, affinity chromatography, or anionic exchange chromatography. In some embodiments, rhGAA is purified by a method comprising anionic exchange chromatography followed by immobilized metal affinity chromatography, optionally followed by purification using a third chromatography column.

[0019] As used herein, the term "α-glucosidase alfa" is intended to refer to a recombinant human acid α-glucosidase identified as [199-arginine,223-histidine]prepro-α-glucosidase (human); Chemical Abstracts Registry Number 420794-05-0. α-glucosidase alfa is approved for marketing in the United States by Genzyme, as the products Lumizyme® and Myozyme®.

[0020] As used herein, the term "ATB200" is intended to refer to a recombinant human acid α-glucosidase described in International Application PCT/US2015/053252.

[0021] As used herein, the term "glycan" is intended to refer to a polysaccharide chain covalently bound to an amino acid residue on a protein or polypeptide. As used herein, the term "N-glycan" or "N-linked glycan" is intended to refer to a polysaccharide chain attached to an amino acid residue on a protein or polypeptide through covalent binding to a nitrogen atom of the amino acid residue. For example, an N-glycan can be covalently bound to the side chain nitrogen atom of an asparagine residue. Glycans may contain one or several monosaccharide units, and the monosaccharide units may be covalently linked to form a straight chain or a branched chain. N-glycan units attached to a rhGAA may comprise one or more monosaccharide units each independently selected from N-acetylglucosamine, mannose, galactose, fucose, mannose-6-phosphate, or sialic acid. The N-glycan units on the protein may be determined by any appropriate analytical technique, such as mass spectrometry. The N-glycan units attached to a rhGAA are determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS) utilizing an instrument such as the Thermo Scientific™ Orbitrap Velos Pro™ Mass Spectrometer, Thermo Scientific™ Orbitrap Fusion™ Lumos Tribid™ Mass Spectrometer or Waters Xevo® G2-XS QToF Mass Spectrometer.

[0022] As used herein, the term "high-mannose N-glycan" is intended to refer to an N-glycan having one to six or more mannose units. A high mannose N-glycan unit may contain a bis(N-acetylglucosamine) chain bonded to an asparagine residue and further bonded to a branched polymannose chain. As used herein interchangeably, the term "M6P" or "mannose-6-phosphate" is intended to refer to a mannose unit phosphorylated at the 6 position, i.e., having a phosphate group bonded to the hydroxyl group at the 6 position. One or more mannose units of one or more N-glycan units are phosphorylated at the 6 position to form mannose-6-phosphate units. The term "M6P" or "mannose-6-phosphate" refers to both a mannose phosphodiester having N-acetylglucosamine (GlcNAc) as a "cap" on the phosphate group, as well as a mannose unit having an exposed phosphate group lacking the GlcNAc cap. In at least one embodiment, the N-glycans

of a protein may have multiple M6P groups, with at least one M6P group having a GlcNAc cap and at least one other M6P group lacking a GlcNAc cap.

[0023] As used herein, the term "complex N-glycan" is intended to refer to an N-glycan comprising types of saccharides other than GlcNAc and mannose, for example, one or more galactose and/or sialic acid units. A complex N-glycan can be a high-mannose N-glycan in which one or mannose units are further bonded to one or more monosaccharide units each independently selected from N-acetylglucosamine, galactose, and sialic acid. As used herein, a "hybrid N-glycan" is intended to refer to an N-glycan comprising at least one high-mannose branch and at least one complex branch. Representative structures for non-phosphorylated, mono-M6P, and bis-M6P N-glycans are shown in Fig. 1A. The mannose-6-phosphate group is shown in Fig. 1B.

[0024] As used herein, "normalization" of lysosomes in a muscle refers to the process of restoring the affected muscle to a lysosomal morphology of a wild-type muscle by reducing the size and number of its accumulated glycogen so that the affected muscle would substantially resemble the normal lysosomal morphology, ultimately leading to reverse disease progression.

[0025] As used herein, "reversal of disease progression" means, among other things, adequately (i) reducing or eliminating glycogen accumulation, (ii) reducing or eliminating lysosomal swelling and/or dysfunction, and (iii) reducing or eliminating the buildup of autophagic debris. Reversal of disease progression may manifest in an ambulatory ERT-experienced Pompe disease patient as two or more of the following "clinical improvements": (a) an average increase in six-minute walk test distance of at least 20 meters, (b) an average improvement in maximum expiratory pressure of at least 16 cmH₂O, and (c) an average decrease in fatigue severity scale score of at least 7. Reversal of disease progression may manifest in a nonambulatory ERT-experienced Pompe disease patient as two or more of the following "clinical improvements": (a) an average improvement in shoulder adduction of at least 8 pounds of force, (b) an average improvement in elbow extension of at least 5 pounds of force, and (c) an average decrease in fatigue severity scale score of at least 3.5. Reversal of disease progression may manifest in an ERT-naïve Pompe disease patient as two or more of the following "clinical improvements": (a) an average increase in six-minute walk test distance of at least 40 meters, (b) an average improvement in upright (sitting) forced vital capacity of at least 4%, (c) an average improvement in maximum inspiratory pressure of at least 11 cmH₂O, and (d) an average decrease in fatigue severity scale score of at least 5.

[0026] An advantage of the method of treatment disclosed herein compared to administration of alglucosidase alfa is that Pompe patients treated with the former exhibit prolonged clinical improvement. For example, improvements may be observed at two to three years from the administration of first treatment or beyond, including, for example, four, five, or six years from the administration of first treatment. In contrast, after two years of enzyme replacement therapy with the standard of care (e.g., alglucosidase alfa), Pompe disease patients either (i) maintain their gains from baseline prior to treatment, but exhibit no discernable improvement beyond the two or three-year mark or (ii) experience a gradual decline and lose any gains achieved through two or three years after treatment with the standard of care. Kuperus et al. 2017. Long-term benefit of enzyme replacement therapy in Pompe disease: A 5-year prospective study. *Neurology*. 89:2365-2373. In contrast, the rhGAA described herein clears lysosomal glycogen more efficiently than does the standard of care and has been shown to elicit improvements in patients (e.g., "ERT-switch

ambulatory," Cohort 1 of Study ATB200-02) not expected to improve after taking an enzyme replacement therapy for at least two years. Clinical data to date using the rhGAA or pharmaceutical composition described herein is expected to deliver continued improvement in patient outcomes even after two-years posttreatment. Thus, in some embodiments, a patient treated with the rhGAA or pharmaceutical composition described herein continues to exhibit progress in one or more clinical improvements for more than two years after treatment (e.g., experiences further gains beyond the gain achieved by or at the two-year mark).

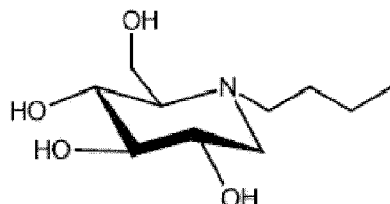
[0027] As used herein, "reversal of lysosomal pathology" means partial or complete clearance of glycogen that had accumulated in the cell due to lack of optimal GAA activity.

[0028] As used herein, forced vital capacity, or "FVC," is the amount of air that can be forcibly exhaled from the lungs of a subject after the subject takes the deepest breath possible.

[0029] As used herein, a "six-minute walk test" (6MWT) is a test for measuring the distance an individual is able to walk over a total of six minutes on a hard, flat surface. The test is conducted by having the individual to walk as far as possible in six minutes.

[0030] As used herein, a "ten-meter walk test" (10MWT) is a test for measuring the time it takes an individual in walking shoes to walk ten meters on a flat surface.

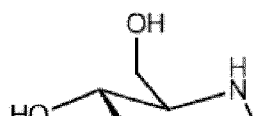
[0031] As used herein, the compound miglustat, also known as N-butyl-1-deoxynojirimycin or NB-DNJ or (2R,3R,4R,5S)-1-butyl-2-(hydroxymethyl)piperidine-3,4,5-triol, is a compound having the following chemical formula:



[0032] One formulation of miglustat is marketed commercially under the trade name Zavesca® as monotherapy for type 1 Gaucher disease. In some embodiments, miglustat is referred to as AT2221.

[0033] As discussed below, pharmaceutically acceptable salts of miglustat may also be used in the present invention. When a salt of miglustat is used, the dosage of the salt will be adjusted so that the dose of miglustat received by the patient is equivalent to the amount which would have been received had the miglustat free base been used.

[0034] As used herein, the compound duvoglustat, also known as 1-deoxynojirimycin or DNJ or (2R,3R,4R,5S)-2-(hydroxymethyl)piperidine-3,4,5-triol, is a compound having the following chemical formula:





[0035] As used herein, the term "pharmacological chaperone" or sometimes simply the term "chaperone" is intended to refer to a molecule that specifically binds to acid α -glucosidase and has one or more of the following effects:

- enhances the formation of a stable molecular conformation of the protein;
- enhances proper trafficking of the protein from the endoplasmic reticulum to another cellular location, preferably a native cellular location, so as to prevent endoplasmic reticulum-associated degradation of the protein;
- prevents aggregation of conformationally unstable or misfolded proteins;
- restores and/or enhances at least partial wild-type function, stability, and/or activity of the protein; and/or
- improves the phenotype or function of the cell harboring acid α -glucosidase.

[0036] Thus, a pharmacological chaperone for acid α -glucosidase is a molecule that binds to acid α -glucosidase, resulting in proper folding, trafficking, non-aggregation, and activity of acid α -glucosidase. As used herein, this term includes but is not limited to active site-specific chaperones (ASSCs) which bind in the active site of the enzyme, inhibitors or antagonists, and agonists. The pharmacological chaperone is an inhibitor or antagonist of acid α -glucosidase. As used herein, the term "antagonist" is intended to refer to any molecule that binds to acid α -glucosidase and either partially or completely blocks, inhibits, reduces, or neutralizes an activity of acid α -glucosidase. The pharmacological chaperone is miglustat.

[0037] As used herein, the term "active site" is intended to refer to a region of a protein that is associated with and necessary for a specific biological activity of the protein. The active site can be a site that binds a substrate or other binding partner and contributes the amino acid residues that directly participate in the making and breaking of chemical bonds. Active sites in this invention can encompass catalytic sites of enzymes, antigen binding sites of antibodies, ligand binding domains of receptors, binding domains of regulators, or receptor binding domains of secreted proteins. The active sites can also encompass transactivation, protein-protein interaction, or DNA binding domains of transcription factors and regulators.

[0038] As used herein, the term "AUC" or "area under the curve" is intended to refer to a mathematical calculation to evaluate the body's total exposure over time to a given drug. In a graph plotting how concentration in the blood of a drug administered to a subject changes with time after dosing, the drug concentration variable lies on the y-axis and time lies on the x-axis. The area between the drug concentration curve and the x-axis for a designated time interval is the AUC. AUCs are used as a guide for dosing schedules and to compare the bioavailability of different drugs' availability in the body.

[0039] As used herein, the term "Cmax" is intended to refer to the maximum plasma concentration

of a drug achieved after administration to a subject.

[0040] As used herein, the term "volume of distribution" or "V" is intended to refer to the theoretical volume that would be necessary to contain the total amount of an administered drug at the same concentration that it is observed in the blood plasma, and represents the degree to which a drug is distributed in body tissue rather than the plasma. Higher values of V indicate a greater degree of tissue distribution. "Central volume of distribution" or "V_c" is intended to refer to the volume of distribution within the blood and tissues highly perfused by blood. "Peripheral volume of distribution" or "V₂" is intended to refer to the volume of distribution within the peripheral tissue.

[0041] As used interchangeably herein, the term "clearance," "systemic clearance," or "CL" is intended to refer to the volume of plasma that is completely cleared of an administered drug per unit time. "Peripheral clearance" is intended to refer to the volume of peripheral tissue that is cleared of an administered drug per unit time.

[0042] As used herein, the term "pharmaceutically acceptable" is intended to refer to molecular entities and compositions that are physiologically tolerable and do not typically produce untoward reactions when administered to a human. Preferably, as used herein, the term "pharmaceutically acceptable" means approved by a regulatory agency of the federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. As used herein, the term "carrier" is intended to refer to a diluent, adjuvant, excipient, or vehicle with which a compound is administered. Suitable pharmaceutical carriers are known in the art and, in at least one embodiment, are described in "Remington's Pharmaceutical Sciences" by E. W. Martin, 18th Edition, or other editions.

[0043] The term "pharmaceutically acceptable salt" as used herein is intended to mean a salt which is, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response, and the like, commensurate with a reasonable benefit/risk ratio, generally water or oil-soluble or dispersible, and effective for their intended use. The term includes pharmaceutically-acceptable acid addition salts and pharmaceutically-acceptable base addition salts. Lists of suitable salts are found in, for example, S. M. Berge et al., J. Pharm. Sci., 1977, 66, pp. 1 -19. The term "pharmaceutically-acceptable acid addition salt" as used herein is intended to mean those salts which retain the biological effectiveness and properties of the free bases and which are not biologically or otherwise undesirable, formed with inorganic acids. The term "pharmaceutically-acceptable base addition salt" as used herein is intended to mean those salts which retain the biological effectiveness and properties of the free acids and which are not biologically or otherwise undesirable, formed with inorganic bases.

[0044] As used herein, the term "buffer" refers to a solution containing a weak acid and its conjugate base that helps to prevent changes in pH.

[0045] As used herein, the terms "therapeutically effective dose" and "effective amount" are intended to refer to an amount of acid α -glucosidase and/or of miglustat and/or of a combination thereof, which is sufficient to result in a therapeutic response in a subject. A therapeutic response may be any response that a user (for example, a clinician) will recognize as an effective response

to the therapy, including any surrogate clinical markers or symptoms described herein and known in the art. Thus, in at least one embodiment, a therapeutic response can be an amelioration or inhibition of one or more symptoms or markers of Pompe disease such as those known in the art. Symptoms or markers of Pompe disease include but are not limited to decreased acid α -glucosidase tissue activity; cardiomyopathy; cardiomegaly; progressive muscle weakness, especially in the trunk or lower limbs; profound hypotonia; macroglossia (and in some cases, protrusion of the tongue); difficulty swallowing, sucking, and/or feeding; respiratory insufficiency; hepatomegaly (moderate); laxity of facial muscles; areflexia; exercise intolerance; exertional dyspnea; orthopnea; sleep apnea; morning headaches; somnolence; lordosis and/or scoliosis; decreased deep tendon reflexes; lower back pain; and failure to meet developmental motor milestones. It should be noted that a concentration of miglustat that has an inhibitory effect on acid α -glucosidase may constitute an "effective amount" for purposes of this invention because of dilution (and consequent shift in binding due to the change in equilibrium and pH), bioavailability, and metabolism of miglustat upon administration in vivo.

[0046] The therapeutic response may also include molecular responses such as glycogen accumulation, lysosomal proliferation, and formation of autophagic zones. The therapeutic responses may be evaluated by comparing physiological and molecular responses of muscle biopsies before and after treatment with a rhGAA described herein. For instance, the amount of glycogen present in the biopsy samples can be used as a marker for determining the therapeutic response. Another example includes biomarkers such as LAMP-1, LC3, and Dysferlin, which can be used as an indicator of lysosomal storage dysfunction. For instance, muscle biopsies collected prior to and after treatment with a rhGAA described herein may be stained with an antibody that recognizes one of the biomarkers.

[0047] As used herein, the term "enzyme replacement therapy" or "ERT" is intended to refer to the introduction of a non-native, purified enzyme into an individual having a deficiency in such enzyme. The administered protein can be obtained from natural sources or by recombinant expression. The term also refers to the introduction of a purified enzyme in an individual otherwise requiring or benefiting from administration of a purified enzyme. In at least one embodiment, such an individual suffers from enzyme insufficiency. The introduced enzyme may be a purified, recombinant enzyme produced in vitro, or a protein purified from isolated tissue or fluid, such as, for example, placenta or animal milk, or from plants.

[0048] As used herein, the term "combination therapy" is intended to refer to any therapy wherein two or more individual therapies are administered concurrently or sequentially. The results of the combination therapy are enhanced as compared to the effect of each therapy when it is performed individually. Enhancement may include any improvement of the effect of the various therapies that may result in an advantageous result as compared to the results achieved by the therapies when performed alone. Enhanced effect or results can include a synergistic enhancement, wherein the enhanced effect is more than the additive effects of each therapy when performed by itself; an additive enhancement, wherein the enhanced effect is substantially equal to the additive effect of each therapy when performed by itself; or less than additive effect, wherein the enhanced effect is lower than the additive effect of each therapy when performed by itself, but still better than the effect of each therapy when performed by itself. Enhanced effect may be measured by any means known in the art by which treatment efficacy or outcome can be measured.

[0049] The term "concurrently" as used herein is intended to mean at the same time as or within a reasonably short period of time before or after, as will be understood by those skilled in the art. For example, if two treatments are administered concurrently with each other, one treatment can be administered before or after the other treatment, to allow for time needed to prepare for the later of the two treatments. Therefore "concurrent administration" of two treatments includes but is not limited to one treatment following the other by about 30 minutes or less, about 30 minutes, 20 minutes or less, about 20 minutes, about 15 minutes, about 10 minutes, about 9 minutes, about 8 minutes, about 7 minutes, about 6 minutes about 5 minutes, about 4 minutes, about 3 minutes, about 2 minutes, about 1 minute, or less than 1 minute.

[0050] "Pompe Disease" refers to an autosomal recessive LSD characterized by deficient acid alpha glucosidase (GAA) activity which impairs lysosomal glycogen metabolism. The enzyme deficiency leads to lysosomal glycogen accumulation and results in progressive skeletal muscle weakness, reduced cardiac function, respiratory insufficiency, and/or CNS impairment at late stages of disease. Genetic mutations in the GAA gene result in either lower expression or produce mutant forms of the enzyme with altered stability, and/or biological activity ultimately leading to disease, (see generally Hirschhorn R, 1995, Glycogen Storage Disease Type II: Acid α -Glucosidase (Acid Maltase) Deficiency, *The Metabolic and Molecular Bases of Inherited Disease*, Scriver et al., eds., McGraw-Hill, New York, 7th ed., pages 2443-2464). The three recognized clinical forms of Pompe Disease (infantile, juvenile and adult) are correlated with the level of residual α -glucosidase activity (Reuser A J et al., 1995, Glycogenosis Type II (Acid Maltase Deficiency), *Muscle & Nerve Supplement* 3, S61-S69). Infantile Pompe disease (type I or A) is most common and most severe, characterized by failure to thrive, generalized hypotonic, cardiac hypertrophy, and cardiorespiratory failure within the second year of life. Juvenile Pompe disease (type II or B) is intermediate in severity and is characterized by a predominance of muscular symptoms without cardiomegaly. Juvenile Pompe individuals usually die before reaching 20 years of age due to respiratory failure. Adult Pompe disease (type III or C) often presents as a slowly progressive myopathy in the teenage years or as late as the sixth decade (Felicia K J et al., 1995, Clinical Variability in Adult-Onset Acid Maltase Deficiency: Report of Affected Sibs and Review of the Literature, *Medicine* 74, 131-135). In Pompe, it has been shown that α -glucosidase is extensively modified post-translationally by glycosylation, phosphorylation, and proteolytic processing. Conversion of the 110 kilodalton (kDa) precursor to 76 and 70 KDa mature forms by proteolysis in the lysosome is required for optimum glycogen catalysis. As used herein, the term "Pompe Disease" refers to all types of Pompe Disease. The formulations and dosing regimens disclosed in this application may be used to treat, for example, Type I, Type II or Type III Pompe Disease.

[0051] A "subject" or "patient" is preferably a human, though other mammals and non-human animals having disorders involving accumulation of glycogen may also be treated. A subject may be a fetus, a neonate, child, juvenile, or an adult with Pompe disease or other glycogen storage or accumulation disorder. One example of an individual being treated is an individual (fetus, neonate, child, juvenile, adolescent, or adult human) having GSD-II (e.g., infantile GSD-II, juvenile GSD-II, or adult-onset GSD-II). The individual can have residual GAA activity, or no measurable activity. For example, the individual having GSD-II can have GAA activity that is less than about 1% of normal GAA activity (infantile GSD-II), GAA activity that is about 1-10% of normal GAA activity (juvenile

GSD-II), or GAA activity that is about 10-40% of normal GAA activity (adult GSD-II). In some embodiments, the subject or patient is an "ERT-experienced" or "ERT-switch" patient, referring to a Pompe disease patient who has previously received enzyme replacement therapy. In some embodiments, the subject or patient is an "ERT-naive" patient, referring to a Pompe disease patient who has not previously received enzyme replacement therapy. In certain embodiments, the subject or patient is ambulatory (e.g., an ambulatory ERT-switch patient or an ambulatory ERT-naive patient). In certain embodiments, the subject or patient is nonambulatory (e.g., a nonambulatory ERT-switch patient). Ambulatory or nonambulatory status may be determined by a six-minute walk test (6MWT). In some embodiments, an ambulatory patient is a Pompe disease patient who is able to walk at least 200 meters in the 6MWT. In some embodiments, a nonambulatory patient is a Pompe disease patient who is unable to walk unassisted or who is wheelchair bound.

[0052] The terms, "treat" and "treatment," as used herein, refer to amelioration of one or more symptoms associated with the disease, prevention or delay of the onset of one or more symptoms of the disease, and/or lessening of the severity or frequency of one or more symptoms of the disease. For example, treatment can refer to improvement of cardiac status (e.g. increase of end-diastolic and/or end-systolic volumes, or reduction, amelioration or prevention of the progressive cardiomyopathy that is typically found in GSD-II) or of pulmonary function (e.g., increase in crying vital capacity over baseline capacity, and/or normalization of oxygen desaturation during crying); improvement in neurodevelopment and/or motor skills (e.g., increase in AIMS score); reduction of glycogen levels in tissue of the individual affected by the disease; or any combination of these effects. In one preferred embodiment, treatment includes improvement of cardiac status, particularly in reduction or prevention of GSD-II-associated cardiomyopathy.

[0053] The terms, "improve," "increase," and "reduce," as used herein, indicate values that are relative to a baseline measurement, such as a measurement in the same individual prior to initiation of the treatment described herein, or a measurement in a control individual (or multiple control individuals) in the absence of the treatment described herein. A control individual is an individual afflicted with the same form of GSD-II (either infantile, juvenile, or adult-onset) as the individual being treated, who is about the same age as the individual being treated (to ensure that the stages of the disease in the treated individual and the control individual(s) are comparable).

[0054] As used herein, the terms "about" and "approximately" are intended to refer to an acceptable degree of error for the quantity measured given the nature or precision of the measurements. For example, the degree of error can be indicated by the number of significant figures provided for the measurement, as is understood in the art, and includes but is not limited to a variation of ± 1 in the most precise significant figure reported for the measurement. Typical exemplary degrees of error are within 20 percent (%), preferably within 10%, and more preferably within 5% of a given value or range of values. Numerical quantities given herein are approximate unless stated otherwise, meaning that the term "about" or "approximately" can be inferred when not expressly stated.

II. Recombinant Human Acid α -Glucosidase (rhGAA)

[0055] In some embodiments, the recombinant human acid α -glucosidase (rhGAA) is an enzyme

having an amino acid sequence as set forth in SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 4, or SEQ ID NO: 5. In some embodiments, the rhGAA is encoded by a nucleotide sequence as set forth in SEQ ID NO: 2.

Table 1. Nucleotide Sequences and Protein Sequences

SEQ ID NO:	Sequences
1	MGVRHPPCSHRLAVCALVSLATAALLGHILLHDFLLVPRELSGSSPVLEETHPAHQQGA SRPGPRDAQAHPRPRAVPTQCDVPPNSRFDCAPDKAITQEQCEARGCCYIPAKQGLQGA QMGQPWCFPPSPYSYKLENLSSEMGYATLTRTPTTFPPKDILTLRLDVMMETENRLH FTIKDPANRRYEVPLETFRVHSRAPSPLYSVEFSEEPFGVIVHRQLDGRVLLNTTVAPLF FADQFLQLSTSLPSQYITGLAEHLSPLMLSTSWTRITLWNRDLAPTGANLYGSHPFYLA LEDGGSAGHVFLNSNAMDVVLQPSALSWRSTGGILDVYIFLGPEPKSVVQQYLDVVGY PFMPYWGFGFHLRCRWGYSSTAITRQVVENMTRAHFPLDVQWNLDDYMDSRDDFTFNKDG FRDFPAMVQELHQGGRRYMMIVDPAISSSGPAGSYRPHYDEGLRRGVFITNETGQPLIGKV WPGSTAFDPDTNPTALA W WEDMVAEFHDQVPFDGMWIDMNEPSNFRGSEDGCPNNELEN PPYVPGVVGGTLQAATICASSHQFLSTHYNLHNLGYLGLTEAIAISHRALVKARGTRPFVISR STFAGHGRIYAGHWTGVDVSSWEQLASSVPEILQFNLLGVPLVGADVCGFLGNTSEELCVR WTQLGAFYPFMRNHSLLSLPQEPYSFSEPAQQAMRKALTLRYALLPHLYTLFHQAHVAG ETVARPLFLEFPKDSSTWTVDHQLLWGEALLITPVLQAGKA EVTGYFPLGTWYDLQTVPI EALGSLPPPPAAPREPAIHSEGQWVTLPAPLDTINVHLRAGYIPLQGPGLTTTESRQQP MALAVALTKGGEARGELFWDDGESLEVLERGAYTQVIFLARNTTIVNELVVRTSEGAGLQ LQKVTVLGVATAPQQVLSNGVPVSNTYSPDTKVLDICVSLLMGEQFLVSWC
2	cagttgggaaagctgaggtgtcgcggggccgcgggtggaggtcggggatgagggcagcaggtaggacagtgacctcggtagcgcgaaggac cccggccacctctagttctctcctcgcgcgggtgttcacgcgagggagggctctgggctcgcgcagctgacgggaaactgagggcagggagcgcg ggcctctagtagctgtccagccatctccaaacctggagtgagggacccggcctgctccaccggcctcctgcccctcgcgcctcgtctccttgc
	gcaaccgctgcaactcctggggcacatcctactcattctctgctgttccccgagagctgagtgctcctccccagtcctggaggagactcacc cagctcaccagcaggggagccagcagaccaggggccccggatgccagccacaccccgccgctccagagcagtgcccacacagtgccagct cccccccaacagccgcttcgattgcgcctcgtacaaggccatcaccaggaacagtgccgagcccgccgctgctgctacatccctgcaaaagcag gggctgcaggggagccagatggggcagccctggtgtcttctccaccagctacccagctgagaaactgagctcctctgaaatggg ctacacggccacccctgacctgaccacccccacccttctcccaaggacatcctgacctcggcgtggagctgagtgatggagactgagaacggcct ccacttcacgatacaagatccagctaacaggcgtacaggggtgcccctggagagccccgcgtgtccacagccgggacccgtcccaactctacagc tggagttctccaggagccctcggggtgctgctgacccggcagctggagcgccgctgctgctgaaacacgacggtggcgccccctgtcttctg gaccagttctctcagctgtccacctcgtgcccctcgcagtgatcacagggcctcggcagacacccagtcacctgctcagcaccagctgagga ggatcacctctgtggaaacgggacgttgcggccacggccggttgcgaacctctacgggtctcacccttctacctggcgctggaggagcggcggtc gcacacgggggtgttctctgctaaacagcaatgcatggtgtggtcctgcagccgagccctgcccctgagctggaggtcgcagacaggtggatcctggat gtctacatcttctggggccagagcccaagagcgtgggtgcagcagctggtgagcttgggataccggttcatgcccactactgggcccgtggct tccacctgtccgctgggggactactcctccaccgctacacccgccaggtgggtggagaacatgaccagggcccacttcccctggagctcgaatgga acgacctggactacatgactcccgaggagcttcacgttcaacaaggatgcttccgggacttcccggccatggtgagagctgcaccagggc ggccggcgctacatgatgaltgtggtatcctgcatcagcagctgggcccctggcgggagctacagggccctacgaggggtctggaggggg gttttcatcacaacagagaccggccagccgctgattgggaaggtatggcccggtctcactgcccctccagctcacaacccccacagccctggcc tgggtgggagacatggtggtgagttcattgaccaggtgcccctgcagggcatgtggtgagatgaacagaccccttcaacttcatcagaggtctg aggagcggctgcccccaaatgagctggagaacccacctacgtgctgggggtggttggggggacccctccagggcggccaccatctgtgctccag ccaccagtttctccacacactacaacctgcacaacctctacggcctgaccgaagccatcgcctccacagggcgctggtggaagctcggggga cacgccatttggatctcccgctcgcactttgtggtccacggccgatacgcgggacactgagcgggggagctgtgagctcctgggagcagctc gcctctcctggtccagaaatcctgcagtttaacctgctgggggtgctctgtgctggggccgagctgtcggcttctggtggcaacacatcagagag ctgtgtgtgctggtggccacagctgggggcttctaccccttcatgaggaaacacacagcctgctcagcttccccaggagccgtacagcttccagc gagccggccagcagggccatgaggaaggccctcaccctggctacgcactcctccccacctctacacactgttccaccagggccacgctcgggg gggagacgttggccggcccccttctctgggttcccccaaggactatgacactggagctgtggaccacacgctcctgtgggggagggccctgctc atcccccagctgctccagggccgggaaggccgaagtgactggctacttcccctgggcacatgtgtacgtcgcagacggtgcgaatagagggccct tggcagcttccacccccacctgcagctccccgtgagccagccatccacagcgaggggcagtggtgagctgcccggccccctgtgacacact caacgtccacctcgggctgggtacatcctccctgcaggggcctggcctcacaacacagagctccgccagcagcccatggccctggctgtgg ccctgaccaggggtgagagggcccgaggggagctgttctgggagcagtgagagagcctggaagtgtcgtgagcagggggcctacacacaggtc atcttcttggcaggaaataacacagctgtgaatgagctgtgactgtgtgacagtgaggagctggcctgacgtgcagaagggtgactgtcctgggc gtggccacggcgccagcaggtcctctccaaagctgtccctgtctcctcaacttccactacagcccgacaccaaaggtcctggacatctgtgtctcgc tgttgatgtgagagcagtttctctgacgtgtgtgttagccggggcggagtggtgtgtctctccagaggggaggtgtgtcccaagggaagcagaccc gtgtgcgggcagcagctgtgtgcccgtgggggttgcagtggtacactggagctgggcaactaacattccaagccggcgcacgtgtgtttccacc tcttggccggggtccttggccccaacgtgtctagagaggttctcctatgacgactgtggccggggcctggagggtgtcctgtgttaataa gattgtaaggttgcctcctcactgttgcggcagtcggggtgattagccacccccctcactgttccagcagcggagaagggggtgctcagg tgagaggtgtgggtatgcacactgagctcctgtctcgcgcctgtcctgcccccaacggcagccgcttccggcgtcccagagggtgtgagctgc cgttccccgagcaagcctgggaactcaggaataatcagagactgggagattctaatttaagtcgaattattttaataaaaggggcatttgggaatc

SEQ ID NO:	Sequences
3	MGVRHPPCSHRLLA VCA LVS LATA ALLGHILLHDFLLVPREL SGSSPVLEETHPAHQQGA SRPGPRDAQAHPRPRAVPTQCDVPPNSRFDCAPDKAITQE QCEARGCCYIPAKQGLQGA QMGQPWCITPPSYPSYKLENLSSEMGYTATLTRTTPITFPKDILTLRLDVMME TENRLH FTIKDPANRRYEVPLETPRVHSRAPSPLYSVEFSEEPFGVIVHRQLDGRVLLNTTVAPLF FADQFLQLSTSLPSQYITGLAEHLSPLMLSTSWTRITLWNRDLAPTPGANLYGSHPFYLA LEDGGS AHGVFLLNSNAMDVVLQPSPALSWRSTGGILDVYIFLGPEPKSVVQQYLDVVG Y PFMPYPWGLGFHL CRWGYSS TAITRQVVENMTRAHFPLDVQWNDLDY MDSRRDFTFNKDG FRDIFPAMVQELHQGGRRYMMIVDPAISSSGPAGSYRPHYDEGLRRGVFITNETGQPLIGKV WPGSTAFPDFTNPTALAWWEDMVAEFHDQVPFDGMWIDMNEPSNFIRGSEDGCPNNELEN PPYVPGVVGGTLQAATICASSHQFLSTHYNLHNL YGLTEAIAASHRALVKARGTRPFVISR STFAGHG RYAGHW TGDVWSSWEQLASSVPEILQFNLLGVPLVGADVCGFLGNTSEELCVR WTQLGAFYPFMRNHNSLLSLPQEPYSFSEPAQQAMRKALTLRYALLPHLYTLFHQAHVAG ETVARPLFLEFPKDSSTWTVDHQLLWGEALLITPVLQAGKAEVTGYFPLGTWYDLQTVPI EALGSLPPPPAAPREPAIHSEGQWVTLPAPLDTINVHLRAGYIIPLQGPGLTTTESRQQP MALAVALTKGGEARGELFWDDGESLEVLERGAYTQVIFLARNTTIVNELVRVTSEGAGLQ LQKVTVLGVATAPQQVLSNGVPVSNFTYSPDTKVLDICVSLLMGEQFLVSWC
4	MGVRHPPCSHRLLA VCA LVS LATA ALLGHILLHDFLLVPREL SGSSPVLEETHPAHQQGA SRPGPRDAQAHPRPRAVPTQCDVPPNSRFDCAPDKAITQE QCEARGCCYIPAKQGLQGA QMGQPWCITPPSYPSYKLENLSSEMGYTATLTRTTPITFPKDILTLRLDVMME TENRLH FTIKDPANRRYEVPLETPRVHSRAPSPLYSVEFSEEPFGVIVRRQLDGRVLLNTTVAPLF FADQFLQLSTSLPSQYITGLAEHLSPLMLSTSWTRITLWNRDLAPTPGANLYGSHPFYLA LEDGGS AHGVFLLNSNAMDVVLQPSPALSWRSTGGILDVYIIFLGPEPKSVVQQYLDVVG Y PFMPYPWGLGFHL CRWGYSS TAITRQVVENMTRAHFPLDVQWNDLDY MDSRRDFTFNKDG FRDFPAMVQELHQGGRRYMMIVDPAISSSGPAGSYRPHYDEGLRRGVFITNETGQPLIGKV WPGSTAFPDFTNPTALAWWEDMVAEFHDQVPFDGMWIDMNEPSNFIRGSEDGCPNNELEN
	PPYVPGVVGGTLQAATICASSHQFLSTHYNLHNL YGLTEAIAASHRALVKARGTRPFVISR STFAGHG RYAGHW TGDVWSSWEQLASSVPEILQFNLLGVPLVGADVCGFLGNTSEELCVR WTQLGAFYPFMRNHNSLLSLPQEPYSFSEPAQQAMRKALTLRYALLPHLYTLFHQAHVAG ETVARPLFLEFPKDSSTWTVDHQLLWGEALLITPVLQAGKAEVTGYFPLGTWYDLQTVPV EALGSLPPPPAAPREPAIHSEGQWVTLPAPLDTINVHLRAGYIIPLQGPGLTTTESRQQP MALAVALTKGGEARGELFWDDGESLEVLERGAYTQVIFLARNTTIVNELVRVTSEGAGLQ LQKVTVLGVATAPQQVLSNGVPVSNFTYSPDTKVLDICVSLLMGEQFLVSWC
5	QQGASRPGPRDAQAHPRPRAVPTQCDVPPNSRFDCAPDKAITQE QCEARGCCYIPAKQG LQGAQMGQPWCFFPPSYPSYKLENLSSEMGYTATLTRTTPITFPKDILTLRLDVMME TE NRLHFTIKDPANRRYEVPLETPRVHSRAPSPLYSVEFSEEPFGVIVHRQLDGRVLLNTTV APLFFADQFLQLSTSLPSQYITGLAEHLSPLMLSTSWTRITLWNRDLAPTPGANLYGSHP FYLALEDGGS AHGVFLLNSNAMDVVLQPSPALSWRSTGGILDVYIIFLGPEPKSVVQQYLD VVGYPFMPYPWGLGFHL CRWGYSS TAITRQVVENMTRAHFPLDVQWNDLDY MDSRRDFTF NKDGFRDIFPAMVQELHQGGRRYMMIVDPAISSSGPAGSYRPHYDEGLRRGVFITNETGQPL IGKVWPGSTAFPDFTNPTALAWWEDMVAEFHDQVPFDGMWIDMNEPSNFIRGSEDGCPNN ELENPPYVPGVVGGTLQAATICASSHQFLSTHYNLHNL YGLTEAIAASHRALVKARGTRPF VISRSTFAGHG RYAGHW TGDVWSSWEQLASSVPEILQFNLLGVPLVGADVCGFLGNTSEE LCVRWTQLGAFYPFMRNHNSLLSLPQEPYSFSEPAQQAMRKALTLRYALLPHLYTLFHQA HVAGETVARPLFLEFPKDSSTWTVDHQLLWGEALLITPVLQAGKAEVTGYFPLGTWYDLQ TVPIEALGSLPPPPAAPREPAIHSEGQWVTLPAPLDTINVHLRAGYIIPLQGPGLTTTES RQQPMALAVALTKGGEARGELFWDDGESLEVLERGAYTQVIFLARNTTIVNELVRVTSEG AGLQLQKVTVLGVATAPQQVLSNGVPVSNFTYSPDTKVLDICVSLLMGEQFLVSWC

[0056] In some embodiments, the rhGAA has a wild-type GAA amino acid sequence as set forth in SEQ ID NO: 1, as described in US Patent No. 8,592,362 and has GenBank accession number

AHE24104.1 (GI:568760974). In some embodiments, the rhGAA has a wild-type GAA amino acid sequence as encoded in SEQ ID NO: 2, the mRNA sequence having GenBank accession number Y00839.1. In some embodiments, the rhGAA has a wild-type GAA amino acid sequence as set forth in SEQ ID NO: 3. In at some embodiments, the rhGAA has a GAA amino acid sequence as set forth in SEQ ID NO: 4, and has National Center for Biotechnology Information (NCBI) accession number NP_000143.2. In some embodiments, the rhGAA is α -glucosidase alfa, the human acid α -glucosidase enzyme encoded by the most predominant of nine observed haplotypes of the GAA gene.

[0057] In some embodiments, the rhGAA is initially expressed as having the full-length 952 amino acid sequence of wild-type GAA as set forth in SEQ ID NO: 1, and the rhGAA undergoes intracellular processing that removes a portion of the amino acids, e.g. the first 56 amino acids. Accordingly, the rhGAA that is secreted by the host cell can have a shorter amino acid sequence than the rhGAA that is initially expressed within the cell. In one embodiment, the shorter protein has the amino acid sequence set forth in SEQ ID NO: 5, which only differs from SEQ ID NO: 1 in that the first 56 amino acids comprising the signal peptide and precursor peptide have been removed, thus resulting in a protein having 896 amino acids. Other variations in the number of amino acids are also possible, such as having 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or more deletions, substitutions and/or insertions relative to the amino acid sequence described by SEQ ID NO: 1 or SEQ ID NO: 5. In some embodiments, the rhGAA product includes a mixture of recombinant human acid α -glucosidase molecules having different amino acid lengths.

[0058] In some embodiments, the rhGAA comprises an amino acid sequence that is at least 90%, 95%, 98% or 99% identical to SEQ ID NO: 1 or SEQ ID NO: 5. Various alignment algorithms and/or programs may be used to calculate the identity between two sequences, including FASTA, or BLAST which are available as a part of the GCG sequence analysis package (University of Wisconsin, Madison, Wis.), and can be used with, e.g., default setting. For example, polypeptides having at least 90%, 95%, 98% or 99% identity to specific polypeptides described herein and preferably exhibiting substantially the same functions, as well as polynucleotide encoding such polypeptides, are contemplated. Unless otherwise indicated a similarity score will be based on use of BLOSUM62. When BLASTP is used, the percent similarity is based on the BLASTP positives score and the percent sequence identity is based on the BLASTP identities score. BLASTP "Identities" shows the number and fraction of total residues in the high scoring sequence pairs which are identical; and BLASTP "Positives" shows the number and fraction of residues for which the alignment scores have positive values and which are similar to each other. Amino acid sequences having these degrees of identity or similarity or any intermediate degree of identity of similarity to the amino acid sequences disclosed herein are contemplated and encompassed by this disclosure. The polynucleotide sequences of similar polypeptides are deduced using the genetic code and may be obtained by conventional means, in particular by reverse translating its amino acid sequence using the genetic code.

[0059] In some embodiments, the rhGAA undergoes post-translational and/or chemical modifications at one or more amino acid residues in the protein. For example, methionine and tryptophan residues can undergo oxidation. As another example, the N-terminal glutamine can form pyro-glutamate. As another example, asparagine residues can undergo deamidation to aspartic acid. As yet another example, aspartic acid residues can undergo isomerization to iso-

aspartic acid. As yet another example, unpaired cysteine residues in the protein can form disulfide bonds with free glutathione and/or cysteine. Accordingly, in some embodiments, the enzyme is initially expressed as having an amino acid sequence as set forth in SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 4, or SEQ ID NO: 5, or an amino acid sequence encoded by SEQ ID NO: 2 and the enzyme undergoes one or more of these post-translational and/or chemical modifications. Such modifications are also within the scope of the present disclosure.

III. N-linked Glycosylation of rhGAA

[0060] There are seven potential N-linked glycosylation sites on a single rhGAA molecule. These potential glycosylation sites are at the following positions of SEQ ID NO: 5: N84, N177, N334, N414, N596, N826, and N869. Similarly, for the full-length amino acid sequence of SEQ ID NO: 1, these potential glycosylation sites are at the following positions: N140, N233, N390, N470, N652, N882, and N925. Other variants of rhGAA can have similar glycosylation sites, depending on the location of asparagine residues. Generally, sequences of Asn-X-Ser or Asn-X-Thr in the protein amino acid sequence indicate potential glycosylation sites, with the exception that X cannot be His or Pro.

[0061] The rhGAA molecules described herein may have, on average, 1, 2, 3, or 4 mannose-6-phosphate (M6P) groups on their N-glycans. For example, only one N-glycan on a rhGAA molecule may bear M6P (mono-phosphorylated or mono-M6P), a single N-glycan may bear two M6P groups (bis-phosphorylated or bis-M6P), or two different N-glycans on the same rhGAA molecule may each bear single M6P groups. In the present invention, the rhGAA molecules described herein on average have 3-4 M6P groups on their N-glycans. Recombinant human acid α -glucosidase molecules may also have N-glycans bearing no M6P groups. In another embodiment, on average the rhGAA comprises greater than 2.5 mol M6P per mol rhGAA and greater than 4 mol sialic acid per mol rhGAA. In some embodiments, on average the rhGAA comprises about 3-3.5 mol M6P per mol rhGAA. In some embodiments, on average the rhGAA comprises about 4-5.4 mol sialic acid per mol rhGAA. On average at least about 3, 4, 5, 6, 7, 8, 9, 10%, or 20% of the total N-glycans on the rhGAA may be in the form of a mono-M6P N-glycan, for example, about 6.25% of the total N-glycans may carry a single M6P group and on average, at least about 0.5, 1, 1.5, 2.0, 2.5, 3.0% of the total N-glycans on the rhGAA are in the form of a bis-M6P N-glycan and on average less than 25% of total rhGAA contains no phosphorylated N-glycan binding to CIMPR. In some embodiments, on average about 10% to about 14% of the total N-glycans on the rhGAA are mono-phosphorylated. In some embodiments, on average about 7% to about 25% of the total N-glycans on the rhGAA are bis-phosphorylated. In some embodiments, on average the rhGAA comprises about 1.3 mol bis-M6P per mol rhGAA.

[0062] The rhGAA described herein may have an average content of N-glycans carrying M6P ranging from 0.5 to 7.0 mol M6P per mol rhGAA or any intermediate value or subrange thereof including 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, or 7.0 mol M6P per mol rhGAA. The rhGAA can be fractionated to provide rhGAA preparations with different average numbers of mono-M6P-bearing or bis-M6P-bearing N-glycans, thus permitting further customization of rhGAA targeting to the lysosomes in target tissues by selecting a particular fraction or by selectively combining different fractions.

[0063] In some embodiments, up to 60% of the N-glycans on the rhGAA may be fully sialylated, for example, up to 10%, 20%, 30%, 40%, 50% or 60% of the N-glycans may be fully sialylated. In some embodiments, no more than 50% of the N-glycans on the rhGAA are fully sialylated. In some embodiments, from 4% to 20% of the total N-glycans are fully sialylated. In other embodiments, no more than 5%, 10%, 20% or 30% of N-glycans on the rhGAA carry sialic acid and a terminal galactose residue (Gal). This range includes all intermediate values and subranges, for example, 7% to 30% of the total N-glycans on the rhGAA can carry sialic acid and terminal galactose. In yet other embodiments, no more than 5%, 10%, 15%, 16%, 17%, 18%, 19%, or 20% of the N-glycans on the rhGAA have a terminal galactose only and do not contain sialic acid. This range includes all intermediate values and subranges, for example, from 8% to 19% of the total N-glycans on the rhGAA in the composition may have terminal galactose only and do not contain sialic acid.

[0064] In some embodiments, 40%, 45%, 50%, or 55% to 60% of the total N-glycans on the rhGAA are complex type N-glycans; or no more than 1%, 2%, 3%, 4%, 5%, 6%, or 7% of total N-glycans on the rhGAA are hybrid-type N-glycans; no more than 5%, 10%, 15%, 20%, or 25% of the high mannose-type N-glycans on the rhGAA are non-phosphorylated; at least 5% or 10% of the high mannose-type N-glycans on the rhGAA are mono-phosphorylated; and/or at least 1% or 2% of the high mannose-type N-glycans on the rhGAA are bis-phosphorylated. These values include all intermediate values and subranges. A rhGAA may meet one or more of the content ranges described above.

[0065] In some embodiments, the rhGAA may bear, on average, 2.0 to 8.0 moles of sialic acid residues per mole of rhGAA. This range includes all intermediate values and subranges thereof, including 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, and 8.0 mol sialic acid residues per mol rhGAA. Without being bound by theory, it is believed that the presence of N-glycan units bearing sialic acid residues may prevent non-productive clearance of the rhGAA by asialoglycoprotein receptors.

[0066] The rhGAA has a certain N-glycosylation profile at certain potential N-glycosylation sites. The rhGAA has seven potential N-glycosylation sites. In some embodiments, at least 20% of the rhGAA is phosphorylated at the first potential N-glycosylation site (e.g., N84 for SEQ ID NO: 5 and N140 for SEQ ID NO: 1). For example, at least 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% of the rhGAA can be phosphorylated at the first potential N-glycosylation site. This phosphorylation can be the result of mono-M6P and/or bis-M6P units. In some embodiments, at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% of the rhGAA bears a mono-M6P unit at the first potential N-glycosylation site. In some embodiments, at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% of the rhGAA bears a bis-M6P unit at the first potential N-glycosylation site. In some embodiments, the rhGAA comprises on average about 1.4 mol M6P (mono-M6P and bis-M6P) per mol rhGAA at the first potential N-glycosylation site. In some embodiments, the rhGAA comprises on average about at least 0.5 mol bis-M6P per mol rhGAA at the first potential N-glycosylation site. In some embodiments, the rhGAA comprises on average about 0.25 mol mono-M6P per mol rhGAA at the first potential N-glycosylation site. In some embodiments, the rhGAA comprises on average about 0.2 mol to about 0.3 mol sialic acid per mol rhGAA at the first potential N-glycosylation site. In at least one embodiment, the rhGAA

comprises a first potential N-glycosylation site occupancy as depicted in Fig. 6A and an N-glycosylation profile as depicted in Fig. 6B. In at least one embodiment, the rhGAA comprises a first potential N-glycosylation site occupancy as depicted in Fig. 32A and an N-glycosylation profile as depicted in Fig. 32B or Fig. 33B.

[0067] In some embodiments, at least 20% of the rhGAA is phosphorylated at the second potential N-glycosylation site (e.g., N177 for SEQ ID NO: 5 and N223 for SEQ ID NO: 1). For example, at least 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% of the rhGAA can be phosphorylated at the second N-glycosylation site. This phosphorylation can be the result of mono-M6P and/or bis-M6P units. In some embodiments, at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% of the rhGAA bears a mono-M6P unit at the second N-glycosylation site. In some embodiments, at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% of the rhGAA bears a bis-M6P unit at the second N-glycosylation site. In some embodiments, the rhGAA comprises on average about 0.5 mol M6P (mono-M6P and bis-M6P) per mol rhGAA at the second potential N-glycosylation site. In some embodiments, the rhGAA comprises on average about 0.4 to about 0.6 mol mono-M6P per mol rhGAA at the second potential N-glycosylation site. In at least one embodiment, the rhGAA comprises a second potential N-glycosylation site occupancy as depicted in Fig. 6A and an N-glycosylation profile as depicted in Fig. 6C. In at least one embodiment, the rhGAA comprises a second potential N-glycosylation site occupancy as depicted in Fig. 32A and an N-glycosylation profile as depicted in Fig. 32C or Fig. 33B.

[0068] In one or more embodiments, at least 5% of the rhGAA is phosphorylated at the third potential N-glycosylation site (e.g., N334 for SEQ ID NO: 5 and N390 for SEQ ID NO: 1). In other embodiments, less than 5%, 10%, 15%, 20%, or 25% of the rhGAA is phosphorylated at the third potential N-glycosylation site. For example, the third potential N-glycosylation site can have a mixture of non-phosphorylated high mannose N-glycans, di-, tri-, and tetra-antennary complex N-glycans, and hybrid N-glycans as the major species. In some embodiments, at least 3%, 5%, 8%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or 50% of the rhGAA is sialylated at the third potential N-glycosylation site. In some embodiments, the rhGAA comprises on average about 0.9 to about 1.2 mol sialic acid per mol rhGAA at the third potential N-glycosylation site. In at least one embodiment, the rhGAA comprises a third potential N-glycosylation site occupancy as depicted in Fig. 6A and an N-glycosylation profile as depicted in Fig. 6D. In at least one embodiment, the rhGAA comprises a third potential N-glycosylation site occupancy as depicted in Fig. 32A and an N-glycosylation profile as depicted in Fig. 32D or Fig. 33B.

[0069] In some embodiments, at least 20% of the rhGAA is phosphorylated at the fourth potential N-glycosylation site (e.g., N414 for SEQ ID NO: 5 and N470 for SEQ ID NO: 1). For example, at least 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% of the rhGAA can be phosphorylated at the fourth potential N-glycosylation site. This phosphorylation can be the result of mono-M6P and/or bis-M6P units. In some embodiments, at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% of the rhGAA bears a mono-M6P unit at the fourth potential N-glycosylation site. In some embodiments, at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% of the rhGAA bears a bis-M6P unit at the fourth potential N-glycosylation site. In some embodiments, at least 3%, 5%, 8%, 10%, 15%, 20%, or 25% of the

rhGAA is sialylated at the fourth potential N-glycosylation site. In some embodiments, the rhGAA comprises on average about 1.4 mol M6P (mono-M6P and bis-M6P) per mol rhGAA at the fourth potential N-glycosylation site. In some embodiments, the rhGAA comprises on average about 0.4 to about 0.6 mol bis-M6P per mol rhGAA at the fourth potential N-glycosylation site. In some embodiments, the rhGAA comprises on average about 0.3 to about 0.4 mol mono-M6P per mol rhGAA at the fourth potential N-glycosylation site. In at least one embodiment, the rhGAA comprises a fourth potential N-glycosylation site occupancy as depicted in Fig. 6A and an N-glycosylation profile as depicted in Fig. 6E. In at least one embodiment, the rhGAA comprises a fourth potential N-glycosylation site occupancy as depicted in Fig. 32A and an N-glycosylation profile as depicted in Fig. 32E or Fig. 33B.

[0070] In some embodiments, at least 5% of the rhGAA is phosphorylated at the fifth potential N-glycosylation site (e.g., N596 for SEQ ID NO: 5 and N692 for SEQ ID NO: 1). In other embodiments, less than 5%, 10%, 15%, 20%, or 25% of the rhGAA is phosphorylated at the fifth potential N-glycosylation site. For example, the fifth potential N-glycosylation site can have fucosylated di-antennary complex N-glycans as the major species. In some embodiments, at least 3%, 5%, 8%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% of the rhGAA is sialylated at the fifth potential N-glycosylation site. In some embodiments, the rhGAA comprises on average about 0.8 to about 0.9 mol sialic acid per mol rhGAA at the fifth potential N-glycosylation site. In at least one embodiment, the rhGAA comprises a fifth potential N-glycosylation site occupancy as depicted in Fig. 6A and an N-glycosylation profile as depicted in Fig. 6F. In at least one embodiment, the rhGAA comprises a fifth potential N-glycosylation site occupancy as depicted in Fig. 32A and an N-glycosylation profile as depicted in Fig. 32F or Fig. 33B.

[0071] In some embodiments, at least 5% of the rhGAA is phosphorylated at the sixth N-glycosylation site (e.g. N826 for SEQ ID NO: 5 and N882 for SEQ ID NO: 1). In other embodiments, less than 5%, 10%, 15%, 20% or 25% of the rhGAA is phosphorylated at the sixth N-glycosylation site. For example, the sixth N-glycosylation site can have a mixture of di-, tri-, and tetra-antennary complex N-glycans as the major species. In some embodiments, at least 3%, 5%, 8%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or 95% of the rhGAA is sialylated at the sixth N-glycosylation site. In some embodiments, the rhGAA comprises on average about 1.5 to about 4.2 mol sialic acid per mol rhGAA at the sixth potential N-glycosylation site. In some embodiments, the rhGAA comprises on average about 0.9 mol acetylated sialic acid per mol rhGAA at the sixth potential N-glycosylation site. In at least one embodiment, the rhGAA comprises a sixth potential N-glycosylation site occupancy as depicted in Fig. 6A and an N-glycosylation profile as depicted in Fig. 6G. In at least one embodiment, the rhGAA comprises a sixth potential N-glycosylation site occupancy as depicted in Fig. 32A and an N-glycosylation profile as depicted in Fig. 32G or Fig. 33B.

[0072] In some embodiments, at least 5% of the rhGAA is phosphorylated at the seventh potential N-glycosylation site (e.g., N869 for SEQ ID NO: 5 and N925 for SEQ ID NO: 1). In other embodiments, less than 5%, 10%, 15%, 20%, or 25% of the rhGAA is phosphorylated at the seventh potential N-glycosylation site. In some embodiments, less than 40%, 45%, 50%, 55%, 60%, or 65% of the rhGAA has any N-glycan at the seventh potential N-glycosylation site. In some embodiments, at least 30%, 35%, or 40% of the rhGAA has an N-glycan at the seventh potential N-

glycosylation site. In some embodiments, the rhGAA comprises on average about 0.86 mol sialic acid per mol rhGAA at the seventh potential N-glycosylation site. In at least one embodiment, all N-glycans identified at the seventh potential N-glycosylation site are complex N-glycans. In at least one embodiment, the rhGAA comprises a seventh potential N-glycosylation site occupancy as depicted in Fig. 6A or as depicted in Fig. 32A and an N-glycosylation profile as depicted in Fig. 32H or Fig. 33B.

[0073] In some embodiments, the rhGAA comprises on average 3-4 M6P residues per rhGAA molecule and about 4 to about 7.3 mol sialic acid per mol rhGAA. In some embodiments, the rhGAA further comprises on average at least about 0.5 mol bis-M6P per mol rhGAA at the first potential N-glycosylation site, about 0.4 to about 0.6 mol mono-M6P per mol rhGAA at the second potential N-glycosylation site, about 0.9 to about 1.2 mol sialic acid per mol rhGAA at the third potential N-glycosylation site, about 0.4 to about 0.6 mol bis-M6P per mol rhGAA at the fourth potential N-glycosylation site, about 0.3 to about 0.4 mol mono-M6P per mol rhGAA at the fourth potential N-glycosylation site, about 0.8 to about 0.9 mol sialic acid per mol rhGAA at the fifth potential N-glycosylation site, and about 1.5 to about 4.2 mol sialic acid per mol rhGAA at the sixth potential N-glycosylation site. In at least one embodiment, the rhGAA further comprises on average about 0.86 mol sialic acid per mol rhGAA at the seventh potential N-glycosylation site. In at least one embodiment, the rhGAA comprises seven potential N-glycosylation sites with occupancy and N-glycosylation profiles as depicted in Figs. 6A-6H. In at least one embodiment, the rhGAA comprises seven potential N-glycosylation sites with occupancy and N-glycosylation profiles as depicted in Figs. 32A-32H and Figs. 33A-33B.

[0074] Methods of making rhGAA are disclosed in U.S. Provisional Patent Application No. 62/057,842, filed September 30, 2014.

[0075] Once inside the lysosome, rhGAA can enzymatically degrade accumulated glycogen. However, conventional rhGAA products have low total levels of mono-M6P- and bis-M6P bearing N-glycans and, thus, target muscle cells poorly, resulting in inferior delivery of rhGAA to the lysosomes. The majority of rhGAA molecules in these conventional products do not have phosphorylated N-glycans, thereby lacking affinity for the CIMPR. Non-phosphorylated high mannose N-glycans can also be cleared by the mannose receptor, which results in non-productive clearance of the ERT (Fig. 2B). In contrast, as shown in Fig. 2A, a rhGAA described herein may contain a higher amount of mono-M6P- and bis-M6P bearing N-glycans, leading to productive uptake of rhGAA into specific tissues such as muscle.

IV. Production and Purification of N-linked Glycosylated rhGAA

[0076] As described in International Application PCT/US2015/053252, cells such as Chinese hamster ovary (CHO) cells may be used to produce the rhGAA described therein. Expressing high M6P rhGAA in CHO cells is advantageous over modifying the glycan profile of an rhGAA post-translationally at least in part because only the former may be converted by glycan degradation to a form of rhGAA with optimal glycogen hydrolysis, thus enhancing therapeutic efficacy.

[0077] The rhGAA is produced by one or more CHO cell lines that are transformed with a DNA

construct encoding the rhGAA described therein. Such CHO cell lines may contain multiple copies of a gene, such as 5, 10, 15, or 20 or more copies, of a polynucleotide encoding GAA. DNA constructs, which express allelic variants of acid α -glucosidase or other variant acid α -glucosidase amino acid sequences such as those that are at least 90%, 95%, 98%, or 99% identical to SEQ ID NO: 1 or SEQ ID NO: 5, may be constructed and expressed in CHO cells. Those of skill in the art may select alternative vectors suitable for transforming CHO cells for production of such DNA constructs.

[0078] Methods for making such CHO cell lines are described in International Application PCT/US2015/053252. Briefly, these methods involve transforming a CHO cell with DNA encoding GAA or a GAA variant, selecting a CHO cell that stably integrates the DNA encoding GAA into its chromosome(s) and that stably expresses GAA, and selecting a CHO cell that expresses GAA having a high content of N-glycans bearing mono-M6P or bis-M6P, and, optionally, selecting a CHO cell having N-glycans with high sialic acid content and/or having N-glycans with a low non-phosphorylated high-mannose content. The selected CHO cell lines may be used to produce rhGAA and rhGAA compositions by culturing the CHO cell line and recovering said composition from the culture of CHO cells. In some embodiments, a rhGAA produced from the selected CHO cell lines contains a high content of N-glycans bearing mono-M6P or bis-M6P that target the CIMPR. In some embodiments, a rhGAA produced as described herein has low levels of complex N-glycans with terminal galactose. In some embodiments, the selected CHO cell lines are referred to as GA-ATB200 or ATB200-X5-14. In some embodiments, the selected CHO cell lines encompass a subculture or derivative of such a CHO cell culture. In some embodiments, a rhGAA produced from the selected CHO cell lines is referred to as ATB200.

[0079] A rhGAA produced as described herein may be purified by following methods described in International Application PCT/US2017/024981 and in U.S. Provisional Application No. 62/506,569. An exemplary process for producing, capturing, and purifying a rhGAA produced from CHO cell lines is shown in Fig. 3.

[0080] Briefly, bioreactor 601 contains a culture of cells, such as CHO cells, that express and secrete rhGAA into the surrounding liquid culture media. The bioreactor 601 may be any appropriate bioreactor for culturing the cells, such as a perfusion, batch or fed-batch bioreactor. The culture media is removed from the bioreactor after a sufficient period of time for cells to produce rhGAA. Such media removal may be continuous for a perfusion bioreactor or may be batch-wise for a batch or fed-batch reactor. The media may be filtered by filtration system 603 to remove cells. Filtration system 603 may be any suitable filtration system, including an alternating tangential flow filtration (ATF) system, a tangential flow filtration (TFF) system, and/or centrifugal filtration system. In various embodiments, the filtration system utilizes a filter having a pore size between about 10 nanometers and about 2 micrometers.

[0081] After filtration, the filtrate is loaded onto a protein capturing system 605. The protein capturing system 605 may include one or more chromatography columns. If more than one chromatography column is used, then the columns may be placed in series so that the next column can begin loading once the first column is loaded. Alternatively, the media removal process can be stopped during the time that the columns are switched.

[0082] In various embodiments, the protein capturing system 605 includes one or more anion exchange (AEX) columns for the direct product capture of rhGAA, particularly rhGAA having a high M6P content. The rhGAA captured by the protein capturing system 605 is eluted from the column(s) by changing the pH and/or salt content in the column. Exemplary conditions for an AEX column are provided in Table 2.

Table 2. Exemplary conditions for an AEX column

Procedure	Buffer	Flow rate (cm/h)	Volume (CV)	Temperature (°C)
Pre-used Sanitization	0.1-10 M NaOH	≤ 25-2500	≥ 1-3 (≥ 10-120 min)	15 - 25
Pre-Equilibration	20-2000 mM phosphate buffer (PB), pH 6.9-7.3	≤ 25-2500	≥ 1-5	15 - 25
Equilibration	4-400 mM PB, pH 6.9-7.3	≤ 25-2500	≥ 1-5	2 - 15
Load	NA	≤ 10-1000	NA	2 - 15
Wash 1	4-400 mM PB, pH 6.9-7.3	≤ 25-2500	≥ 2-10	2 - 15
Wash2	4-400 mM PB, pH 6.9-7.3	≤ 25-2500	≥ 2-10	15 - 25
Elution	4-400 mM PB, 20-2000 mM NaCl, pH 6.1-6.5	≤ 25-2500	NA	15 - 25
Strip	4-400 mM PB, 0.1-10 M NaCl, pH 6.1-6.5	≤ 25-2500	≥ 1-5	15 - 25
Post-use Sanitization	0.1-10 M NaOH	≤ 25-2500	≥ 1-3 (≥ 10-120 min)	15 - 25
Storage	0.01-1.0 M NaOH	≤ 25-2500	≥ 1-5	15 - 25

[0083] The eluted rhGAA can be subjected to further purification steps and/or quality assurance steps. For example, the eluted rhGAA may be subjected to a virus kill step 607. Such a virus kill 607 may include one or more of a low pH kill, a detergent kill, or other technique known in the art. The rhGAA from the virus kill step 607 may be introduced into a second chromatography system 609 to further purify the rhGAA product. Alternatively, the eluted rhGAA from the protein capturing system 605 may be fed directly to the second chromatography system 609. In various embodiments, the second chromatography system 609 includes one or more immobilized metal affinity chromatography (IMAC) columns for further removal of impurities. Exemplary conditions for an IMAC column are provided in Table 3 below.

Table 3. Exemplary conditions for an IMAC column

Procedure	Buffer	Flow rate (cm/h)	Vol (CV)
Rinse	4-400 mM PB, pH 6.3-6.7	≤ 25-2500	≥ 1-5
Pre-use	0.01-1.0 M NaOH	≤ 25-2500	≥ 1-3 (10 -

Procedure	Buffer	Flow rate (cm/h)	Vol (CV)
Sanitization			30 min)
Equilibration	4-400 mM PB, pH 6.5	$\leq 25-2500$	$\geq 1-5$
Wash with WFI	Water For Injection (WFI)	$\leq 25-2500$	$\geq 1-3$
Chelating	0.01-1.0 M Copper Acetate	$\leq 25-2500$	$\geq 1-5$
Wash with WFI	WFI	$\leq 25-2500$	$\geq 2-10$
Wash with acidic buffer	2-200 mM Sodium Acetate, 0.05-5 M NaCl, pH 3.5-4.5	$\leq 25-2500$	$\geq 2-10$
Equilibration	4-400 mM PB, pH 6.3-6.7	$\leq 25-2500$	$\geq 1-5$
Blank run with elution buffer	4-400 mM PB, 15-1500 mM Glycine, pH 6.1-6.5	$\leq 25-2500$	$\geq 2-20$
Equilibration	4-400 mM PB, pH 6.3-6.7	$\leq 25-2500$	$\geq 1-5$
Load	NA	$\leq 25-2500$	$\geq 1-5$
Wash1	4-400 mM PB, pH 6.3-6.7	$\leq 25-2500$	$\geq 2-10$
Wash2	4-400 mM PB, 0.1-10 M NaCl, 5-30% propylene glycol, pH 6.3-6.7	$\leq 25-2500$	$\geq 2-10$
Wash3	4-400 mM PB, pH 6.3-6.7	$\leq 25-2500$	$\geq 2-10$
Elution	4-400 mM PB, 15-1500 mM Glycine, pH 6.1-6.5	$\leq 25-2500$	NA
Strip	4-400 mM PB, 50-5000 mM imidazole, pH 6.3-6.7	$\leq 25-2500$	$\geq 1-5$
Post-use Sanitization	0.01-1M NaOH	$\leq 25-2500$	$\geq 1-3$ (10 - 30 min)
Rinse	4-400 mM PB, pH 6.3-6.7	$\leq 25-2500$	$\geq 1-5$
Storage	5-30% ethanol	$\leq 25-2500$	$\geq 1-5$

[0084] After the rhGAA is loaded onto the second chromatography system 609, the recombinant protein is eluted from the column(s). The eluted rhGAA can be subjected to a virus kill step 611. As with virus kill 607, virus kill 611 may include one or more of a low pH kill, a detergent kill, or other technique known in the art. In some embodiments, only one of virus kill 607 or 611 is used, or the virus kills are performed at the same stage in the purification process.

[0085] The rhGAA from the virus kill step 611 may be introduced into a third chromatography system 613 to further purify the recombinant protein product. Alternatively, the eluted recombinant protein from the second chromatography system 609 may be fed directly to the third chromatography system 613. In various embodiments, the third chromatography system 613 includes one or more cation exchange chromatography (CEX) columns and/or size exclusion chromatography (SEC) columns for further removal of impurities. The rhGAA product is then eluted from the third chromatography system 613. Exemplary conditions for a CEX column are provided in Table 4 below.

Table 4. Exemplary conditions for a CEX column

Procedure	Buffer	Flow rate (cm/h)	Vol (CV)
Pre-used Sanitization	0.1-10 M NaOH	≤ 25-2500	≥ 1-3 (≥ 10-120 min)
Equilibration	2-200 mM Sodium citrate, pH 4.0-5.0	≤ 30-3000	≥ 2-10
Load	NA	≤ 30-3000	NA
Wash	2-200 mM Sodium citrate, pH 4.0-5.0	≤ 30-3000	≥ 2-10
Elution	2-200 mM Sodium citrate, 15-1500 mM NaCl, pH 4.0-5.0	≤ 30-3000	≥ 2-10
Strip	2-200 mM Sodium citrate, 0.1-10 M NaCl, pH 4.0-5.0	≤ 30-3000	≥ 1-5
Post-use Sanitization	0.1-10 M NaOH	≤ 25-2500	≥ 1-3 (≥ 10-120 min)
Storage	0.01-1.0 M NaOH	≤ 30-3000	≥ 1-5

[0086] The rhGAA product may also be subjected to further processing. For example, another filtration system 615 may be used to remove viruses. In some embodiments, such filtration can utilize filters with pore sizes between 5 and 50 μm . Other product processing can include a product adjustment step 617, in which the recombinant protein product may be sterilized, filtered, concentrated, stored, and/or have additional components for added for the final product formulation.

[0087] As used herein, the term "ATB200" refers to a rhGAA with a high content of N-glycans bearing mono-M6P and bis-M6P, which is produced from a GA-ATB200 cell line and purified using methods described herein.

V. Pharmaceutical Composition

[0088] In various embodiments, a pharmaceutical composition comprising the rhGAA described herein, either alone or in combination with other therapeutic agents, and/or a pharmaceutically acceptable carrier, is provided.

[0089] In one or more embodiments, a pharmaceutical composition described herein comprises a pharmaceutically acceptable salt.

[0090] In some embodiments, the pharmaceutically acceptable salt used herein is a pharmaceutically-acceptable acid addition salt. The pharmaceutically-acceptable acid addition salt may include, but is not limited to, hydrochloric acid, hydrobromic acid, sulfuric acid, sulfamic acid, nitric acid, phosphoric acid, and the like, and organic acids including but not limited to acetic acid, trifluoroacetic acid, adipic acid, ascorbic acid, aspartic acid, benzenesulfonic acid, benzoic acid, butyric acid, camphoric acid, camphorsulfonic acid, cinnamic acid, citric acid, digluconic acid,

ethanesulfonic acid, glutamic acid, glycolic acid, glycerophosphoric acid, hemisulfic acid, hexanoic acid, formic acid, fumaric acid, 2-hydroxyethanesulfonic acid (isethionic acid), lactic acid, hydroxymaleic acid, malic acid, malonic acid, mandelic acid, mesitylenesulfonic acid, methanesulfonic acid, naphthalenesulfonic acid, nicotinic acid, 2-naphthalenesulfonic acid, oxalic acid, pantoic acid, pectinic acid, phenylacetic acid, 3-phenylpropionic acid, pivalic acid, propionic acid, pyruvic acid, salicylic acid, stearic acid, succinic acid, sulfanilic acid, tartaric acid, p-toluenesulfonic acid, undecanoic acid, and the like.

[0091] In some embodiments, the pharmaceutically acceptable salt used herein is a pharmaceutically-acceptable base addition salt. The pharmaceutically-acceptable base addition salt may include, but is not limited to, ammonia or the hydroxide, carbonate, or bicarbonate of ammonium or a metal cation such as sodium, potassium, lithium, calcium, magnesium, iron, zinc, copper, manganese, aluminum, and the like. Salts derived from pharmaceutically-acceptable organic nontoxic bases include, but are not limited to, salts of primary, secondary, and tertiary amines, quaternary amine compounds, substituted amines including naturally occurring substituted amines, cyclic amines and basic ion-exchange resins, such as methylamine, dimethylamine, trimethylamine, ethylamine, diethylamine, triethylamine, isopropylamine, tripropylamine, tributylamine, ethanolamine, diethanolamine, 2-dimethylaminoethanol, 2-diethylaminoethanol, dicyclohexylamine, lysine, arginine, histidine, caffeine, hydrabamine, choline, betaine, ethylenediamine, glucosamine, methylglucamine, theobromine, purines, piperazine, piperidine, N-ethylpiperidine, tetramethylammonium compounds, tetraethylammonium compounds, pyridine, N,N-dimethylaniline, N-methylpiperidine, N-methylmorpholine, dicyclohexylamine, dibenzylamine, N,N-dibenzylphenethylamine, 1-phenylamine, N,N'-dibenzylethylenediamine, polyamine resins, and the like.

[0092] In some embodiments, the rhGAA or a pharmaceutically acceptable salt thereof may be formulated as a pharmaceutical composition adapted for intravenous administration. In some embodiments, the pharmaceutical composition is a solution in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic to ease pain at the site of the injection. The ingredients of the pharmaceutical composition may be supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampule or sachet indicating the quantity of active agent. Where the composition is to be administered by infusion, it may be dispensed with an infusion bottle containing sterile pharmaceutical grade water, saline or dextrose/water. In some embodiments, the infusion may occur at a hospital or clinic. In some embodiments, the infusion may occur outside the hospital or clinic setting, for example, at a subject's residence. Where the composition is administered by injection, an ampule of sterile water for injection or saline may be provided so that the ingredients may be mixed prior to administration.

[0093] In some embodiments, the rhGAA or a pharmaceutically acceptable salt thereof may be formulated for oral administration. Orally administrable compositions may be formulated in a form of tablets, capsules, ovules, elixirs, solutions or suspensions, gels, syrups, mouth washes, or a dry powder for reconstitution with water or other suitable vehicle before use, optionally with flavoring and coloring agents for immediate-, delayed-, modified-, sustained-, pulsed-, or controlled-release applications. Solid compositions such as tablets, capsules, lozenges, pastilles, pills, boluses, powder, pastes, granules, bullets, dragees, or premix preparations can also be used. Solid and

liquid compositions for oral use may be prepared according to methods well known in the art. Such compositions can also contain one or more pharmaceutically acceptable carriers and excipients which can be in solid or liquid form. Tablets or capsules can be prepared by conventional means with pharmaceutically acceptable excipients, including but not limited to binding agents, fillers, lubricants, disintegrants, or wetting agents. Suitable pharmaceutically acceptable excipients are known in the art and include but are not limited to pregelatinized starch, polyvinylpyrrolidone, povidone, hydroxypropyl methylcellulose (HPMC), hydroxypropyl ethylcellulose (HPEC), hydroxypropyl cellulose (HPC), sucrose, gelatin, acacia, lactose, microcrystalline cellulose, calcium hydrogen phosphate, magnesium stearate, stearic acid, glyceryl behenate, talc, silica, corn, potato or tapioca starch, sodium starch glycolate, sodium lauryl sulfate, sodium citrate, calcium carbonate, dibasic calcium phosphate, glycine croscarmellose sodium, and complex silicates. Tablets can be coated by methods well known in the art.

[0094] In some embodiments, a pharmaceutical composition described herein may be formulated according to International Application PCT/US2017/024982 and U.S. Provisional Application No. 62/506,574. For instance, in some embodiments, the pH of a pharmaceutical composition described herein is from about 5.0 to about 7.0 or about 5.0 to about 6.0. In some embodiments, the pH ranges from about 5.5 to about 6.0. In some embodiments, the pH of the pharmaceutical composition is 6.0. In some embodiments, the pH may be adjusted to a target pH by using pH adjusters (e.g., alkalinizing agents and acidifying agents) such as sodium hydroxide and/or hydrochloric acid.

[0095] The pharmaceutical composition described herein may comprise a buffer system such as a citrate system, a phosphate system, and a combination thereof. The citrate and/or phosphate may be a sodium citrate or sodium phosphate. Other salts include potassium and ammonium salts. In one or more embodiments, the buffer comprises a citrate. In further embodiments, the buffer comprises sodium citrate (e.g., a mixture of sodium citrate dehydrate and citric acid monohydrate). In one or more embodiments, buffer solutions comprising a citrate may comprise sodium citrate and citric acid. In some embodiments, both a citrate and phosphate buffer are present.

[0096] In some embodiments, a pharmaceutical composition described herein comprises at least one excipient. The excipient may function as a tonicity agent, bulking agent, and/or stabilizer. Tonicity agents are components which help to ensure the formulation has an osmotic pressure similar to or the same as human blood. Bulking agents are ingredients which add mass to the formulations (e.g. lyophilized) and provide an adequate structure to the cake. Stabilizers are compounds that can prevent or minimize the aggregate formation at the hydrophobic air-water interfacial surfaces. One excipient may function as a tonicity agent and bulking agent at the same time. For instance, mannitol may function as a tonicity agent and also provide benefits as a bulking agent.

[0097] Examples of tonicity agents include sodium chloride, mannitol, sucrose, and trehalose. In some embodiments, the tonicity agent comprises mannitol. In some embodiments, the total amount of tonicity agent(s) ranges in an amount of from about 10 mg/mL to about 50 mg/mL. In further embodiments, the total amount of tonicity agent(s) ranges in an amount of from about 10, 11, 12, 13, 14, or 15 mg/mL to about 16, 20, 25, 30, 35, 40, 45, or 50 mg/mL.

[0098] In some embodiments, the excipient comprises a stabilizer. In some embodiments, the stabilizer is a surfactant. In some embodiments, the stabilizer is polysorbate 80. In one or more embodiments, the total amount of stabilizer ranges from about 0.1 mg/mL to about 1.0 mg/mL. In further embodiments, the total amount of stabilizer ranges from about 0.1, 0.2, 0.3, 0.4, or 0.5 mg/mL to about 0.5, 0.6, 0.7, 0.8, 0.9, or 1.0 mg/mL. In yet further embodiments, the total amount of stabilizer is about 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, or 1.0 mg/mL.

[0099] In some embodiments, a pharmaceutical composition comprises (a) a rhGAA (such as ATB200), (b) at least one buffer selected from the group consisting of a citrate, a phosphate, and a combination thereof, and (c) at least one excipient selected from the group consisting of mannitol, polysorbate 80, and a combination thereof, and has a pH of (i) from about 5.0 to about 6.0, or (ii) from about 5.0 to about 7.0. In some embodiments, the composition further comprises water. In some embodiments, the composition may further comprise an acidifying agent and/or alkalizing agent.

[0100] In some embodiments, the pharmaceutical composition comprises (a) a rhGAA (such as ATB200) at a concentration of about 5-50 mg/mL, about 5-30 mg/mL, or about 15 mg/mL, (b) sodium citrate buffer at a concentration of about 10-100 mM or about 25 mM, (c) mannitol at a concentration of about 10-50 mg/mL, or about 20 mg/mL, (d) polysorbate 80, present at a concentration of about 0.1-1 mg/mL, about 0.2-0.5 mg/mL, or about 0.5 mg/mL, and (e) water, and has a pH of about 6.0. In at least one embodiment, the pharmaceutical composition comprises (a) 15 mg/mL rhGAA (such as ATB200) (b) 25 mM sodium citrate buffer, (c) 20 mg/mL mannitol (d) 0.5 mg/mL polysorbate 80, and (e) water, and has a pH of about 6.0. In some embodiments, the composition may further comprise an acidifying agent and/or alkalizing agent.

[0101] In some embodiments, the pharmaceutical composition comprising rhGAA is diluted prior to administration to a subject in need thereof.

[0102] In some embodiments, a pharmaceutical composition described herein comprises a chaperone. In some embodiments, the chaperone is miglustat or a pharmaceutically acceptable salt thereof. In another embodiment, the chaperone is duvoglustat or a pharmaceutically acceptable salt thereof.

[0103] In some embodiments, a rhGAA described herein is formulated in one pharmaceutical composition while a chaperone such as miglustat is formulated in another pharmaceutical composition. In some embodiments, the pharmaceutical composition comprising miglustat is based on a formulation available commercially as Zavesca[®] (Actelion Pharmaceuticals).

[0104] In some embodiments, the pharmaceutical composition described herein may undergo lyophilization (freeze-drying) process to provide a cake or powder. Accordingly, another aspect of the invention pertains to a pharmaceutical composition after lyophilization. The lyophilized mixture may comprise the rhGAA described herein (e.g., ATB200), buffer selected from the group consisting of a citrate, a phosphate, and combinations thereof, and at least one excipient selected from the group consisting of trehalose, mannitol, polysorbate 80, and a combination thereof. In some embodiments, other ingredients (e.g., other excipients) may be added to the lyophilized mixture. The pharmaceutical composition comprising the lyophilized formulation may be provided

vial, which then can be stored, transported, reconstituted and/or administered to a patient.

VI. Methods of Treatment

A. Treatment of Diseases

[0105] Another aspect of the disclosure pertains to a method of treatment of a disease or disorder related to glycogen storage dysregulation by administering the rhGAA or pharmaceutical composition described herein. In some examples, the disease is Pompe disease (also known as acid maltase deficiency (AMD) and glycogen storage disease type II (GSD II)). In some embodiments, the rhGAA is ATB200. In some embodiments, the pharmaceutical composition comprises ATB200.

[0106] The rhGAA or pharmaceutical composition described herein is administered by an appropriate route. The rhGAA or pharmaceutical composition is administered intravenously in the present invention.

[0107] In some embodiments, the therapeutic effects of the rhGAA or pharmaceutical composition described herein may be assessed based on one or more of the following criteria: (1) cardiac status (e.g., increase of end-diastolic and/or end-systolic volumes, or reduction, amelioration or prevention of the progressive cardiomyopathy that is typically found in GSD-II), (2) pulmonary function (e.g., increase in crying vital capacity over baseline capacity, and/or normalization of oxygen desaturation during crying), (3) neurodevelopment and/or motor skills (e.g., increase in AIMS score), and (4) reduction of glycogen levels in tissue of the individual affected by the disease.

[0108] In some embodiments, the cardiac status of a subject is improved by 10%, 20%, 30%, 40%, or 50% (or any percentage in-between) after administration of one or more dosages of the rhGAA or pharmaceutical composition described herein, as compared to that of a subject treated with a vehicle or that of a subject prior to treatment. The cardiac status of a subject may be assessed by measuring end-diastolic and/or end-systolic volumes and/or by clinically evaluating cardiomyopathy. In some embodiments, the pulmonary function of a subject is improved by 10%, 20%, 30%, 40%, or 50% (or any percentage in-between) after administration of one or more dosages of ATB200 or pharmaceutical composition comprising ATB200, as compared to that of a subject treated with a vehicle or that of a subject prior to treatment. In certain embodiments, the improvement is achieved after 1 week, 2 weeks, 3 weeks, 1 month, 2 months, or more from administration (or any time period in between). In certain embodiments, ATB200 or pharmaceutical composition comprising ATB200 improves the pulmonary function of a subject after 1 week, 2 weeks, 3 weeks, 1 month, 2 months, or more from administration (or any time period in between).

[0109] In some embodiments, the pulmonary function of a subject is improved by 10%, 20%, 30%, 40%, or 50% (or any percentage in-between) after administration of one or more dosages of the rhGAA or pharmaceutical composition described herein, as compared to that of a subject treated with a vehicle or that of a subject prior to treatment. The pulmonary function of a subject may be

assessed by crying vital capacity over baseline capacity, and/or normalization of oxygen desaturation during crying. In some embodiments, the pulmonary function of a subject is improved by 10%, 20%, 30%, 40%, or 50% (or any percentage in-between) after administration of one or more dosages of ATB200 or pharmaceutical composition comprising ATB200, as compared to that of a subject treated with a vehicle or that of a subject prior to treatment. In certain embodiments, the improvement is achieved after 1 week, 2 weeks, 3 weeks, 1 month, 2 months, or more from administration (or any time period in between). In certain embodiments, ATB200 or pharmaceutical composition comprising ATB200 improves the pulmonary function of a subject after 1 week, 2 weeks, 3 weeks, 1 month, 2 months, or more from administration (or any time period in between).

[0110] In some embodiments, the neurodevelopment and/or motor skills of a subject is improved by 10%, 20%, 30%, 40%, or 50% (or any percentage in-between) after administration of one or more dosages of the rhGAA or pharmaceutical composition described herein, as compared to that of a subject treated with a vehicle or that of a subject prior to treatment. The neurodevelopment and/or motor skills of a subject may be assessed by determining an AIMS score. The AIMS is a 12-item anchored scale that is clinician-administered and scored (see Rush JA Jr., Handbook of Psychiatric Measures, American Psychiatric Association, 2000, 166-168). Items 1-10 are rated on a 5-point anchored scale. Items 1-4 assess orofacial movements. Items 5-7 deal with extremity and truncal dyskinesia. Items 8-10 deal with global severity as judged by the examiner, and the patient's awareness of the movements and the distress associated with them. Items 11-12 are yes/no questions concerning problems with teeth and/or dentures (such problems can lead to a mistaken diagnosis of dyskinesia). In some embodiments, the neurodevelopment and/or motor skills of a subject is improved by 10%, 20%, 30%, 40%, or 50% (or any percentage in-between) after administration of one or more dosages of ATB200 or pharmaceutical composition comprising ATB200, as compared to that of a subject treated with a vehicle or that of a subject prior to treatment. In certain embodiments, the improvement is achieved after 1 week, 2 weeks, 3 weeks, 1 month, 2 months, or more from administration (or any time period in between). In certain embodiments, ATB200 or pharmaceutical composition comprising ATB200 improves the neurodevelopment and/or motor skills of a subject after 1 week, 2 weeks, 3 weeks, 1 month, 2 months, or more from administration (or any time period in between).

[0111] In some embodiments, the glycogen level of a certain tissue of a subject is reduced by 10%, 20%, 30%, 40%, or 50% (or any percentage in-between) after administration of one or more dosages of the rhGAA or pharmaceutical composition described herein, as compared to that of a subject treated with a vehicle or that of a subject prior to treatment. In some embodiment, the tissue is muscle such as quadriceps, triceps, and gastrocnemius. The glycogen level of a tissue can be analyzed using methods known in the art. The determination of glycogen levels is well known based on amyloglucosidase digestion, and is described in publications such as: Amalfitano et al. (1999), "Systemic correction of the muscle disorder glycogen storage disease type ii after hepatic targeting of a modified adenovirus vector encoding human acid-alpha-glucosidase," Proc Natl Acad Sci USA, 96:8861-8866. In some embodiments, the glycogen level in muscle of a subject is reduced by 10%, 20%, 30%, 40%, or 50% (or any percentage in between) after administration of one or more dosages of ATB200 or pharmaceutical composition comprising ATB200, as compared to that of a subject treated with a vehicle or that of a subject prior to treatment. In certain embodiments, the reduction is achieved after 1 week, 2 weeks, 3 weeks, 1 month, 2 months, or more from administration (or any time period in between). In certain embodiments, ATB200 or

pharmaceutical composition comprising ATB200 reduces the glycogen level in muscle of a subject after 1 week, 2 weeks, 3 weeks, 1 month, 2 months, or more from administration (or any time period in between).

B. Biomarkers

[0112] Biomarkers of glycogen accumulation in a muscle fiber in a subject, such as urine hexose tetrasaccharide (Hex4), may be used to assess and compare the therapeutic effects of enzyme replacement therapy in a subject with Pompe disease. In some embodiments, the therapeutic effect of the rhGAA or a pharmaceutical composition comprising rhGAA on glycogen accumulation is assessed by measuring the levels of Hex4 in a subject.

[0113] Biomarkers of muscle injury or damage such as creatine kinase (CK), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) may be used to assess and compare the therapeutic effects of enzyme replacement therapy in a subject with Pompe disease. In some embodiments, the therapeutic effect of the rhGAA or a pharmaceutical composition comprising rhGAA on muscle damage is assessed by measuring the levels of CK, ALT, and/or AST in a subject. In at least one embodiment, the therapeutic effect of the rhGAA or a pharmaceutical composition comprising rhGAA on muscle damage is assessed by measuring the levels of CK in a subject.

[0114] Biomarkers such as LAMP-1, LC3, and Dysferlin may also be used to assess and compare the therapeutic effects of the rhGAA or pharmaceutical composition described herein. In Pompe disease, the failure of GAA to hydrolyze lysosomal glycogen leads to the abnormal accumulation of large lysosomes filled with glycogen in some tissues. (Raben et al., JBC 273: 19086-19092, 1998.) Studies in a mouse model of Pompe disease have shown that the enlarged lysosomes in skeletal muscle cannot adequately account for the reduction in mechanical performance, and that the presence of large inclusions containing degraded myofibrils (i.e., autophagic buildup) contributes to the impairment of muscle function. (Raben et al., Human Mol Genet 17: 3897-3908, 2008.) Reports also suggest that impaired autophagy flux is associated with poor therapeutic outcome in Pompe patients. (Nascimbeni et al., Neuropathology and Applied Neurobiology doi: 10.1111/nan.12214, 2015; Fukuda et al., Mol Ther 14: 831-839, 2006.) In addition, late-onset Pompe disease is prevalent in unclassified limb-girdle muscular dystrophies (LGMDs) (Preisler et al., Mol Genet Metab 110: 287-289, 2013), which is a group of genetically heterogeneous neuromuscular diseases with more than 30 genetically defined subtypes of varying severity. IHC examination revealed substantially elevated sarcoplasmic presence of dysferlin in the skeletal muscle fibers of Gaa KO mice.

[0115] Various known methods can be used to measure the gene expression level and/or protein level of such biomarkers. For instance, a sample from a subject treated with the rhGAA or pharmaceutical composition described herein can be obtained, such as biopsy of tissues, in particular muscle. In some embodiments, the sample is a biopsy of muscle in a subject. In some embodiments, the muscle is selected from quadriceps, triceps, and gastrocnemius. The sample obtained from a subject may be stained with one or more antibodies or other detection agents that detect such biomarkers or be identified and quantified by mass spectrometry. The samples may

also or alternatively be processed for detecting the presence of nucleic acids, such as mRNAs, encoding the biomarkers via, e.g., RT-qPCR methods.

[0116] In some embodiments, the gene expression level and/or protein level of one or more biomarkers is measured in a muscle biopsy obtained from an individual prior to and post treatment with the rhGAA or pharmaceutical composition described herein. In some embodiments, the gene expression level and/or protein level of one or more biomarkers is measured in a muscle biopsy obtained from an individual treated with a vehicle. In some embodiments, the gene expression level and/or protein level of one or more biomarkers is reduced by 10%, 20%, 30%, 40%, or 50% (or any percentage in-between) after administration of one or more dosages of the rhGAA or pharmaceutical composition described herein, as compared to that of a subject treated with a vehicle or that of a subject prior to treatment. In some embodiments, the gene expression level and/or protein level of one or more biomarkers is reduced by 10%, 20%, 30%, 40%, or 50% (or any percentage in-between) after administration of one or more dosages of ATB200 or pharmaceutical composition comprising ATB200, as compared to that of a subject treated with a vehicle or that of a subject prior to treatment. In certain embodiments, the reduction is achieved after 1 week, 2 weeks, 3 weeks, 1 month, 2 months, or more from administration (or any time period in between). In certain embodiments, ATB200 or pharmaceutical composition comprising ATB200 reduces the gene expression level and/or protein level of one or more biomarkers after 1 week, 2 weeks, 3 weeks, 1 month, 2 months, or more from administration (or any time period in between).

C. Dosages of rhGAA

[0117] The pharmaceutical formulation or reconstituted composition is administered in a therapeutically effective amount (e.g., a dosage amount that, when administered at regular intervals, is sufficient to treat the disease, such as by ameliorating symptoms associated with the disease, preventing or delaying the onset of the disease, and/or lessening the severity or frequency of symptoms of the disease). The amount which is therapeutically effective in the treatment of the disease may depend on the nature and extent of the disease's effects, and can be determined by standard clinical techniques. In addition, *in vitro* or *in vivo* assays may optionally be employed to help identify optimal dosage ranges. In at least one embodiment, a rhGAA described herein or pharmaceutical composition comprising the rhGAA is administered at a dose of about 1 mg/kg to about 100 mg/kg, such as about 5 mg/kg to about 30 mg/kg, typically about 5 mg/kg to about 20 mg/kg. In at least one embodiment, the rhGAA or pharmaceutical composition described herein is administered at a dose of about 5 mg/kg, about 10 mg/kg, about 15 mg/kg, about 20 mg/kg, about 25 mg/kg, about 30 mg/kg, about 35 mg/kg, about 40 mg/kg, about 50 mg/kg, about 50 mg/kg, about 60 mg/kg, about 70 mg/kg, about 80 mg/kg, about 90 mg/kg, or about 100 mg/kg. In some embodiments, the rhGAA is administered at a dose of 5 mg/kg, 10 mg/kg, 20 mg/kg, 50 mg/kg, 75 mg/kg, or 100 mg/kg. In at least one embodiment, the rhGAA or pharmaceutical composition is administered at a dose of about 20 mg/kg. In some embodiments, the rhGAA or pharmaceutical composition is administered concurrently or sequentially with a pharmacological chaperone. In some embodiments, the pharmacological chaperone is miglustat. In at least one embodiment, the miglustat is administered as an oral dose of about 260 mg. The effective dose for a particular individual can be varied (e.g. increased or decreased) over time, depending on the

needs of the individual. For example, in times of physical illness or stress, or if anti-acid α -glucosidase antibodies become present or increase, or if disease symptoms worsen, the amount can be increased.

[0118] In some embodiments, the therapeutically effective dose of the rhGAA or pharmaceutical composition described herein is lower than that of conventional rhGAA products. For instance, if the therapeutically effective dose of a conventional rhGAA product is 20 mg/kg, the dose of the rhGAA or pharmaceutical composition described herein required to produce the same as or better therapeutic effects than the conventional rhGAA product may be lower than 20 mg/kg. Therapeutic effects may be assessed based on one or more criteria discussed above (e.g., cardiac status, glycogen level, or biomarker expression). In some embodiments, the therapeutically effective dose of the rhGAA or pharmaceutical composition described herein is at least about 5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more lower than that of conventional rhGAA products.

[0119] In some embodiments, the therapeutic effect of the rhGAA or pharmaceutical composition described herein comprises an improvement in motor function, an improvement in muscle strength (upper-body, lower-body, or total-body), an improvement in pulmonary function, decreased fatigue, reduced levels of at least one biomarker of muscle injury, reduced levels of at least one biomarker of glycogen accumulation, or a combination thereof. In some embodiments, the therapeutic effect of the rhGAA or pharmaceutical composition described herein comprises a reversal of lysosomal pathology in a muscle fiber, a faster and/or more effective reduction in glycogen content in a muscle fiber, an increase in six-minute walk test distance, a decrease in timed up and go test time, a decrease in four-stair climb test time, a decrease in ten-meter walk test time, a decrease in gait-stair-gower-chair score, an increase in upper extremity strength, an improvement in shoulder adduction, an improvement in shoulder abduction, an improvement in elbow flexion, an improvement in elbow extension, an improvement in upper body strength, an improvement in lower body strength, an improvement in total body strength, an improvement in upright (sitting) forced vital capacity, an improvement in maximum expiratory pressure, an improvement in maximum inspiratory pressure, a decrease in fatigue severity scale score, a reduction in urine hexose tetrasaccharide levels, a reduction in creatine kinase levels, a reduction in alanine aminotransferase levels, a reduction in aspartate aminotransferase levels, or any combination thereof.

[0120] In some embodiments, the rhGAA or pharmaceutical composition described herein achieves desired therapeutic effects faster than conventional rhGAA products when administered at the same dose. Therapeutic effects may be assessed based on one or more criteria discussed above (e.g., cardiac status, glycogen level, or biomarker expression). For instance, if a single dose of a conventional rhGAA product decreases glycogen levels in tissue of a treated individual by 10% in a week, the same degree of reduction may be achieved in less than a week when the same dose of the rhGAA or pharmaceutical composition described herein is administered. In some embodiments, when administered at the same dose, the rhGAA or pharmaceutical composition described herein may achieve desired therapeutic effects at least about 1.25, 1.5, 1.75, 2.0, 3.0, or more faster than conventional rhGAA products.

[0121] In some embodiments, the therapeutically effective amount of rhGAA (or composition or

medicament comprising rhGAA) is administered more than once. In some embodiments, the rhGAA or pharmaceutical composition described herein is administered at regular intervals, depending on the nature and extent of the disease's effects, and on an ongoing basis. Administration at a "regular interval," as used herein, indicates that the therapeutically effective amount is administered periodically (as distinguished from a one-time dose). The interval can be determined by standard clinical techniques. In certain embodiments, rhGAA is administered bimonthly, monthly, bi-weekly, weekly, twice weekly, or daily. In some embodiments, the rhGAA is administered intravenously twice weekly, weekly, or every other week. The administration interval for a single individual need not be a fixed interval, but can be varied over time, depending on the needs of the individual. For example, in times of physical illness or stress, if anti-rhGAA antibodies become present or increase, or if disease symptoms worsen, the interval between doses can be decreased.

[0122] In some embodiments, when used at the same dose, the rhGAA or pharmaceutical composition as described herein may be administered less frequently than conventional rhGAA products and yet capable of producing the same as or better therapeutic effects than conventional rhGAA products. For instance, if a conventional rhGAA product is administered at 20 mg/kg weekly, the rhGAA or pharmaceutical composition as described herein may produce the same as or better therapeutic effects than the conventional rhGAA product when administered at 20 mg/kg, even though the rhGAA or pharmaceutical composition is administered less frequently, e.g., biweekly or monthly. Therapeutic effects may be assessed based on one or more criterion discussed above (e.g., cardiac status, glycogen level, or biomarker expression). In some embodiments, an interval between two doses of the rhGAA or pharmaceutical composition described herein is longer than that of conventional rhGAA products. In some embodiments, the interval between two doses of the rhGAA or pharmaceutical composition is at least about 1.25, 1.5, 1.75, 2.0, 3.0, or more longer than that of conventional rhGAA products.

[0123] In some embodiments, under the same treatment condition (e.g., the same dose administered at the same interval), the rhGAA or pharmaceutical composition described herein provides therapeutic effects at a degree superior than that provided by conventional rhGAA products. Therapeutic effects may be assessed based on one or more criteria discussed above (e.g., cardiac status, glycogen level, or biomarker expression). For instance, when compared to a conventional rhGAA product administered at 20 mg/kg weekly, the rhGAA or pharmaceutical composition administered at 20 mg/kg weekly may reduce glycogen levels in tissue of a treated individual at a higher degree. In some embodiments, when administered under the same treatment condition, the rhGAA or pharmaceutical composition described herein provides therapeutic effects that are at least about 1.25, 1.5, 1.75, 2.0, 3.0, or more greater than those of conventional rhGAA products.

D. Combination Therapy

[0124] In the invention, the rhGAA or pharmaceutical composition comprising the rhGAA described herein is administered concurrently or sequentially with a pharmacological chaperone. The pharmacological chaperone is administered orally while the rhGAA or pharmaceutical composition is administered intravenously.

[0125] In the invention, the pharmacological chaperone is miglustat and is administered at an oral dose of about 260 mg.

[0126] In the present invention, the rhGAA is administered intravenously at a dose of about 20 mg/kg and the miglustat is administered orally at a dose of about 260 mg.

[0127] In some embodiments, the miglustat and the rhGAA are administered concurrently. For instance, the miglustat may be administered within 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 minute(s) before or after administration of the rhGAA. In some embodiments, the miglustat is administered within 5, 4, 3, 2, or 1 minute(s) before or after administration of the rhGAA.

[0128] In some embodiments, the miglustat and the rhGAA are administered sequentially. In at least one embodiment, the miglustat is administered prior to administration of the rhGAA. In at least one embodiment, the miglustat is administered less than three hours prior to administration of the rhGAA. In at least one embodiment, the miglustat is administered about two hours prior to administration of the rhGAA. For instance, the miglustat may be administered about 1.5 hours, about 1 hour, about 50 minutes, about 30 minutes, or about 20 minutes prior to administration of the rhGAA. In at least one embodiment, the miglustat is administered about one hour prior to administration of the rhGAA.

[0129] In some embodiments, the miglustat is administered after administration of the rhGAA. In at least one embodiment, the miglustat is administered within three hours after administration of the rhGAA. In at least one embodiment, the miglustat is administered within two hours after administration of the rhGAA. For instance, the miglustat may be administered within about 1.5 hours, about 1 hour, about 50 minutes, about 30 minutes, or about 20 minutes after administration of the rhGAA.

[0130] In some embodiments, the subject fasts for at least two hours before and at least two hours after administration of miglustat.

E. Kit

[0131] Another aspect of the disclosure pertains to kits comprising the rhGAA or pharmaceutical composition described herein. In one or more examples, the kit comprises a container (e.g., vial, tube, bag, etc.) comprising the rhGAA or pharmaceutical composition (either before or after lyophilization) and instructions for reconstitution, dilution and administration.

EXAMPLES

Example 1: Preparation of CHO Cells producing rhGAA having a high content of mono- or bis-M6P-bearing N-glycans.

[0132] DG44 CHO (DHFR-) cells were transfected with a DNA construct that expresses rhGAA. The DNA construct is shown in Fig. 4. After transfection, CHO cells containing a stably integrated GAA gene were selected with hypoxanthine/thymidine deficient (-HT) medium). GAA expression in these cells was induced by methotrexate treatment (MTX, 500 nM).

[0133] Cell pools that expressed high amounts of GAA were identified by GAA enzyme activity assays and were used to establish individual clones producing rhGAA. Individual clones were generated on semisolid media plates, picked by ClonePix system, and were transferred to 24-deep well plates. The individual clones were assayed for GAA enzyme activity to identify clones expressing a high level of GAA. Conditioned media for determining GAA activity used a 4-MU- α -Glucosidase substrate. Clones producing higher levels of GAA as measured by GAA enzyme assays were further evaluated for viability, ability to grow, GAA productivity, N-glycan structure and stable protein expression. CHO cell lines, including CHO cell line GA-ATB200, expressing rhGAA with enhanced mono-M6P or bis-M6P N-glycans were isolated using this procedure.

Example 2: Purification of rhGAA

[0134] Multiple batches of the rhGAA according to the invention were produced in shake flasks and in perfusion bioreactors using CHO cell line GA-ATB200, the product of which is referred to as "ATB200." Weak anion exchange ("WAX") liquid chromatography was used to fractionate ATB200 rhGAA according to terminal phosphate and sialic acid. Elution profiles were generated by eluting the ERT with increasing amount of salt. The profiles were monitored by UV (A280nm). Similar CIMPR receptor binding (at least -70%) profiles were observed for purified ATB200 rhGAA from different production batches (Fig. 5), indicating that ATB200 rhGAA can be consistently produced.

Example 3: Oligosaccharide Characterization of ATB200 rhGAA

[0135] ATB200 rhGAA was analyzed for site-specific N-glycan profiles using different LC-MS/MS analytical techniques. The results of the first two LC-MS/MS methods are shown in Figs. 6A-6H. The results of a third LC-MS/MS method with 2-AA glycan mapping is shown in Figs. 32A-32H, Fig. 33A-33B, and Table 5.

[0136] In the first LC-MS/MS analysis, the protein was denatured, reduced, alkylated, and digested prior to LC-MS/MS analysis. During protein denaturation and reduction, 200 μ g of protein sample, 5 μ L of 1 mol/L tris-HCl (final concentration 50 mM), 75 μ L of 8 mol/L guanidine HCl (final concentration 6 M), 1 μ L of 0.5 mol/L EDTA (final concentration 5 mM), 2 μ L of 1 mol/L DTT (final concentration 20 mM), and Milli-Q[®] water were added to a 1.5 mL tube to provide a total volume of 100 μ L. The sample was mixed and incubated at 56°C for 30 minutes in a dry bath. During alkylation, the denatured and reduced protein sample was mixed with 5 μ L of 1 mol/L iodoacetamide (IAM, final concentration 50 mM), then incubated at 10-30°C in the dark for 30 minutes. After alkylation, 400 μ L of precooled acetone was added to the sample and the mixture was frozen at -80°C refrigeration for 4 hours. The sample was then centrifuged for 5 min at 13000

rpm at 4°C and the supernatant was removed. 400 µL of precooled acetone was added to the pellets, which was then centrifuged for 5 min at 13000 rpm at 4°C and the supernatant was removed. The sample was then air dried on ice in the dark to remove acetone residue. Forty microliters of 8M urea and 160 µL of 100 mM NH₄HCO₃ were added to the sample to dissolve the protein. During trypsin digestion, 50 µg of the protein was then added with trypsin digestion buffer to a final volume of 100 µL, and 5 µL of 0.5 mg/mL trypsin (protein to enzyme ratio of 20/1 w/w) was added. The solution was mixed well and incubated overnight (16 ± 2 hours) at 37°C. Two and a half microliters of 20% TFA (final concentration 0.5%) were added to quench the reaction. The sample was then analyzed using the Thermo Scientific™ Orbitrap Velos Pro™ Mass Spectrometer.

[0137] In the second LC-MS/MS analysis, the ATB200 sample was prepared according to a similar denaturation, reduction, alkylation, and digestion procedure, except that iodoacetic acid (IAA) was used as the alkylation reagent instead of IAM, and then analyzed using the Thermo Scientific™ Orbitrap Fusion™ Lumos Tribid™ Mass Spectrometer.

[0138] The results of the first and second analyses are shown in Figs. 6A-6H. In Figs. 6A-6H, the results of the first analysis are represented by left bar (dark grey) and the results from the second analysis are represented by the right bar (light grey). The symbol nomenclature for glycan representation is in accordance with Varki, A., Cummings, R.D., Esko J.D., et al., *Essentials of Glycobiology*, 2nd edition (2009).

[0139] As can be seen from Figs. 6A-6H, the two analyses provided similar results, although there was some variation between the results. This variation can be due to a number of factors, including the instrument used and the completeness of N-glycan analysis. For example, if some species of phosphorylated N-glycans were not identified and/or not quantified, then the total number of phosphorylated N-glycans may be underrepresented, and the percentage of rhGAA bearing the phosphorylated N-glycans at that site may be underrepresented. As another example, if some species of non-phosphorylated N-glycans were not identified and/or not quantified, then the total number of non-phosphorylated N-glycans may be underrepresented, and the percentage of rhGAA bearing the phosphorylated N-glycans at that site may be overrepresented.

[0140] Fig. 6A shows the N-glycosylation site occupancy of ATB200. As can be seen from Fig. 6A, the first, second, third, fourth, fifth, and sixth N-glycosylation sites are mostly occupied, with both analyses detecting around or over 90% and up to about 100% of the ATB200 enzyme having an N-glycan detected at each potential N-glycosylation site. However, the seventh potential N-glycosylation site is N-glycosylated about half of the time.

[0141] Fig. 6B shows the N-glycosylation profile of the first potential N-glycosylation site, N84. As can be seen from Fig. 6B, the major N-glycan species is bis-M6P N-glycans. Both the first and second analyses detected over 75% of the ATB200 having bis-M6P at the first site, corresponding to an average of about 0.8 mol bis-M6P per mol ATB200 at the first site.

[0142] Fig. 6C shows the N-glycosylation profile of the second potential N-glycosylation site, N177. As can be seen from Fig. 6C, the major N-glycan species are mono-M6P N-glycans and non-phosphorylated high mannose N-glycans. Both the first and second analyses detected over 40% of

the ATB200 having mono-M6P at the second site, corresponding to an average of about 0.4 to about 0.6 mol mono-M6P per mol ATB200 at the second site.

[0143] Fig. 6D shows the N-glycosylation profile of the third potential N-glycosylation site, N334. As can be seen from Fig. 6D, the major N-glycan species are non-phosphorylated high mannose N-glycans, di-, tri-, and tetra-antennary complex N-glycans, and hybrid N-glycans. Both the first and second analyses detected over 20% of the ATB200 having a sialic acid residue at the third site, corresponding to an average of about 0.9 to about 1.2 mol sialic acid per mol ATB200 at the third site.

[0144] Fig. 6E shows the N-glycosylation profile of the fourth potential N-glycosylation site, N414. As can be seen from Fig. 6E, the major N-glycan species are bis-M6P and mono-M6P N-glycans. Both the first and second analyses detected over 40% of the ATB200 having bis-M6P at the fourth site, corresponding to an average of about 0.4 to about 0.6 mol bis-M6P per mol ATB200 at the fourth site. Both the first and second analyses also detected over 25% of the ATB200 having mono-M6P at the fourth site, corresponding to an average of about 0.3 to about 0.4 mol mono-M6P per mol ATB200 at the fourth site.

[0145] Fig. 6F shows the N-glycosylation profile of the fifth potential N-glycosylation site, N596. As can be seen from Fig. 6F, the major N-glycan species are fucosylated di-antennary complex N-glycans. Both the first and second analyses detected over 70% of the ATB200 having a sialic acid residue at the fifth site, corresponding to an average of about 0.8 to about 0.9 mol sialic acid per mol ATB200 at the fifth site.

[0146] Fig. 6G shows the N-glycosylation profile of the sixth potential N-glycosylation site, N826. As can be seen from Fig. 6G, the major N-glycan species are di-, tri-, and tetra-antennary complex N-glycans. Both the first and second analyses detected over 80% of the ATB200 having a sialic acid residue at the sixth site, corresponding to an average of about 1.5 to about 1.8 mol sialic acid per mol ATB200 at the sixth site.

[0147] An analysis of the N-glycosylation at the seventh site, N869, showed approximately 40% N-glycosylation, with the most common N-glycans being A4S3S3GF (12%), A5S3G2F (10%), A4S2G2F (8%) and A6S3G3F (8%).

[0148] Fig. 6H shows a summary of the phosphorylation at each of the seven potential N-glycosylation sites. As can be seen from Fig. 6H, both the first and second analyses detected high phosphorylation levels at the first, second, and fourth potential N-glycosylation sites. Both analyses detected over 80% of the ATB200 was mono- or bis-phosphorylated at the first site, over 40% of the ATB200 was mono-phosphorylated at the second site, and over 80% of the ATB200 was mono- or bis-phosphorylated at the fourth site.

[0149] Another N-glycosylation analysis of ATB200 was performed according to an LC-MS/MS method as described below. This analysis yielded an average N-glycosylation profile over ten lots of ATB200 (Figs. 32A-32H, Figs. 33A-33B).

[0150] N-linked glycans from ATB200 were released enzymatically with PNGase-F and labeled with

2-Anthranilic acid (2-AA). The 2-AA labeled N-glycans were further processed by solid phase extraction (SPE) to remove excess salts and other contaminants. The purified 2-AA N-glycans were dissolved in acetonitrile/water (20/80; v/v), and 10 micrograms were loaded on an amino-polymer analytical column (apHera™, Supelco) for High Performance Liquid Chromatography with Fluorescence detection (HPLC-FLD) and High Resolution Mass Spectrometry (HRMS) analysis.

[0151] The liquid chromatographic (LC) separation was performed under normal phase conditions in a gradient elution mode with mobile phase A (2% acetic acid in acetonitrile) and mobile phase B (5% acetic acid; 20 millimolar ammonium acetate in water adjusted to pH 4.3 with ammonium hydroxide). The initial mobile phase composition was 70% A/30% B. For the fluorescence detection, the parameters for the detector (RF-20Axs, Shimadzu) were Excitation (Ex):320 nm; Emission (Em):420 nm. The HRMS analysis was carried out using a Quadrupole Time of Flight mass spectrometer (Sciex X500B QTOF) operating in Independent Data Acquisition (IDA) mode. The acquired datafiles were converted into mzML files using MSConvert from ProteoWizard, and then GRITS Toolbox 1.2 Morning Blend software (UGA) was utilized for glycan database searching and subsequent annotation of identified N-glycans. The N-glycans were identified using both precursor monoisotopic masses (m/z) and product ion m/z . Experimental product ions and fragmentation patterns were confirmed in-silico using the GlycoWorkbench 2 Application.

[0152] To determine the relative quantitation of N-linked glycans from ATB200, data acquired from the HPLC-FLD-QTOF MS/MS experiment was processed as follows. All of the N-glycan peaks in the FLD chromatogram were integrated, and each peak was assigned a percentage of the total area of all peaks in the FLD chromatogram. The fluorescent signal, expressed as a peak area, is a quantitative measure of the amount of each N-glycan in the sample (Fig. 33A). However, in most cases, multiple N-glycan species were contained in the same FLD peak. Therefore, the mass spectrometer data was also required to obtain relative quantitation of each N-glycan species (Table 5). The ion intensity signal for each N-glycan was "extracted" from the data to create a chromatographic peak called an extracted ion chromatogram (XIC). The XIC aligned with the FLD chromatographic peak and was specific to only one N-glycan species. The XIC peak created from the ion intensity signal was then integrated and this peak area is a relative quantitative measure of the amount of glycan present. Both the FLD peak areas and mass spectrometer XIC peak areas were used to enable relative quantitation of all the N-linked glycan species of ATB200 reported herein.

[0153] The results of this LC-MS/MS analysis are provided in Table 5 below. The symbol nomenclature for glycan representation is in accordance with Wopereis W, et al. 2006. Abnormal glycosylation with hypersialylated O-glycans in patients with Sialuria. *Biochimica et Biophysica Acta*. 1762:598-607; Gornik O, et al. 2007. Changes of serum glycans during sepsis and acute pancreatitis. *Glycobiology*. 17:1321-1332 <https://doi.org/10.1093/glycob/cwm106>; Kattla JJ, et al. 2011. Biologic protein glycosylation. In: Murray Moo-Young (ed.), *Comprehensive Biotechnology*, Second Edition, 3:467-486; Tharmalingam-Jaikaran T, et al. N-glycan profiling of bovine follicular fluid at key dominant follicle developmental stages. 2014. *Reproduction*. 148:569-580; Clerc F, et al. Human plasma protein N-glycosylation. 2015. *Glycoconj J*. DOI 10.1007/s10719-015-9626-2; and Blackler RJ, et al. 2016. Single-chain antibody-fragment M6P-1 possesses a mannose 6-phosphate monosaccharide-specific binding pocket that distinguishes N-glycan phosphorylation in a branch-specific manner. *Glycobiology*. 26-2:181-192.

Table 5: Type and Prevalence of Oligosaccharides identified on ATB200 based on 2-AA glycan mapping and LC-MS/MS identification

High Mannose N-Glycans	% Total	Complex N-Glycans	% Total	Complex N-Glycans	% Total	Complex N-Glycans	% Total
2P-M7	11.39	FA2G251	3.89	A3G351+1Ac	0.65	FA2G2S1+1Ac	0.29
P-M7	7.97	FA2G252	3.42	A3G2S2+1Ac	0.64	A4G3	0.29
M6	6.89	A2G252	3.32	A1G151	0.63	A4G4+3KDN	0.29
P-M6	3.42	FA2G2	2.77	A4G351	0.61	A4G453	0.28
M5	2.06	FA4G453	2.26	FA3G3	0.61	FA5G4	0.24
P-M5	1.67	A2G251	2.25	A1G1	0.6	A4G352	0.21
2P-M8	1.27	FA3G351	2.12	FA2G252+1Ac	0.57	FA1	0.21
P-M8	1.17	A3G352	1.8	A3G251	0.57	FA4G4	0.21
BP-M6	0.9	FA2G1	1.66	A3G251	0.56	A3G1	0.21
M7	0.81	A2G2	1.46	A2G2S2+1Ac	0.5	FA4G352	0.21
BP-M7	0.69	FA3G351	1.42	FA3G2	0.45	FA3G252	0.21
M4	0.14	A4G451	1.28	A3G3+3KDN	0.45	A1	0.2
BP2-M5	0.04	FA3G352	1.25	A4G351	0.45	A4G2	0.19
BP2-M6	0.01	FA4G4(1LN)S3	1.1	A2G151	0.41	FA4G3	0.19
Hybrid N-Glycans	% Total	FA4G4S1	1.08	A3G2	0.4	FAB	0.18
FA1P-M6	2.16	A3G3	1.08	FA4G4S1+LN	0.4	A1G151	0.18
M5A1G151	1.56	FA4G454	1.07	FA3G251	0.39	A4G 151	0.16
FP-M6A1G151	0.42	FA3G353	1.04	FA2	0.38	FA1G1	0.15
A1MS	0.36	FA4G4S2	0.94	FA4G4S2+LN	0.38	FA3G1	0.14
A1G1M5	0.32	A2G1	0.94	A3G252	0.37	FA5G452	0.12
P-M6A1G151	0.17	FA2G151	0.94	A2	0.34	A3G151	0.11
Summary	Total	A4G4	0.91	FA4G4(2LN)53	0.33	A3	0.11
High Mannose N-Glycans	38%	FA 1G 151	0.91	FA2G25g1	0.32	FA3G3S3+1Ac	0.1
Hybrid N-Glycans	5%	FA2G2S2+2Ac	0.76	FA4G4(1LN)S4	0.31	A2G251+1Ac	0.09
Complex N-Glycans	57%	A4G452	0.69	A3G353	0.29	FA3G151	0.06

[0154] Based on this 2-AA and LC-MS/MS analysis, and as further summarized in Fig. 33C, the ATB200 tested has an average M6P content of 3-5 mol per mol of ATB200 (accounting for both

mono-M6P and bis-M6P) and sialic acid content of 4-7 mol per mol of ATB200.

[0155] As shown in Figs. 32A-32H and summarized in Fig. 33B, the first potential N-glycosylation site of ATB200 has an average M6P content of about 1.4 mol M6P/mol ATB200, accounting for an average mono-M6P content of about 0.25 mol mono-M6P/mol ATB200 and an average bis-M6P content of about 0.56 mol bis-M6P/mol ATB200; the second potential N-glycosylation site of ATB200 has an average M6P content of about 0.5 mol M6P/mol ATB200, with the primary phosphorylated N-glycan species being mono-M6P N-glycans; the third potential N-glycosylation site of ATB200 has an average sialic acid content of about 1 mol sialic acid/mol ATB200; the fourth potential N-glycosylation site of ATB200 has an average M6P content of about 1.4 mol M6P/mol ATB200, accounting for an average mono-M6P content of about 0.35 mol mono-M6P/mol ATB200 and an average bis-M6P content of about 0.52 mol bis-M6P/mol ATB200; the fifth potential N-glycosylation site of ATB200 has an average sialic acid content of about 0.86 mol sialic acid/mol ATB200; the sixth potential N-glycosylation site of ATB200 has an average sialic acid content of about 4.2 mol sialic acid/mol ATB200; and the seventh potential N-glycosylation site of ATB200 has an average sialic acid content of about 0.86 mol sialic acid/mol ATB200.

[0156] Also according to this 2-AA and LC-MS/MS analytical technique, an average of about 65% of the N-glycans at the first potential N-glycosylation site of ATB200 are high mannose N-glycans, about 89% of the N-glycans at the second potential N-glycosylation site of ATB200 are high mannose N-glycans, over half of the N-glycans at the third potential N-glycosylation site of ATB200 are sialylated (with nearly 20% fully sialylated) and about 85% of the N-glycans at the third potential N-glycosylation site of ATB200 are complex N-glycans, about 84% of the N-glycans at the fourth potential N-glycosylation site of ATB200 are high mannose N-glycans, about 70% of the N-glycans at the fifth potential N-glycosylation site of ATB200 are sialylated (with about 26% fully sialylated) and about 100% of the N-glycans at the fifth potential N-glycosylation site of ATB200 are complex N-glycans, about 85% of the N-glycans at the sixth potential N-glycosylation site of ATB200 are sialylated (with nearly 27% fully sialylated) and about 98% of the N-glycans at the sixth potential N-glycosylation site of ATB200 are complex N-glycans, and about 87% of the N-glycans at the seventh potential N-glycosylation site of ATB200 are sialylated (with nearly 8% fully sialylated) and about 100% of the N-glycans at the seventh potential N-glycosylation site of ATB200 are complex N-glycans.

Example 4: Analytical Comparison of ATB200 and Myozyme[®]/Lumizyme[®]

[0157] Purified ATB200 and Lumizyme[®] N-glycans were evaluated by MALDI-TOF to determine the individual N-glycan structures found on each ERT. Lumizyme[®] was obtained from a commercial source. As shown in Fig. 7, ATB200 exhibited four prominent peaks eluting to the right of Lumizyme[®]. This confirms that ATB200 was phosphorylated to a greater extent than Lumizyme[®] since this evaluation is by terminal charge rather than CIMPR affinity. As summarized in Fig. 8, ATB200 samples were found to contain lower amounts of non-phosphorylated high-mannose type N-glycans than Lumizyme[®].

[0158] To evaluate the ability of the conventional rhGAAs in Myozyme[®] and Lumizyme[®] to interact with the CIMPR, the two conventional rhGAA preparations were injected onto a CIMPR affinity column (which binds rhGAA having M6P groups) and the flow through collected. The bound material was eluted with a free M6 gradient. Fractions were collected in 96-well plate and GAA activity assayed by 4MU- α -glucosidase substrate. The relative amounts of unbound (flow through) and bound (M6P eluted) rhGAA were determined based on GAA activity and reported as the fraction of total enzyme. Figs. 9A and 9B show the binding profile of rhGAAs in Myozyme[®] and Lumizyme[®]: 73% of the rhGAA in Myozyme[®] (Fig. 9B) and 78% of the rhGAA in Lumizyme[®] (Fig. 9A) did not bind to the CIMPR. Indeed, only 27% of the rhGAA in Myozyme[®] and 22% of the rhGAA in Lumizyme[®] contained M6P that can be productive to target it to the CIMPR on muscle cells. In contrast, as shown in Fig. 5, under the same condition, more than 70% of the rhGAA in ATB200 was found to bind to the CIMPR.

[0159] In addition to having a greater percentage of rhGAA that can bind to the CIMPR, it is important to understand the quality of that interaction. Lumizyme[®] and ATB200 receptor binding was determined using a CIMPR plate binding assay. Briefly, CIMPR-coated plates were used to capture GAA. Varying concentrations of rhGAA were applied to the immobilized receptor and unbound rhGAA was washed off. The amount of remaining rhGAA was determined by GAA activity. As shown in Fig. 10A, ATB200 bound to CIMPR significantly better than Lumizyme[®]. Fig. 10B shows the relative content of bis-M6P N-glycans in Lumizyme[®] (a conventional rhGAA product) and ATB200 according to the invention. For Lumizyme[®], there is on average only 10% of molecules having a bis-phosphorylated N-glycan. In contrast, on average every rhGAA molecule in ATB200 has at least one bis-phosphorylated N-glycan.

[0160] Overall, the higher content of M6P N-glycans in ATB200 than in Lumizyme[®] indicates that the higher portion of rhGAA molecules in ATB200 can target muscle cells. As shown above, the high percentage of mono-phosphorylated and bis-phosphorylated structures determined by MALDI agree with the CIMPR profiles which illustrated significantly greater binding of ATB200 to the CIMPR receptor. N-glycan analysis via MALDI-TOF mass spectrometry confirmed that on average each ATB200 molecule contains at least one natural bis-M6P N-glycan structure. This higher bis-M6P N-glycan content on ATB200 directly correlated with high-affinity binding to CIMPR in M6P receptor plate binding assays (K_D about 2-4 nM).

[0161] The relative cellular uptake of ATB200 and Lumizyme[®] rhGAA were compared using normal and Pompe fibroblast cell lines. Comparisons involved 5-100 nM of ATB200 according to the invention with 10-500 nM conventional rhGAA product Lumizyme[®]. After 16-hr incubation, external rhGAA was inactivated with TRIS base and cells were washed 3-times with PBS prior to harvest. Internalized GAA measured by 4MU- α -Glucoside hydrolysis and was graphed relative to total cellular protein and the results appear in Figs. 11A-11C.

[0162] ATB200 was also shown to be efficiently internalized into cells. As depicted in Figs. 11A-11B, ATB200 is internalized into both normal and Pompe fibroblast cells and is internalized to a greater degree than the conventional rhGAA product Lumizyme[®]. ATB200 saturates cellular receptors at

about 20 nM, while about 250 nM of Lumizyme® is needed to saturate cellular receptors. The uptake efficiency constant (K_{uptake}) extrapolated from these results is 2-3 nM for ATB200 and 56 nM for Lumizyme®, as shown by Fig. 11C. These results suggest that ATB200 is a well-targeted treatment for Pompe disease.

Example 5: ATB200 and Pharmacological Chaperone

[0163] The stability of ATB200 in acidic or neutral pH buffers was evaluated in a thermostability assay using SYPRO Orange, as the fluorescence of the dye increases when proteins denature. As shown in Fig. 12, the addition of AT2221 stabilized ATB200 at pH 7.4 in a concentration-dependent manner, comparable to the stability of ATB200 at pH 5.2, a condition that mimics the acidic environment of the lysosome. As summarized in Table 6, the addition of AT2221 increased the melting temperature (T_m) of ATB200 by nearly 10°C.

Table 6. Stability of ATB200 In Combination with AT2221

Test Condition	T_m (°C)
pH 7.4	56.2
pH 7.4 + 10 μ M AT2221	61.6
pH 7.4 + 30 μ M AT2221	62.9
pH 7.4 + 100 μ M AT2221	66.0
pH 5.2	67.3

Example 6: Co-administration of ATB200 and AT2221 in *Gaa* KO Mice

[0164] The therapeutic effects of ATB200 and AT2221 were evaluated and compared against those of Alglucosidase alfa in *Gaa* KO mice. For the study, male *Gaa* KO (3- to 4-month old) and age-matched wild-type (WT) mice were used. Alglucosidase alfa was administered via bolus tail vein intravenous (IV) injection. In the co-administration regimen, AT2221 was administered via oral gavage (PO) 30 minutes prior to the IV injection of ATB200. Treatment was given biweekly. Treated mice were sacrificed after 14 days from the last administration and various tissues were collected for further analysis. Table 7 summarizes the study design:

Table 7. Co-administration Study Design

Genotype	Treatment	Drug Dosage per Administration (bi-weekly)	Number of Administration
<i>Gaa</i> KO	Vehicle	N/A	6
<i>Gaa</i> KO	Alglucosidase alfa	20 mg/kg	6
<i>Gaa</i> KO	ATB200/AT2221	20 mg/kg (ATB200)	6
		10 mg/kg (AT2221)	
WT (Sve	Not Treated	N/A	N/A

Genotype	Treatment	Drug Dosage per Administration (bi-weekly)	Number of Administration
129)			

[0165] Tissue glycogen content in tissues samples was determined using amyloglucosidase digestion, as discussed above. As shown in Fig. 13, a combination of 20 mg/kg ATB200 and 10 mg/kg AT2221 significantly decreased the glycogen content in four different tissues (quadriceps, triceps, gastrocnemius, and heart) as compared to the same dosage of alglucosidase alfa.

[0166] Tissue samples were also analyzed for biomarker changes following the methods discussed in: Khanna R, et al. (2012), "The pharmacological chaperone AT2220 increases recombinant human acid α -glucosidase uptake and glycogen reduction in a mouse model of Pompe disease," Plos One 7(7): e40776; and Khanna, R et al. (2014), "The Pharmacological Chaperone AT2220 Increases the Specific Activity and Lysosomal Delivery of Mutant Acid α -Glucosidase, and Promotes Glycogen Reduction in a Transgenic Mouse Model of Pompe Disease," PLoS ONE 9(7): e102092. As shown in Fig. 14, a profound increase in and enlargement of LAMP1-positive vesicles was seen in muscle fibers of *Gaa* KO animals compared to WT, indicative of lysosomal proliferation. Co-administration of ATB200 / AT2221 led to more fibers with normalized LAMP1 level, while the remaining LAMP1-positive vesicles also reduced in size (insets).

[0167] Similarly, intense LC3-positive aggregates in the muscle fibers of untreated *Gaa* KO mice signify the presence of autophagic zones and autophagy build-up. LC3-positive aggregates (red) were preferentially reduced in mice treated with ATB200 / AT2221 co-administration as compared to mice treated with alglucosidase alfa (Fig. 15A). A similar observation was made when the expression of LC3 was assessed using western blot. As shown in Fig. 15B, the majority of animals treated with ATB200 / AT2221 showed a significant decrease in levels of LC3 II, the lipidated form that is associated with autophagosomes, suggesting an improved autophagy flux. In comparison, the effect of alglucosidase alfa on autophagy was modest.

[0168] Dysferlin, a protein involved in membrane repair and whose deficiency/mistrafficking is associated with a number of muscular dystrophies, was also assessed. As shown in Fig. 16, dysferlin (brown) was heavily accumulated in the sarcoplasm of *Gaa* KO mice. Compared to alglucosidase alfa, ATB200 / AT2221 was able to restore dysferlin to the sarcolemma in a greater number of muscle fibers.

[0169] These data are consistent with improvements at the cellular level demonstrated in human Pompe disease patients treated with ATB200 and miglustat, (e.g., the patients exhibit reduced levels of biomarkers of glycogen accumulation and muscle injury), leading not only to effective treatment of Pompe disease but also a reversal in disease progression. Clinical data in human Pompe disease patients are summarized in Examples 8-13, below.

Example 7: Single Fiber Analysis

[0170] As shown in Fig. 17, majority of the vehicle-treated mice showed grossly enlarged lysosomes (green) (see, for example "B") and the presence of massive autophagic buildup (red) (see, for example "A"). Myozyme®-treated mice did not show any significant difference as compared to vehicle-treated mice. In contrast, most fibers isolated from mice treated with ATB200 showed dramatically decreased lysosome size (see, for example, "C"). Furthermore, the area with autophagic buildup was also reduced to various degrees (see, for example, "C"). As a result, a significant portion of muscle fibers analyzed (36-60%) from ATB200-treated mice appeared normal or near-normal. Table 8 below summarizes the single fiber analysis shown in Fig. 17.

Table 8. Single Fiber Analysis

Treatment	Animal Analyzed	Total Number of Fibers Analyzed (n)	Lysosome Enlargement	Fibers with Autophagy Buildup	Fibers with Normal or Near-normal Appearance
WT	2	65	-	-	100%
Vehicle	2	65	+	>90%	<10%
Alglucosidase alfa	4	150	+	>90%	<10%
ATB200	5	188	Dramatic size decrease in most fibers	40-64%*	36-60%
* This included fibers with varying degree of reduction in autophagic buildup. Overall, the extent of the buildup was smaller in ATB200-treated group compared to Vehicle- or alglucosidase alfa-treated group.					

[0171] Overall, the data indicate that ATB200, with its higher M6P content, both alone and further stabilized by the pharmacological chaperone AT2221 at the neutral pH of blood, is more efficient in tissue targeting and lysosomal trafficking compared to alglucosidase alfa when administered to *Gaa* KO mice, consistent with the stabilization of ATB200 by AT2221 as depicted in Fig. 18. As a result, administration of ATB200 and co-administration of ATB200/AT2221 was more effective than alglucosidase alfa in correcting some of the disease-relevant pathologies, such as glycogen accumulation, lysosomal proliferation, and formation of autophagic zones. Due to these positive therapeutic effects, administration of ATB200 and ATB200/AT2221 co-administration is shown to improve the chance of muscle fiber recovery from damage and even to reverse damage by clearing glycogen that had accumulated in the cell due to lack of optimal GAA activity. As with Example 6, these data are also consistent with improvements at the cellular level demonstrated in human Pompe disease patients that lead to both effective treatment of Pompe disease and reversal in disease progression following administration of ATB200 and miglustat. Clinical data in human Pompe disease patients are summarized in Examples 8-13, below.

Example 8: The ATB200-02 Trial: an in-human study of ATB200/AT2221 in patients with Pompe disease

[0172] Preclinical studies were conducted in *Gaa* knockout mice to evaluate the pharmacokinetics

(PK) and efficiency of glycogen reduction at varying ATB200 enzyme replacement therapy (ERT) and AT2221 chaperone doses. These data were used to estimate the comparable AT2221 chaperone doses in humans. Study ATB200-02 (NCT02675465) was then designed as an open-label fixed-sequence, ascending dose, first-in-human, phase 1/2 study to evaluate the safety, tolerability, PK, pharmacodynamics (PD), and efficacy of ATB200 co-administered with AT2221 in patients with Pompe disease. Figs. 19A-19B present the ATB200-02 study design. Ambulatory patients who have previously received enzyme replacement therapy with alglucosidase alfa are referred to as ambulatory ERT-switch (or ERT-switch ambulatory) patients or Cohort 1 patients. Nonambulatory patients who have previously received enzyme replacement therapy with alglucosidase alfa are referred to as nonambulatory ERT-switch (or ERT-switch nonambulatory) patients or Cohort 2 patients. Ambulatory patients who have not previously received enzyme replacement therapy with alglucosidase alfa are referred to as ERT-naïve (or ERT-naïve ambulatory) patients or Cohort 3 patients.

[0173] Sixteen clinical sites in five countries participated in the ATB200-02 study. The study employed the following key inclusion criteria: males and females aged 18-65 years who were diagnosed with Pompe disease based on documented deficiency of GAA enzyme activity or by GAA phenotyping, and who had received enzyme replacement therapy with alglucosidase alfa for 2-6 years (or ≥ 2 years for Cohort 2) prior to trial initiation (Cohort 1). Eligible subjects were those currently receiving alglucosidase alfa at a frequency of every other week and having completed the last 2 infusions without a drug-related adverse event (AE) resulting in dose interruption (Cohorts 1 and 2). Subjects had to be able to walk between 200 and 500 meters on the 6-Minute Walk Test (6MWT) (Cohorts 1 and 3), have an upright forced vital capacity (FVC) of 30-80% of predicted normal value (Cohorts 1 and 3), or be wheelchair-bound and unable to walk unassisted (Cohort 2). Protocols for the 6MWT and FVC test can be found, for example, in Lachman and Schoser, *Journal of Rare Diseases*, 2013, 8:160, and in Bittner and Singh, *The 6 Minute Walk Test, Cardiology Advisor*, 2013. Fig. 19C provides the baseline characteristics for 20 subjects. Safety, tolerability, and biomarkers were assessed for Cohorts 1, 2 and 3. The following functional assessments were assessed for Cohorts 1 and 3: 6MWT, other motor function tests (time tests and gait-stair-gower-chair (GSGC)), manual muscle test, and pulmonary function (FVC, maximal inspiratory pressure (MIP)/ maximal expiratory pressure (MEP)). Protocols for the time tests and GSGC tests can be found, for example in Lachman and Schoser, *Journal of Rare Diseases*, 2013, 8:160. For Cohort 2, the functional assessments included muscle strength tests.

Example 9: Interim PK Results from the ATB200-02 Trial

[0174] A summary of pharmacokinetics data for AT2221 is provided in Fig. 20. Total GAA protein concentrations in plasma for ATB200 at 5 mg/kg, 10 mg/kg, and 20 mg/kg were determined by validated LC-MS/MS quantification of rhGAA-specific "signature" peptide(s) T09 (primary) and T50 (confirmatory) for 11 Cohort 1 patients who completed Stages 1 and 2, as well as for five Cohort 2 patients who completed the PK study in Stage 3. For Stage 1, blood samples for plasma total GAA protein concentration were collected prior to the start of ATB200 infusion and at 1, 2, 3, 3.5, 4, 4.5, 5, 6, 8, 10, 12, and 24 hour(s) after the start of infusion. For Stage 2 and Stage 3, blood samples for plasma total GAA protein concentration were collected prior to the start of infusion and at 1, 2, 3, 3.5, 4, 4.5, 5, 6, 8, 10, 12, and 24 hour(s) after the start of infusion.

[0175] AT2221 PK analyses were also performed for 11 Cohort 1 patients who completed Stages 1 and 2, as well as for five Cohort 2 patients who completed the PK study in Stage 3. Blood samples for plasma AT2221 concentrations were taken just prior to AT2221 oral administration (time 0) and at 1, 1.5, 2, 2.5, 3, 4, 5, 6, 9, 11, and 25 hour(s) after AT2221 oral administration. Plasma AT2221 was determined by a validated LC-MS/MS assay.

[0176] As shown in Fig. 21, levels of ATB200 increased in a slightly greater-than-dose proportional manner when administered alone. Co-administration of ATB200 at 20 mg/kg with a single high dose (260 mg) of AT2221 increased the total GAA protein exposure area under the curve (AUC) by approximately 17%, compared to ATB200 at 20 mg/kg administered alone (Fig. 21, Fig. 22C). Co-administration of ATB200 at 20 mg/kg with multiple high doses (260 mg) of AT2221 increased the total GAA protein exposure area under the curve (AUC) by approximately 29%, compared to ATB200 at 20 mg/kg administered alone (Fig. 21, Fig. 22D). Increases in the distribution half-life and partial AUC-24h were observed on the log scale, during the terminal elimination phase (Fig. 21, Fig. 22A, Fig. 22B). As shown in Fig. 21, the distribution half-life (α -phase) increased by 40%, consistent with the stabilizing effect of high-dose AT2221 on ATB200 in plasma. The increase in the distribution half-life was accompanied by an increase in partial AUC from time to maximum plasma concentration to 24 hours post-dose by 42.2% (Fig. 21, Fig. 22B). Further evidence of ATB200 stabilization by AT2221 was observed in 12- and 24-hour post-dose comparisons of low- and high-dose AT2221 vs ATB200 alone (Figs. 22E and 22F). There was no statistically significant difference in plasma total GAA protein exposure between ERT-naïve (Cohort 3) and ERT-switch patients (Cohort 1) (Fig. 23). The PK disposition of signature peptide T50 did not differ from that of signature peptide T09 (AUC ratio: 1.00).

Example 10: Interim Efficacy Results From the ATB200-02 Trial

[0177] As shown in Figs. 24A and 24B, 6MWT improved for ambulatory ERT-switch patients and ERT-naïve patients at month 6 with continued benefit observed to month 12. 6MWT increased in 7/10, 8/10, and 8/8 ERT-switch patients at months 6, 9, and 12, respectively. 6MWT increased in 5/5, 5/5, and 2/2 ERT-naïve patients at Months 6, 9, and 12, respectively.

[0178] As shown in Fig. 24C, Fig. 26A, and Fig. 26C, improvements in motor function tests and manual muscle strength, along with 6MWT, were consistent with an overall improvement in muscle function for both ERT-switch and ERT-naïve patients over 12 months.

[0179] As shown in Fig. 25, and Fig. 26B, consistent and substantial increases were observed in upper extremity strength in all nonambulatory ERT-switch patients at month 6 and month 9.

[0180] As shown in Fig. 27, FVC was stable or increased in 5/9, 6/9, and 3/7 ERT-switch patients at months 6, 9, and 12 respectively and FVC was stable or increased in 5/5, 5/5, and 2/2 ERT-naïve patients at months 6, 9, and 12, respectively. Also as shown in Fig. 27, maximal inspiratory pressure (MIP) was stable and maximal expiratory pressure (MEP) increased in ERT-switch ambulatory patients, while MIP increased and MEP was stable in ERT-naïve patients.

[0181] The Fatigue Severity Scale ("FSS") is a self-assessment questionnaire consisting of nine questions, each scored on a scale of 1 to 7. The total score ranges from 9 to 63, with higher values representing higher level of fatigue due to the disease condition. The normative value in the healthy population is approximately 21 (Grace J et al. *Parkinsonism Relat Disord.* 2007;13:443-445). As shown in Fig. 28, all cohorts were significantly impacted by fatigue at baseline, and all cohorts demonstrated an improvement in their FSS after receiving ATB200/AT2221.

Example 11: Interim Results From the ATB200-02 Trial: Markers of Muscle Injury

[0182] The following muscle damage markers were assessed: creatine kinase (CK) enzyme, alanine aminotransferase (ALT), and aspartate aminotransferase (AST). Results available after nine months of the clinical trial are reported in Figs. 29A-29C (data from a maximum of 58 weeks, 24 weeks, and 36 weeks for Cohorts 1, 2, and 3, respectively; lower n values reflect that some data were either unable to be analyzed or were not yet analyzed). Mean reductions from baseline observed at these respective time points were approximately 30-35% for the ambulatory ERT-switch patients (n=9), 5-20% for the nonambulatory ERT-switch patients (n=4), and 40-55% for the ERT-naïve patients (n=5). Results for CK enzyme available after twelve months of the clinical trial are reported in Fig. 29D (data from a maximum of 12 months for Cohorts 1, 2, and 3; lower n values reflect that some data were either unable to be analyzed or were not yet analyzed).

[0183] Urine hexose tetrasaccharide (Hex4) was assessed as a marker of glycogen accumulation. Results for Hex4 available after twelve months of the clinical trial are reported in Fig. 29D (data from a maximum of 12 months for Cohorts 1, 2, and 3; lower n values reflect that some data were either unable to be analyzed or were not yet analyzed).

Example 12: Interim Safety Results From the ATB200-02 Trial

[0184] The longest duration of treatment was over 20 months. Adverse events (AEs) were generally mild and transient, with a very low rate of infusion-associated reactions (less than 1%) after over 400 total infusions across all three Cohorts. These incidences were controlled by standard premedication.

[0185] The most common AEs reported as treatment-related at up to 72 weeks were nausea (3/20), tremor (3/20), headache (3/20), fatigue (3/20), diarrhea (2/20), muscle spasm (2/20), and joint swelling (2/20).

[0186] The most common AEs reported as treatment-related at up to 20+ months were abdominal pain (including upper and lower abdominal pain) (8/20), diarrhea (8/20), nasopharyngitis (6/20), nausea (5/20), headache (5/20), and upper respiratory tract infection (5/20) (Fig. 30). One serious AE was reported, which was unrelated to the study drug (hospitalization for lower respiratory tract infection). No patients discontinued the study due to an AE.

[0187] There were three incidents of infusion-associated reactions (IARs) in 550+ infusions, which

were controlled by standard premedication. One IAR event (skin discoloration) occurred in a nonambulatory ERT-switch patient (Cohort 2). Two IAR events (localized pruritus, erythema, and burning sensation) occurred in an ERT-naïve patient (Cohort 3) (Fig. 30).

Example 13: Summary and Conclusions of Interim Results From the ATB200-02 Trial

[0188] As summarized in Fig. 31, there is concordance in the interim data from the ATB200-02 trial showing significant and unexpected parallel improvements in markers of muscle injury and substrate accumulation, muscle function tests (timed tests and endurance), manual muscle strength, and stabilization and/or improvement in respiratory function tests across the different cohorts. Muscle function improved in 16/18 and 10/10 patients at months 6 and 9, respectively. Increases in 6MWT distance were consistent and durable in ERT-switch ambulatory and ERT-naïve patients out to month 12, as were the improvements in other motor function tests in ERT-switch ambulatory and ERT-naïve patients. Qualitative and quantitative measures showed increases in upper extremity strength in nonambulatory ERT-switch patients at months 6 and 9. FVC, MIP, and MEP were generally stable in ERT-switch patients and increased in ERT-naïve patients. An improvement in fatigue score was observed in all cohorts. Biomarker levels (e.g., levels of CK and Hex4) decreased in all cohorts and ATB200/AT2221 was generally well tolerated.

[0189] Thus, the multi-dimensional impact of the therapy suggests that the combination regimen of ATB200/AT2221 has the potential to be an important treatment option for patients with Pompe disease. These clinical results support the results from the single fiber analysis studies described in Example 7, which demonstrate that the treatment is effective at clearing pathology from muscle fibers. Further study of the clinical trial is ongoing.

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This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

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Patentkrav

1. Sammensætning, som omfatter en population af rekombinante humane sure α -glucosidase (rhGAA)-molekyler til anvendelse til
5 behandling af Pompes sygdom hos et individ, der har behov for det, hvor rhGAA-molekylerne er dannet af kinesisk hamsterovarier (CHO)-celler;
hvor rhGAA-molekylerne omfatter 7 potentielle N-glycosyleringssteder;
10 hvor rhGAA-molekylerne i gennemsnit omfatter 3-4 mannose-6-phosphat (M6P)-rester;
hvor rhGAA-molekylerne i gennemsnit omfatter mindst ca. 0,5 mol bis-mannose-6-phosphat (bis-M6P) pr. mol rhGAA i det første potentielle N-glycosyleringssted bestemt ved anvendelse
15 af væskekromatografi-tandem massespektrometri (LC-MS/MS);
hvor populationen af rhGAA-molekyler administreres samtidigt eller sekventielt med en farmakologisk chaperone, og hvor den farmakologiske chaperone er miglustat eller et farmaceutisk acceptabelt salt deraf;
20 hvor populationen af rhGAA-molekyler administreres intravenøst ved en dosis på ca. 20 mg/kg, og miglustat eller et farmaceutisk acceptabelt salt deraf administreres oralt ved en dosis på ca. 260 mg; og hvor
(i) individet er en ERT-skift-patient, og individets
25 lungefunktion målt ved hjælp af en oprejst forceret vitalkapacitets (FVC)-test er stabil eller forbedret 6 måneder efter behandling sammenlignet med baseline; og/eller
(ii) individet er en ambulant ERT-skift-patient, og individets kreatinkinaseniveauer 6 måneder efter behandling er reduceret
30 mindst 15 % sammenlignet med baseline; og/eller
(iii) individet er en ikke-ambulant ERT-skift-patient, og individets kreatinkinaseniveauer 6 måneder efter behandling er reduceret mindst 20 % sammenlignet med baseline; og/eller
(iv) individet er en ERT-skift-patient, og individets niveauer
35 af urinhexosetetrasaccharid 6 måneder efter behandling er reduceret mindst 35 % sammenlignet med baseline.

2. Sammensætning til anvendelse ifølge krav 1, hvor

miglustat eller et farmaceutisk acceptabelt salt deraf administreres før administration af rhGAA.

3. Sammensætning til anvendelse ifølge krav 1 eller krav 2,
5 hvor rhGAA-molekylerne omfatter en aminosyresekvens, der er mindst 95 % identisk med SEQ ID NO: 1 eller SEQ ID NO: 5.

4. Sammensætning til anvendelse ifølge et hvilket som helst
af kravene 1 til 3, hvor rhGAA-molekylerne i gennemsnit
10 omfatter fra ca. 0,5 mol til ca. 7,0 mol mono-M6P eller bis-M6P pr. mol rhGAA bestemt ved anvendelse af LC-MS/MS.

5. Sammensætning til anvendelse ifølge et hvilket som helst
af kravene 1 til 4, hvor rhGAA-molekylerne i gennemsnit
15 omfatter mindst 2,5 mol M6P pr. mol rhGAA og mindst 4 mol sialinsyre pr. mol rhGAA bestemt ved anvendelse af LC-MS/MS.

6. Sammensætning til anvendelse ifølge et hvilket som helst
af kravene 1 til 5, hvor rhGAA-molekylerne i gennemsnit pr.
20 mol rhGAA omfatter:

(a) ca. 0,4 til ca. 0,6 mol mono-M6P i det andet potentielle N-glycosyleringssted;

(b) ca. 0,4 til ca. 0,6 mol bis-M6P i det fjerde potentielle N-glycosyleringssted; og

25 (c) ca. 0,3 til ca. 0,4 mol mono-M6P i det fjerde potentielle N-glycosyleringssted;

hvor (a)-(c) er bestemt ved anvendelse af LC-MS/MS.

7. Sammensætning til anvendelse ifølge et hvilket som helst
30 af kravene 1 til 6, hvor rhGAA-molekylerne yderligere omfatter ca. 4 mol til ca. 7,3 mol sialinsyre pr. mol rhGAA; og hvor rhGAA-molekylerne i gennemsnit pr. mol rhGAA omfatter:

(a) ca. 0,9 til ca. 1,2 mol sialinsyre i det tredje potentielle N-glycosyleringssted;

35 (b) ca. 0,8 til ca. 0,9 mol sialinsyre i det femte potentielle N-glycosyleringssted; og

(c) ca. 1,5 til ca. 4,2 mol sialinsyre i det sjette potentielle N-glycosyleringssted;

hvor (a)-(c) er bestemt ved anvendelse af LC-MS/MS.

8. Sammensætning til anvendelse ifølge et hvilket som helst af kravene 1 til 7, hvor populationen af rhGAA-molekyler er
5 formuleret i en farmaceutisk sammensætning, og hvor den farmaceutiske sammensætning yderligere omfatter mindst én buffer valgt fra gruppen, der består af en citrat, en fosfat og en kombination deraf, og mindst ét hjælpestof valgt fra gruppen, der består af mannitol, polysorbat 80 og en
10 kombination deraf; hvor den farmaceutiske sammensætning har et pH på ca. 5,0 til ca. 7,0.

9. Sammensætning til anvendelse ifølge et hvilket som helst af kravene 1 til 8, hvor populationen af rhGAA-molekyler er
15 til stede i den farmaceutiske sammensætning ved en koncentration på ca. 5-50 mg/ml, den mindst ene buffer er en natriumcitratbuffer, der er til stede ved en koncentration på ca. 10-100 mM, det mindst ene hjælpestof er mannitol, der er til stede ved en koncentration på ca. 10-50 mg/ml, og
20 polysorbat 80, der er til stede ved en koncentration på ca. 0,1-1 mg/ml, og den farmaceutiske sammensætning yderligere omfatter vand og eventuelt omfatter et forsuringsmiddel og/eller alkaliseringsmiddel; hvor den farmaceutiske sammensætning har et pH på ca. 6,0.

DRAWINGS

Structure and Receptor Affinity for High Mannose and
Phosphorylated Oligosaccharides

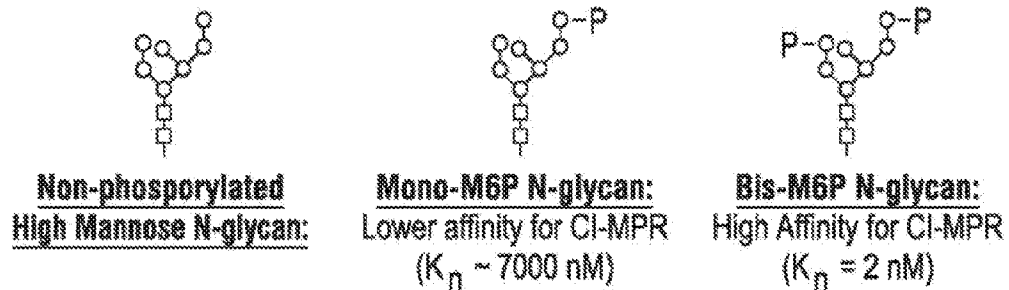


Fig. 1A

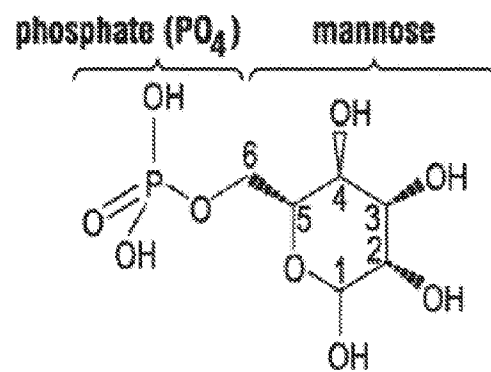


Fig. 1B

Comparison of Productive and Non-Productive Targeting Glycan Specific Receptors

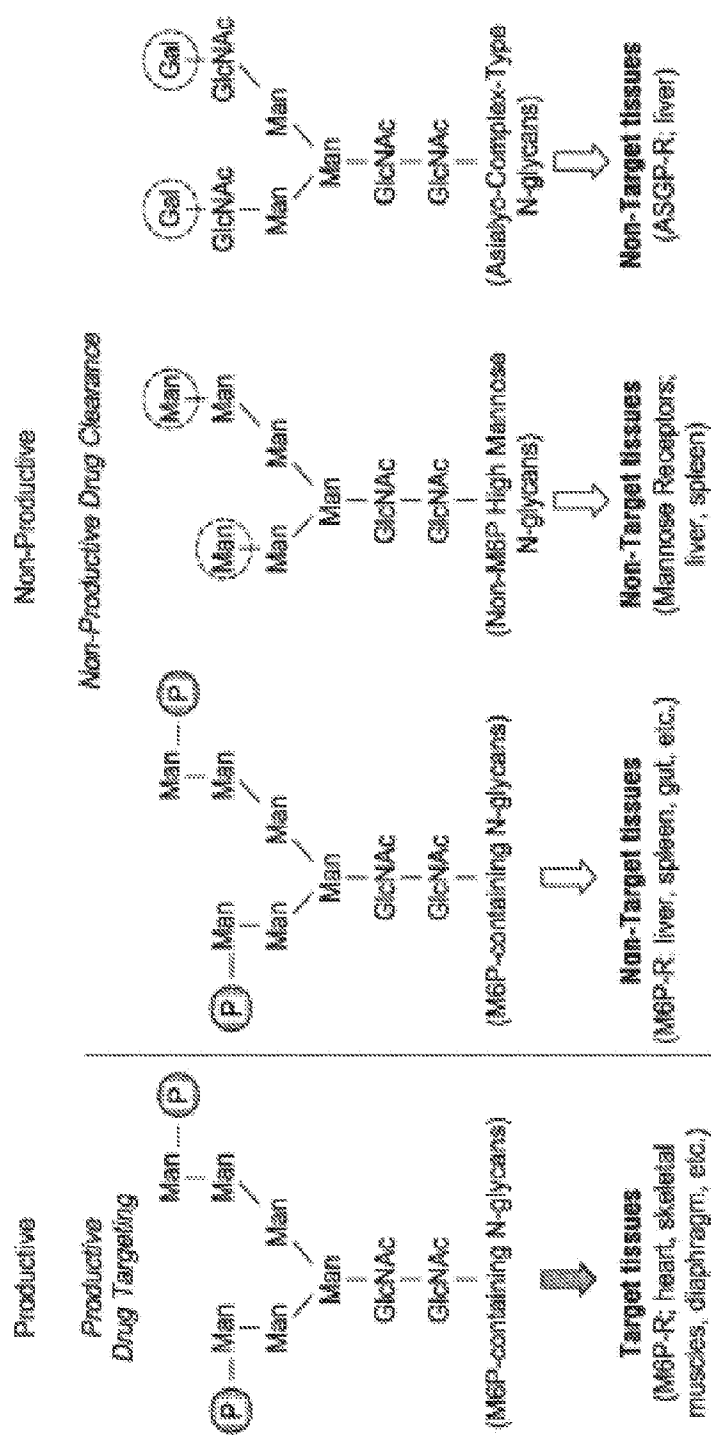


Fig. 2A

Fig. 2B

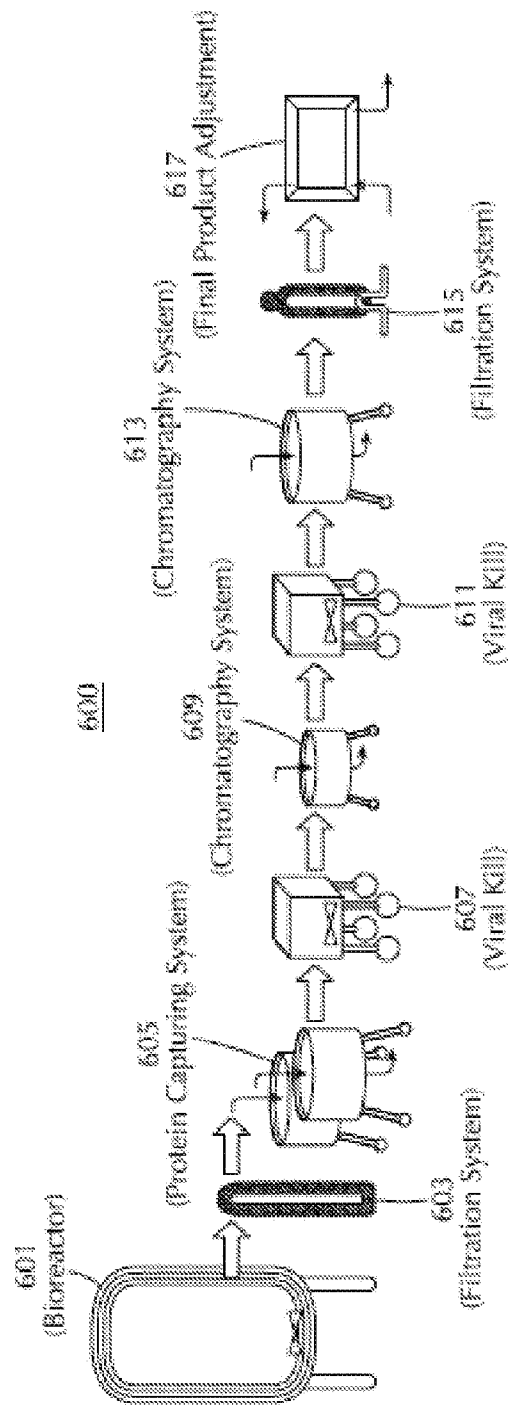
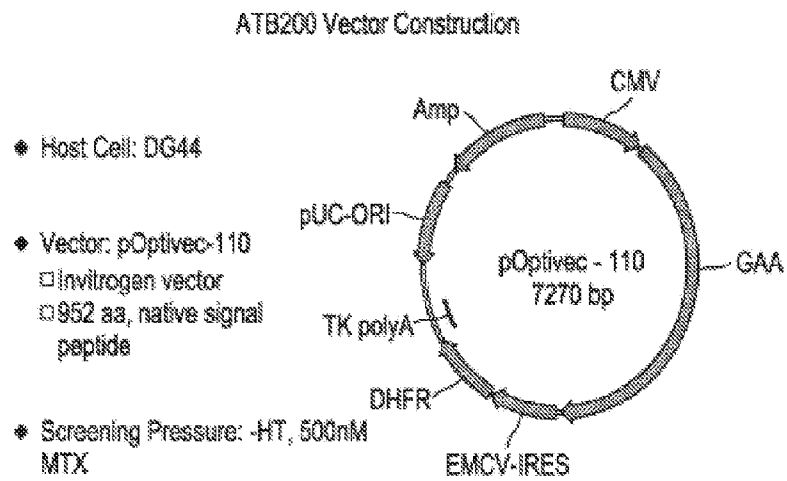


Fig. 3

**Fig. 4**

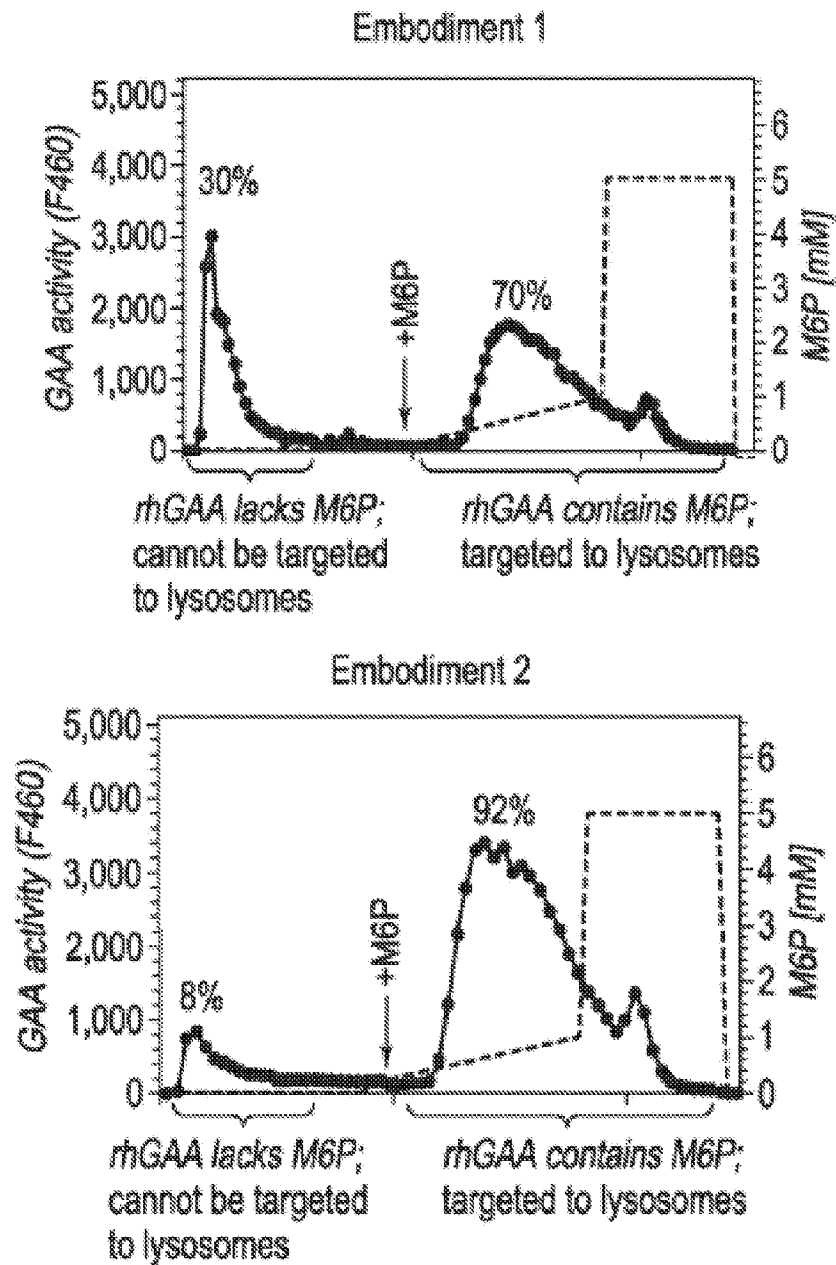
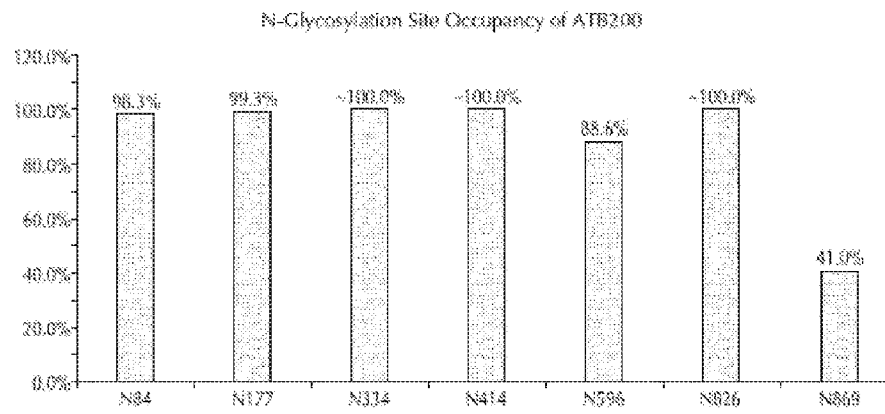


Fig. 5

**Fig. 6A**

N-Glycosylation Profile of N414

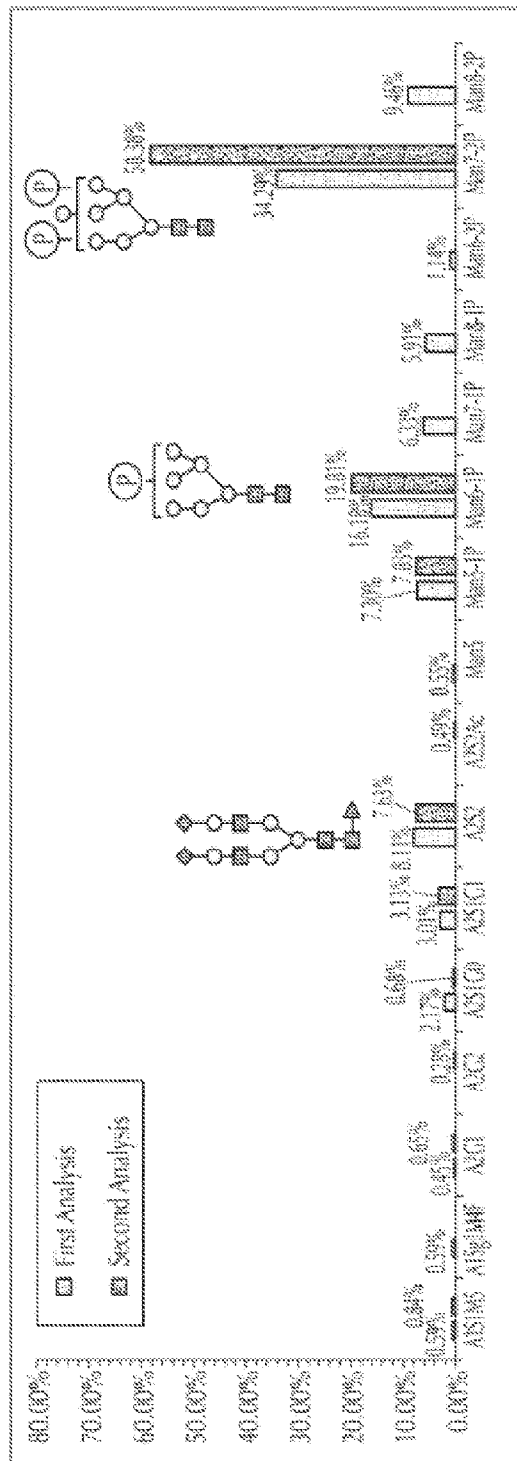


Fig. 6E

Relative Percent Bis-M6P and Mono-M6P in Each N-glycosylation Site

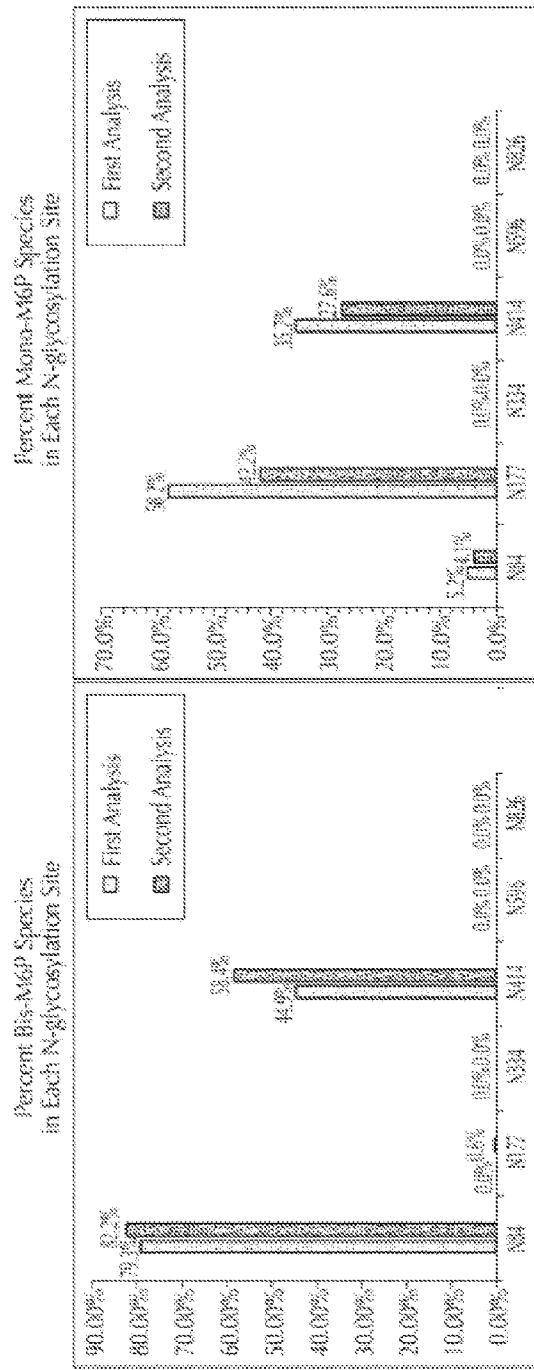
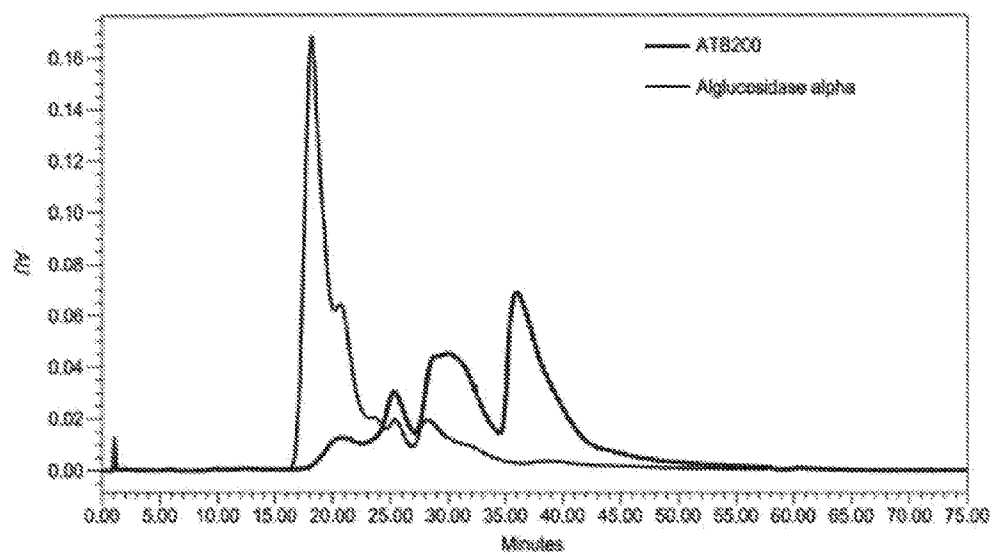


Fig. 6H

**Fig. 7**

Distribution of N-Glycans on rhGAA Preparations

	Lumizyme	BP-rhGAA*	1	2	ATB200
Complex Type N-Glycans	70.7%	48.9%	51.0%		47.5%
Hybrid Type N-Glycans	6.7%	9.7%	4.4%		3.7%
High Mannose Type N-Glycans:					
Non-phosphorylated	15.8%	23.7%	14.0%		9.9%
Mono-M6P	5.2%	10.4%	13.4%		14.2%
Bis-M6P	1.6%	6.6%	17.2%		24.7%

Fig. 8

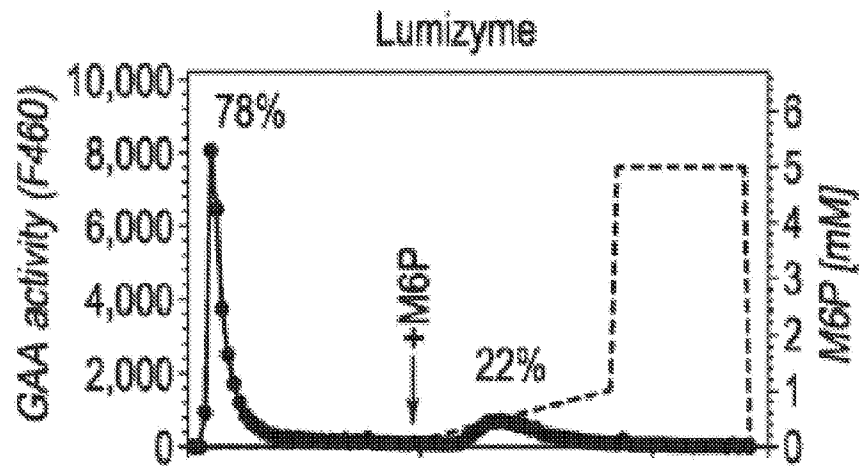


Fig. 9A

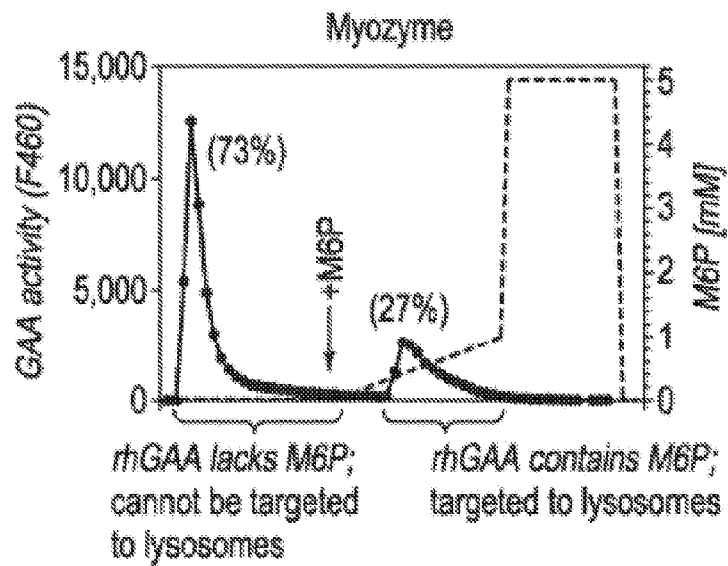


Fig. 9B

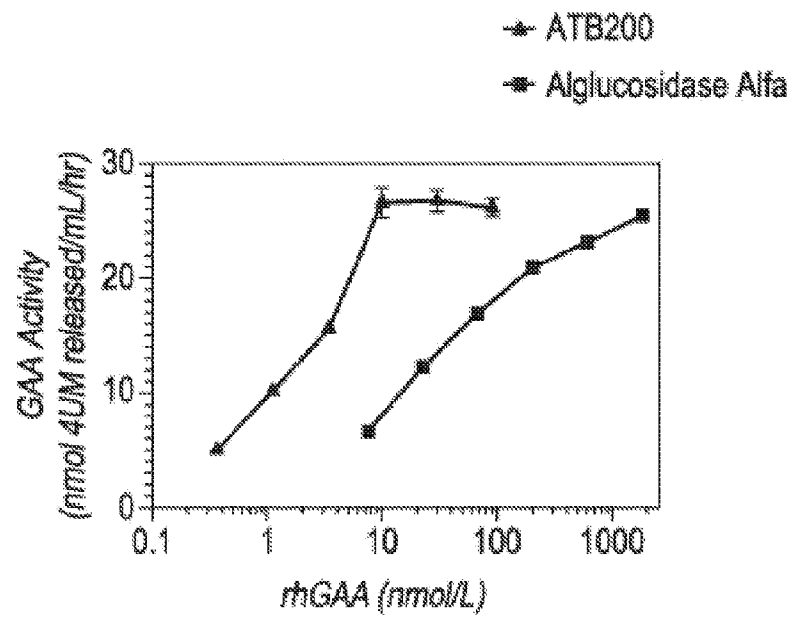


Fig. 10A

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

Fig. 10B

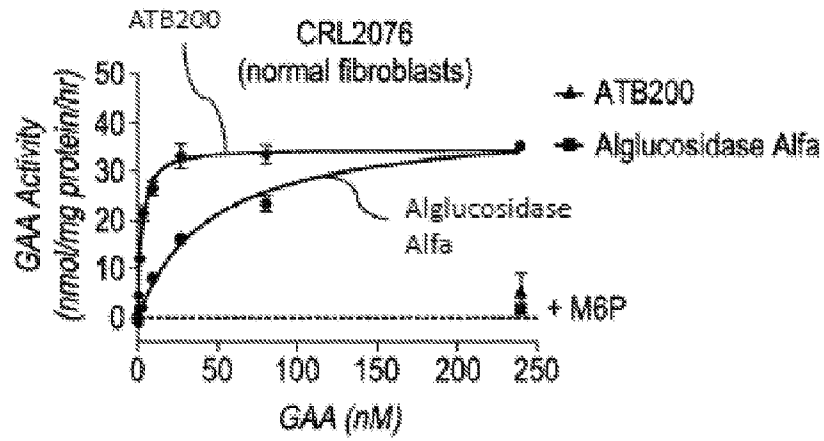


Fig. 11A

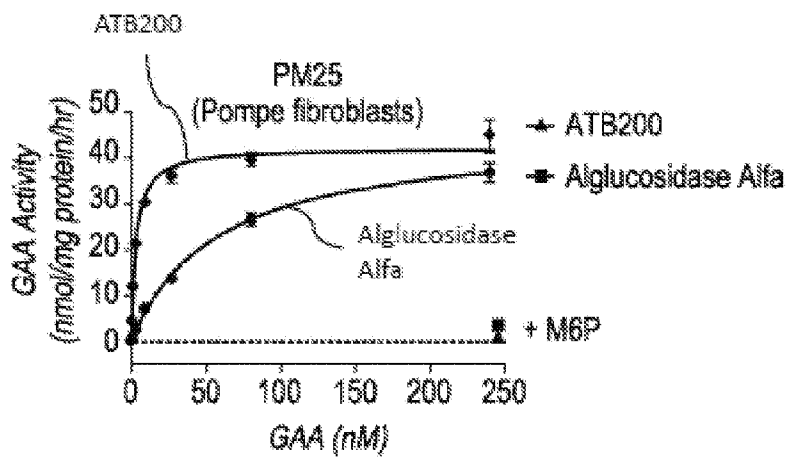
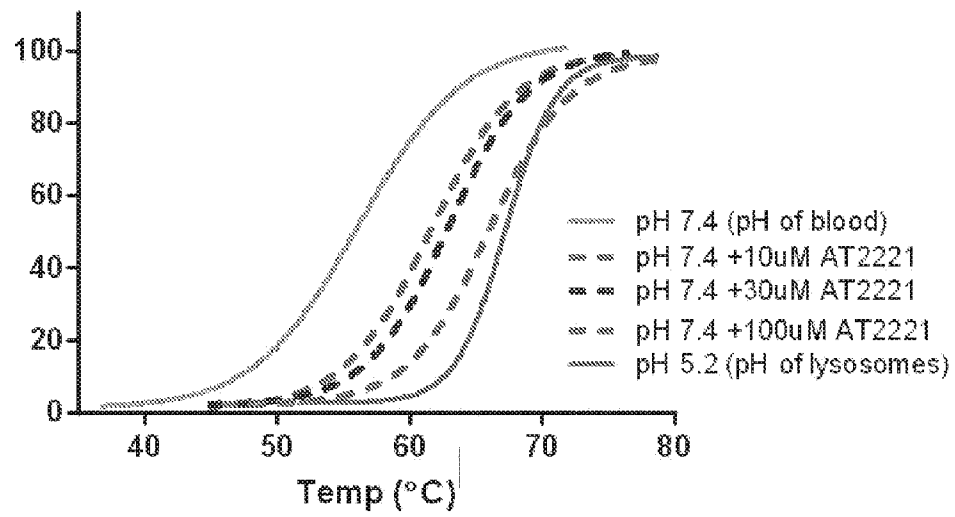


Fig. 11B

Cell Line	K _{uptake} (nM)	
	AT200	Lumizyme
normal	2	56
Pompe	3	57

Fig. 11C

**Fig. 12**

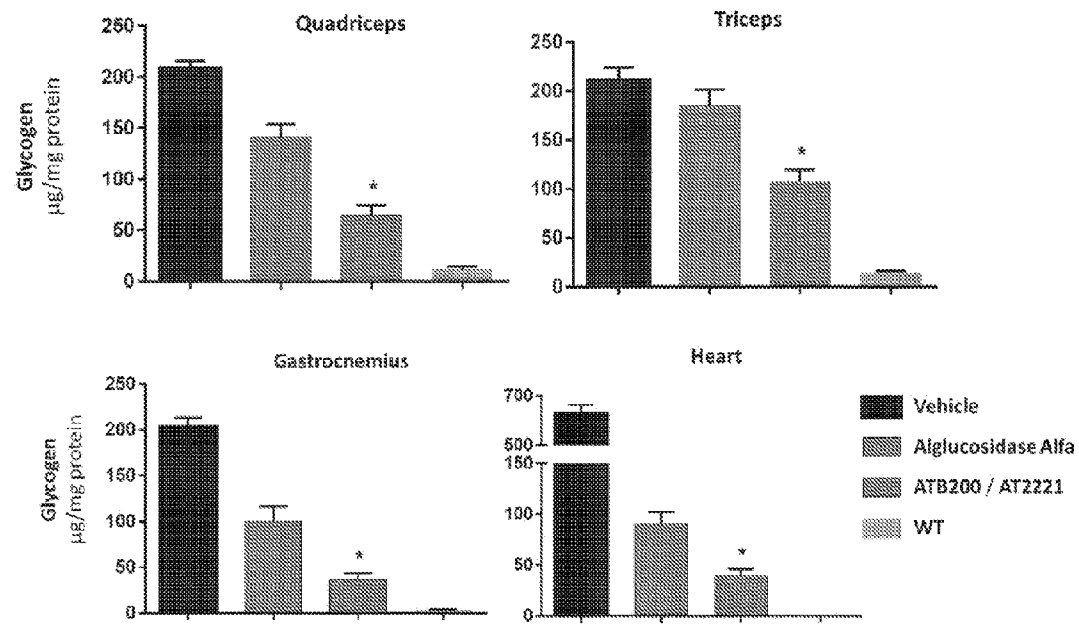


Fig. 13

IHC Examination of LAMP1 in *vastus lateralis* of Quadriceps

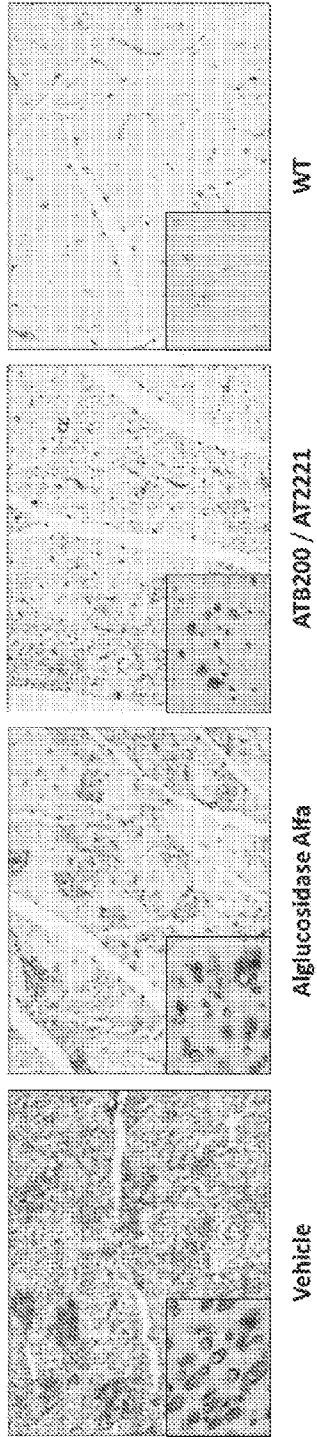
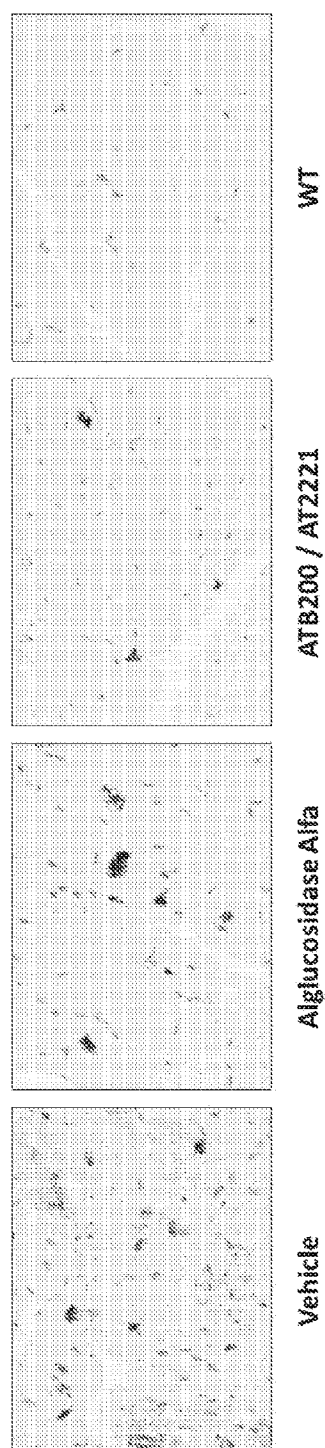


Fig. 14

IHC Examination of LC3 in *vastus lateralis* of Quadriceps**Fig. 15A**

WB Examination of LC3 in Quadriceps

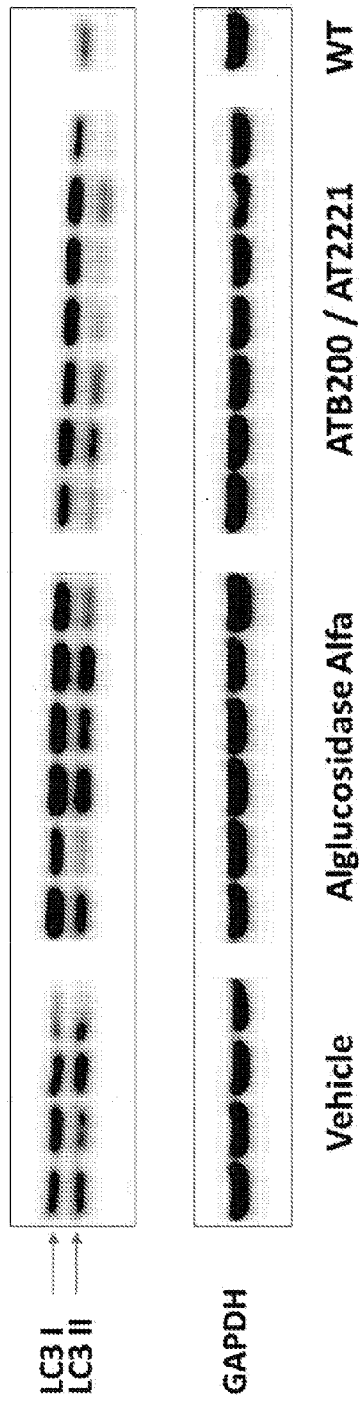


Fig. 15B

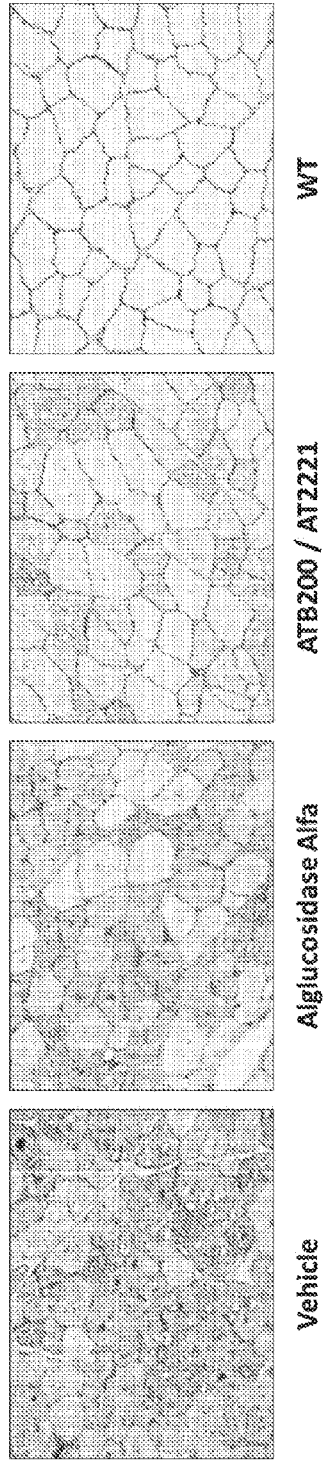
IHC Examination of Dysferlin in *vastus lateralis* of Quadriceps

Fig. 16

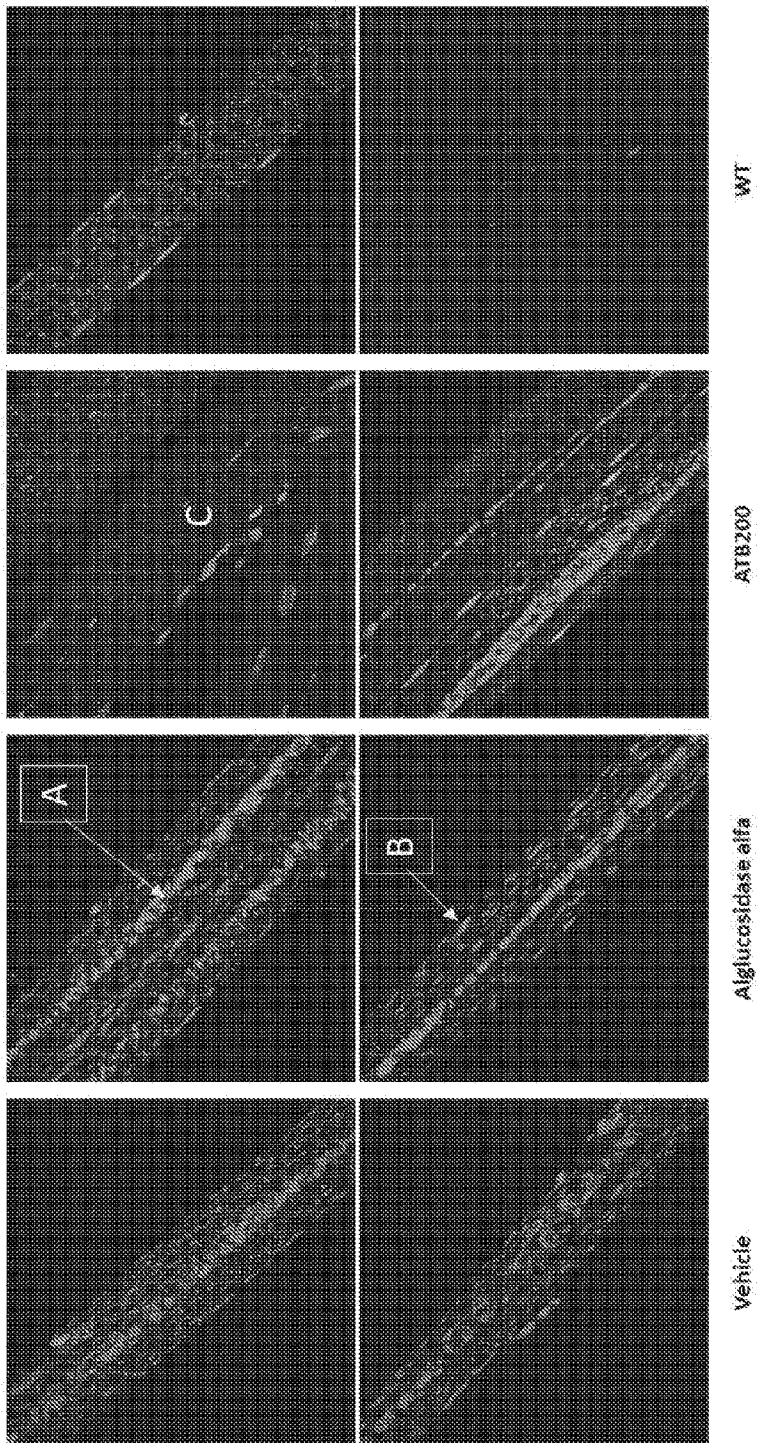
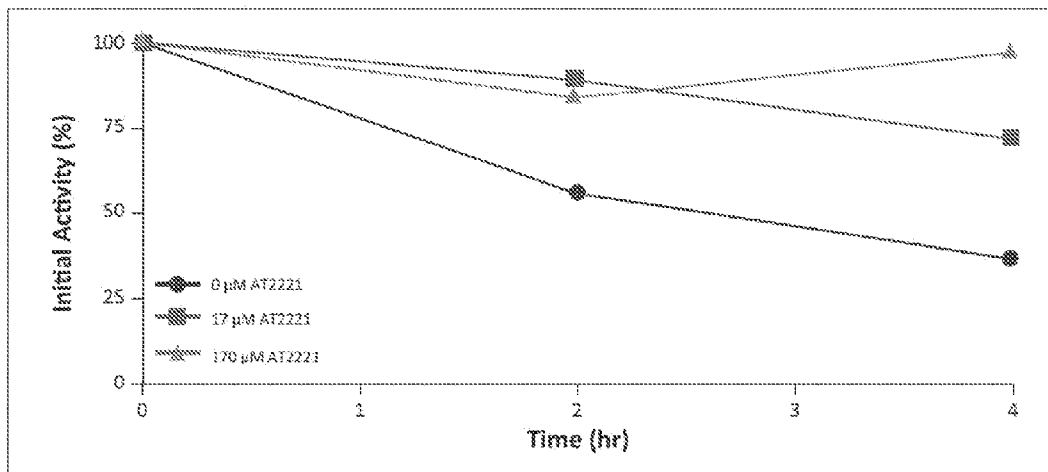
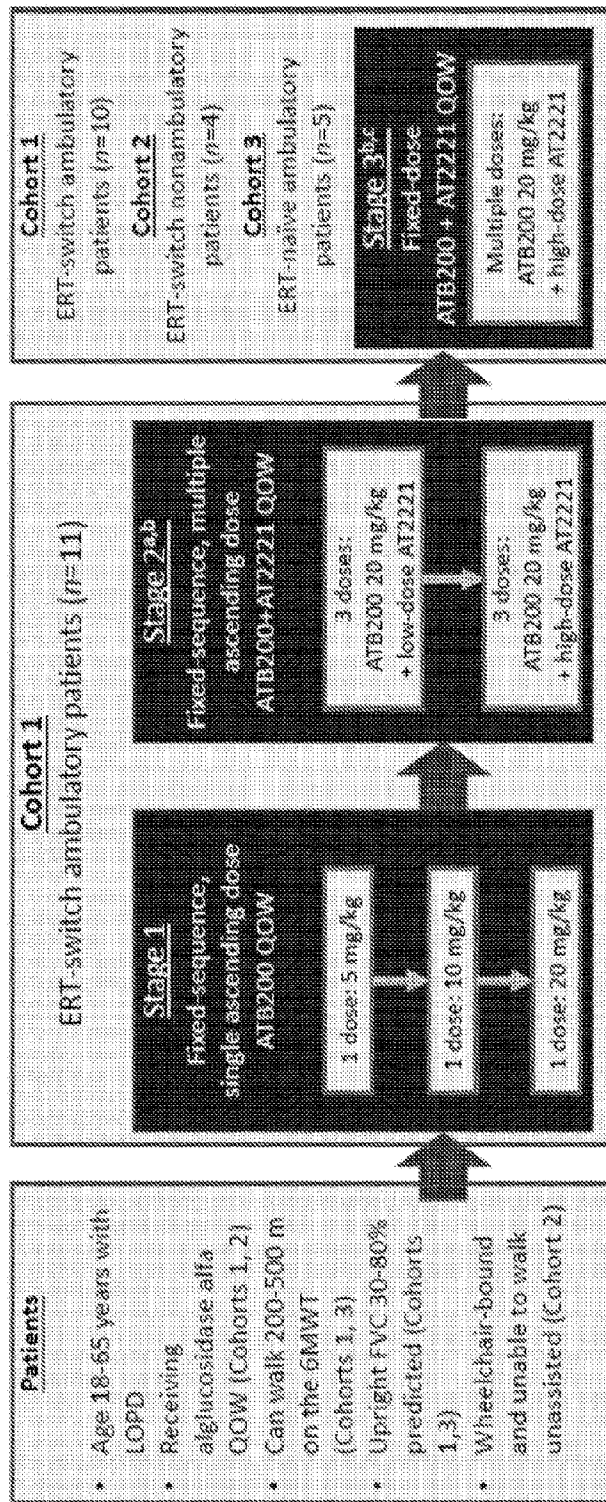


Fig. 17

Stabilization of ATB200 by AT2221

**Fig. 18**



6-hourly intravenous infusion; FVC: forced vital capacity; QOW: every other week.

*Safety data from 2 ambulatory patients from Cohort 1 were reviewed at each dose level before dosing in Cohorts 1 and 2. Younger stages 2 and 3. Younger stages 2 and 3. AT2221 was administered orally prior to the start of ATB200 intravenous infusion. For all doses, ATB200 was infused intravenously in a 4-hour period. *The first 2 patients in Cohorts 2 and 3 served as sentinel patients for their respective cohorts.

1. Johnson P, et al. Presented at the 13th Annual WCKD Symposium[®], February 13-17, 2017, San Diego, CA, USA. Poster no. LB-28.2. Robert M, et al. Presented at the 22nd International Congress of the World Muscle Society, October 8-9, 2017, St. Malo, France. Poster no. LB-P-3.

Fig. 19A

Phase 1/2 Clinical Study to Evaluate Safety, Tolerability, Pharmacokinetics (PK), and Pharmacodynamics (PD) of ATB200 + Chaperone (ATB200/AT2221) at 16 Sites in 5 Countries

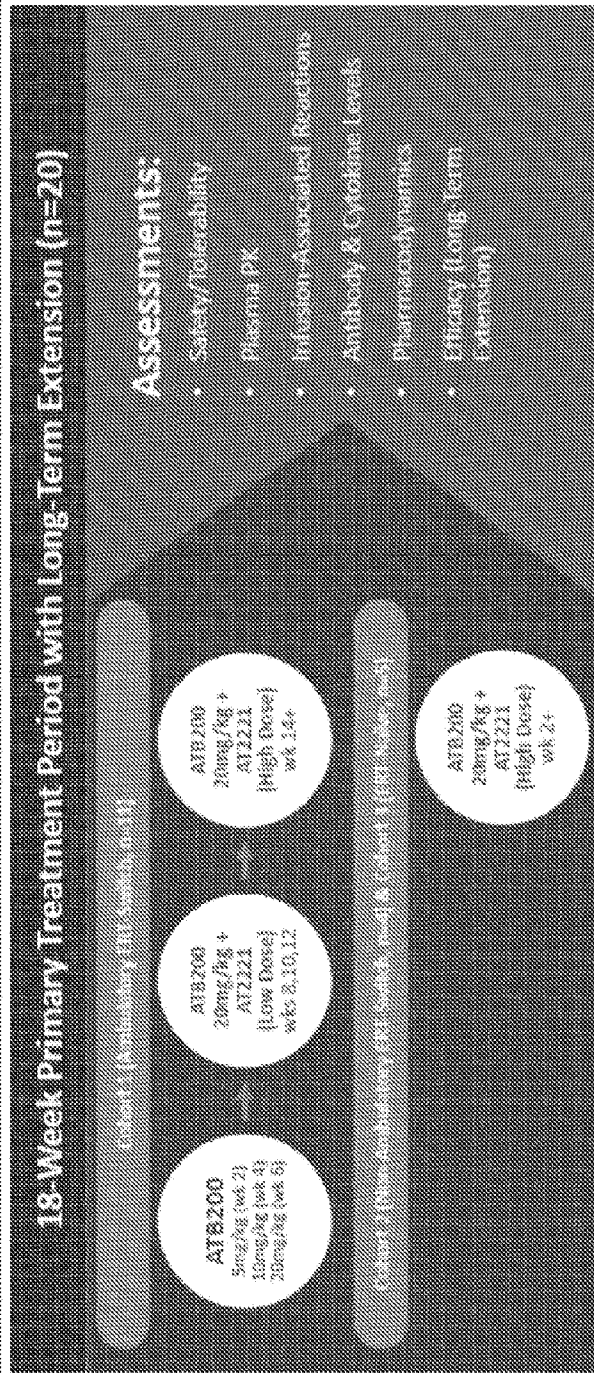


Fig. 19B

Baseline Characteristics (N=20)

The patients enrolled across the three cohorts are representative of the overall LCPD population, with significant impairment at baseline			
	Cohort 1 ERT-Switch Ambulatory (N=11)	Cohort 2 ERT-Switch Non-ambulatory (N=4)	Cohort 3 ERT-Valve (N=5)
Age, years, mean (min, max)	49.4 (28, 66)	36.0 (18, 56)	49.4 (24, 65)
Sex, M:F	9:2	3:1	1:4
Time on alglucosidase alfa, years, mean (SD)	4.8 (1.42) ^a	8.9 (3.8)	NA
6MWT, meters, mean (SD)	392.0 (93.4)	NA	399.5 (83.5)
FVC Upright, % predicted, mean (SD)	52.3 (13.2)	NA	53.4 (20.3)

NA not applicable; SD standard deviation.

^aCohort 1 patients were required to be on ERT on alglucosidase alfa for 2-6 years at baseline.

Fig. 19C

AT2221 Pharmacokinetics Summary

Treatment	C _{max} (ng/mL) ^a	t _{max} (h) ^b	AUC _{0-∞} (ng·h/mL) ^a	AUC ₀₋₂₄ (ng·h/mL) ^a	t _{1/2} (h) ^c	CL/F (L/h) ^f	V _d /F (L) ^e
Low Dose MD (N=11)	1504 (23.9)	3.0 (1.5-4.0)	11,968 (24.5)	12,913 (25.6)	6.5 (29.3)	10.3 (21.3)	97.3 (39.8)
High Dose MD (N=16)	3086 (29.4)	3.0 (1.0-4.0)	24,095 (25.9)	25,506 (25.9)	5.9 (18.3)	10.5 (22.9)	90.5 (34.1)

CL/F=plasma clearance adjusted for AT2221 oral bioavailability; V_d/F=apparent terminal phase volume of distribution adjusted for AT2221 oral bioavailability.

^aGeometric mean (CV%), ^bMedian (min-max), ^cArithmetic mean (CV%).

Fig. 20

Total GAA Protein by Signature Peptide T09

Cohort	Treatment	C _{max} (ng/mL) ^a	t _{max} (hr) ^a	AUC t _{max-24h} (ng·hr/mL) ^a	AUC _{0-24h} (ng·hr/mL) ^a	F _{rel} ^a	αt _{1/2} (hr) ^a	CL _T (L/hr) ^a
1	ATB200 5 mg/kg alone ^d	58.4 (19.1)	4.0 (3.0-4.0)	108 (25.1)	211 (17.2)	—	3.1 (10.2)	2.2 (16.9)
1	ATB200 10 mg/kg alone ^d	135 (18.3)	4.0 (3.5-4.0)	287 (25.6)	538 (24.4)	2.6 (9.4)	1.3 (10.6)	1.7 (22.4)
1	ATB200 20 mg/kg alone ^d	325 (13.5)	4.0 (3.5-4.0)	844 (20.8)	1418 (16.9)	6.9 (7.4)	1.5 (8.5)	1.3 (18.4)
1	ATB200 20 mg/kg + AT2221 low dose MD ^e	335 (15.4)	4.0 (3.5-5.0)	1062 (23.8)	1652 (20.5)	1.17 (7.7)	1.8 (21.8)	1.1 (20.5)
1	ATB200 20 mg/kg + AT2221 high dose MD ^e	345 (18.5)	4.0 (3.5-4.0)	1203 (24.2)	1871 (21.5)	1.28 (9.4)	2.1 (16.1)	1.0 (22.7)
3	ATB200 20 mg/kg + AT2221 high dose MD ^e	322 (14.3)	4.0 (4.0-4.5)	1147 (20.9)	1775 (19.3)	N/A	2.2 (9.9)	0.8 (28.4)

AUC=area under the curve; CL_T=total body clearance; C_{max}=maximum drug concentration; CV=coefficient of variability; MD=multiple doses; t_{1/2}=half life; t_{max}=time to maximum drug concentration; F_{rel}=AUC Ratio of 20 mg/kg and 10 mg/kg vs 5 mg/kg, and 20 mg/kg + low dose or high dose AT2221 vs 20 mg/kg alone.

^aGeometric mean (CV%). ^bMedian (min-max). ^cArithmetic mean (CV%). ^dn=11. ^en=5.

Fig. 21

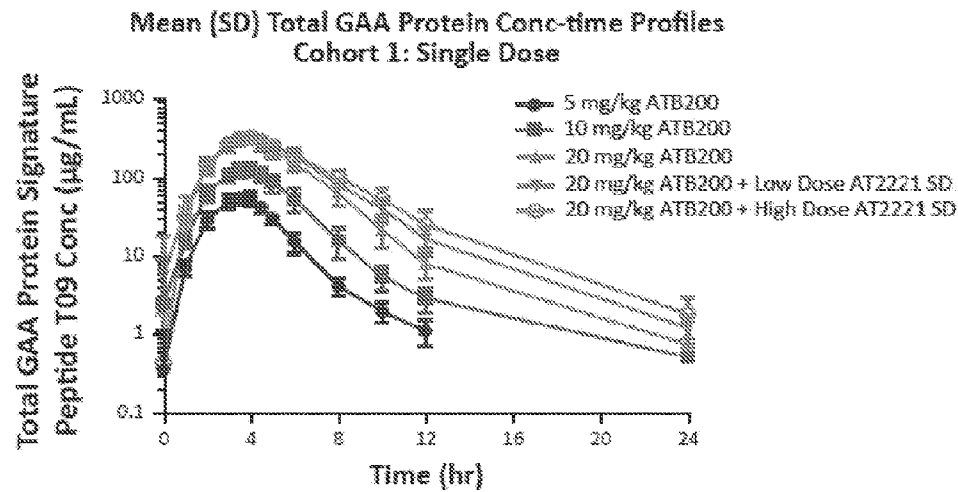


Fig. 22A

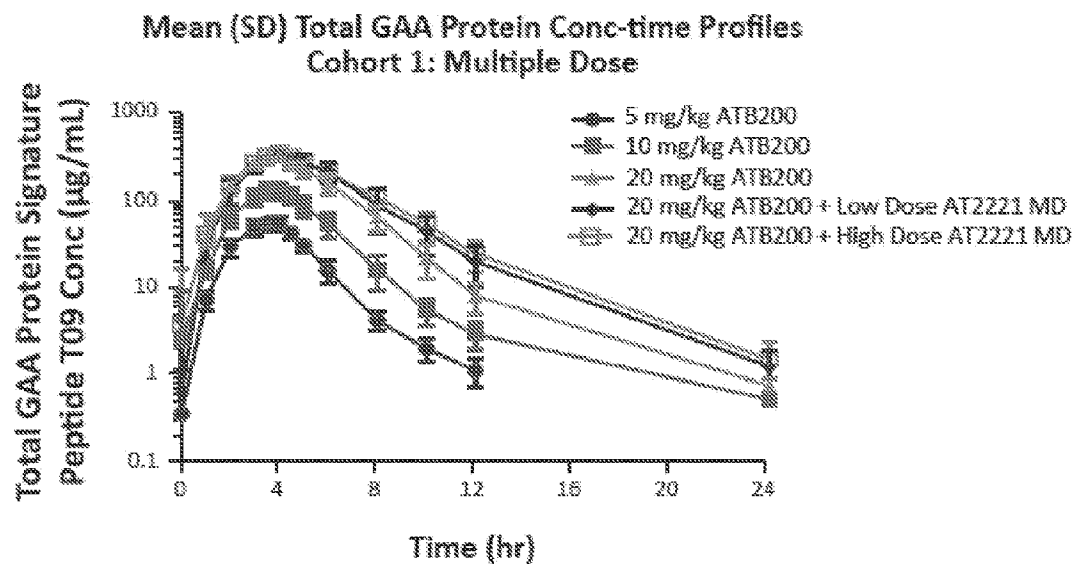


Fig. 22B

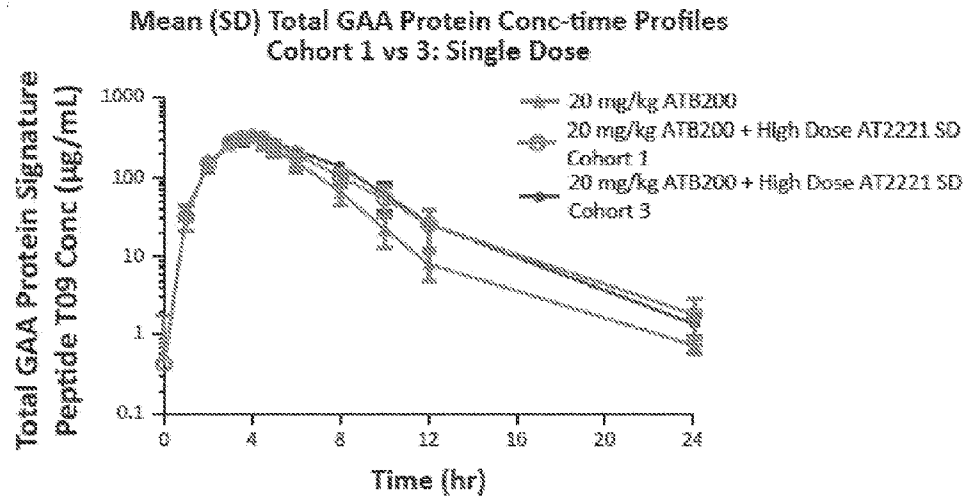


Fig. 22C

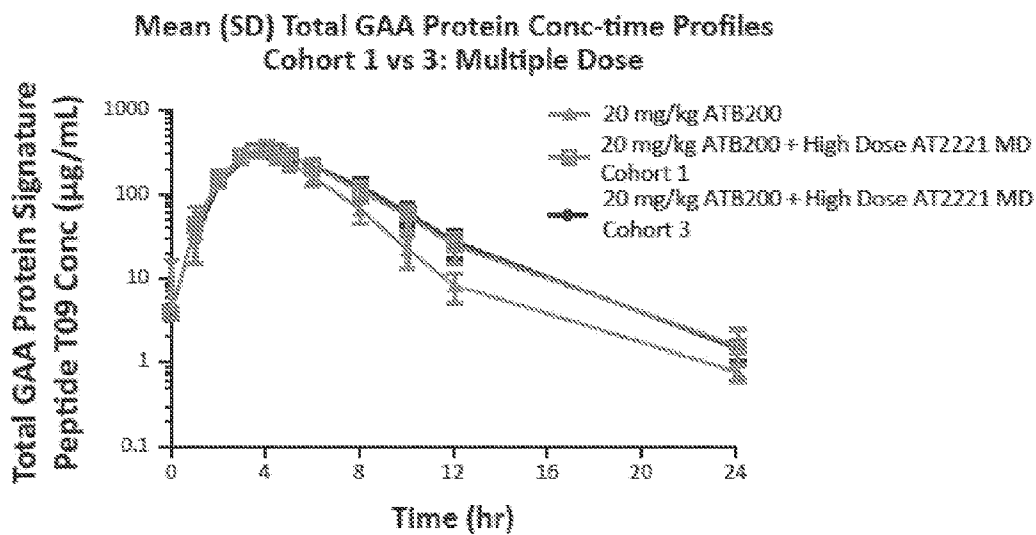
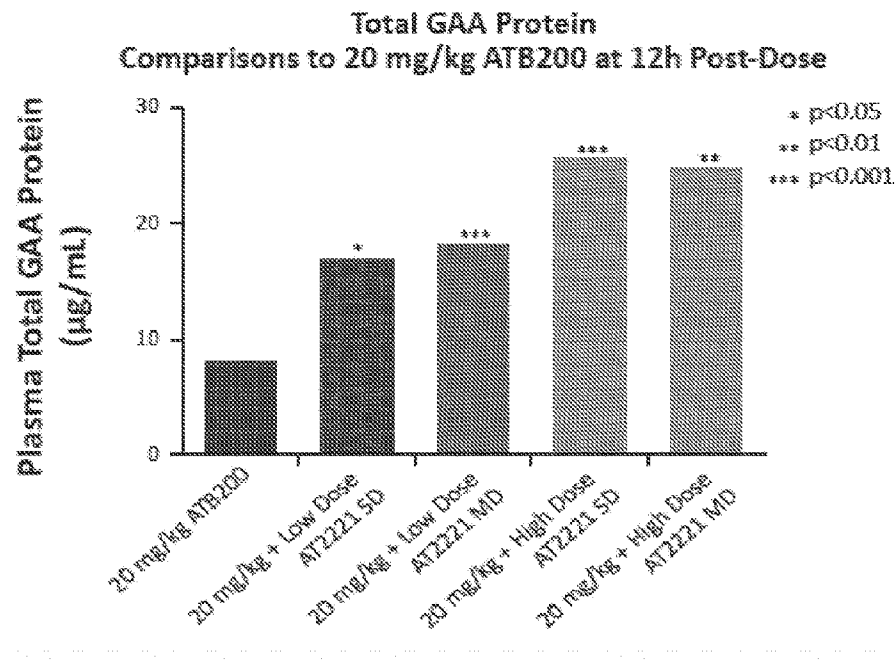
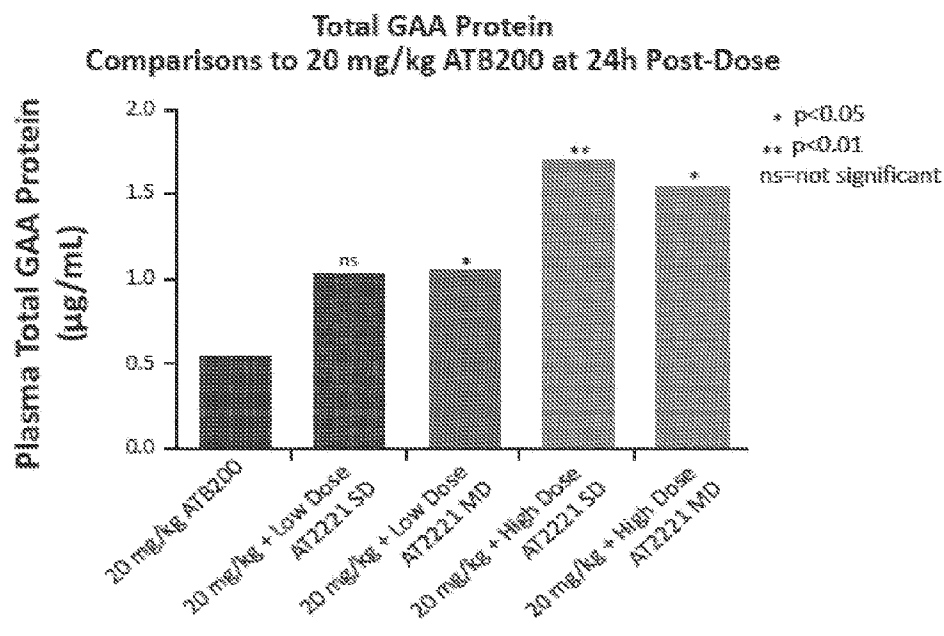


Fig. 22D

**Fig. 22E****Fig. 22F**

ANOVA for Total GAA Protein by Signature Peptide T09

Reference	Test	AUC Ratio % Ref	90% Lower-bound CI	90% Upper-bound CI
Cohort 1 SD	Cohort 1 MD	98.0	94.9	101.2
Cohort 1 SD	Cohort 3 SD	100.1	82.3	121.8
Cohort 1 MD	Cohort 3 MD	97.5	80.2	118.6

AUC=area under the curve; CI=confidence interval.

Fig. 23

6-Minute Walk Test

6MWT Improved for Both ERT-switch Ambulatory and ERT-naïve Patients
at Months 6, 9 and 12 Compared to Baseline

6-Minute Walk Test (m); mean (SD)

Cohort 1 ERT-Switch Ambulatory	Baseline (n=10)	CPBL M6 (n=10)	CPBL M9 (n=10)	CPBL M12 (n=8)
	397.2 (96.8)	+23.9 (52.2)	+24.5 (40.8)	+57.4 (34.4)
Cohort 3 ERT-Naïve	Baseline (n=5)	CPBL M6 (n=5)	CPBL M9 (n=5)	CPBL M12 (n=2)
	399.5 (83.5)	+41.8 (29.4)	+63.5 (23.1)	+86.8 (11.1)

- 6MWT increased in 7/10, 8/10, and 8/8 ERT-switch patients at Months 6, 9 and 12 respectively
- 6MWT increased in 5/5, 5/5, and 2/2 ERT-naïve patients at Months 6, 9 and 12 respectively

Fig. 24A

Patient	Baseline	Change From Baseline		
		Month 6	Month 9	Month 12
Cohort 1 Ambulatory ERT-Switch				
1	544	+51	+56	+112
2	379	+125	+110	+103
3	339	+21	+45	+73
4	332	+8	+26	+45
5	456	-5	+8	+41
6	500	+55	+28	+33
7	220	+29	+21	+30
8	410	+38	+13	+22
9	464	-4	-9	---
10	328	-78	-43	---
Mean (SD)	397.2 (96.8)	+23.9 (52.2)	+24.5 (40.8)	+57.4 (34.4)
Cohort 3 ERT-Naïve				
1	480	+41	+72	+95
2	384	+62	+78	+79
3	460	+79	+69	---
4	406	+14	+44	---
5	267	+13	+35	---
Mean (SD)	399.5 (83.5)	+41.8 (29.4)	+63.5 (23.1)	+86.8 (11.1)

Fig. 24B

Other Motor Function Tests

	Assessment, sec	Baseline, Mean (SD)	Change From Baseline to Month 6, Mean (SD)	Change From Baseline to Month 9, Mean (SD)	Change From Baseline to Month 12, Mean (SD)
Cohort 1 ERT-Switch		n=10	n=10	n=10	n=8
	Timed Up and Go	10.5 (6.6)	-1.8 (3.5)	-1.2 (3.3)	-1.6 (2.2)
	4-Stair Climb	4.1 (2.7)	-0.6 (1.6)	-0.4 (1.6)	-1.0 (1.5)
	10M Walk	7.4 (3.0)	+0.1 (1.9)	-0.1 (1.6)	-0.5 (1.7)
	Gowers ^a	7.9 (2.9)	-1.1 (3.8)	4.5 ^b (13.4)	-2.6 (1.9)
	GSGC Score	12.6 (4.8)	+0.1 (3.9)	+0.5 (4.6)	-1.9 (2.2)
Cohort 3 ERT-Native		n=5	n=5	n=5	n=2
	Timed Up and Go	9.4 (2.9)	-1.0 (1.1)	-0.6 (1.4)	-1.8 (0.5)
	4-Stair Climb	4.2 (1.5)	-0.5 (0.3)	0.0 (1.5)	-0.4 (0.4)
	10M Walk	7.9 (3.0)	-0.7 (1.1)	-1.3 (1.0)	-0.6 (0.0)
	Gowers	13.9 (11.0)	7.9 ^c (20.9)	-1.6 (3.9)	-2.1 (1.3)
	GSGC Score	12.2 (3.6)	-1.8 (3.8)	-2.4 (3.4)	0.0 (1.4)

GSGC=Gait, Stairs, Gowers, Chair

GSGC is an observer-rated combined score of 4 motor function assessments: Gait (10m walk), 4-Stair Climb, Gowers (stand from floor), and Rising From Chair. Each test is scored from 1 (normal) to 3 (cannot perform, max score of 6 for Rising From Chair). Total scores range from 4 to 27.

^a6/9, missing values not obtained due to patient refusal to perform test.

^bMedian change from baseline was +1.5, and 7/9 patients had a decrease.

^cMedian change from baseline was -0.8, and 4/5 patients had a decrease.

Fig. 24C

Muscle Strength Testing (QMT) in Cohort 2 Patients

	Muscle Group Tested	Baseline, Mean (SD) n=4	Change From Baseline to Month 6, Mean (SD) n=4	Change from Baseline to Month 9, Mean (SD) n=4
Quantitative Muscle Testing— Dynamometer pounds force	Shoulder Adduction*	5.7 (8.8)	+8.1 (12.8)	+9.6 (12.3)
	Shoulder Abduction	16.7 (18.1)	+1.0 (6.6)	+0.5 (9.3)
	Elbow Flexion	12.7 (13.7)	+2.4 (15.9)	+6.0 (19.3)
	Elbow Extension	12.3 (13.9)	+5.5 (4.7)	+7.5 (8.2)
Manual Muscle Testing, manual score [†]	Shoulder Adduction*	5.7 (8.8)	+8.1 (12.8)	+9.6 (12.3)
	Shoulder Abduction	16.7 (18.1)	+1.0 (6.6)	+0.5 (9.3)
	Elbow Flexion	12.7 (13.7)	+2.4 (15.9)	+6.0 (19.3)
	Elbow Extension	12.3 (13.9)	+5.5 (4.7)	+7.5 (8.2)

Measure is pounds of force for right and left sides combined.

*Shoulder adduction not available for 1 patient.

[†]Scoring: (1) Visible muscle movement, but no movement at the joint; (2) Movement at the joint, but not against gravity; (3) Movement against gravity, but not against added resistance; (4) Movement against resistance, but less than normal; (5) Normal strength.

Fig. 25

Manual Muscle Test Score in Cohort 1: ERT-Switch Ambulatory Patients

Increases were observed in manual muscle strength in ERT-switch patients
at Months 6, 9, and 12

	Body Area	Baseline		Change From Baseline					
				Month 6		Month 9		Month 12	
		mean (SD)	n	mean (SD)	n	mean (SD)	n	mean (SD)	n
Cohort 1 ERT-Switch Ambulatory	Upper Body Max score 40	35.4 (5.7)	10	+1.2 (2.4)	10	+1.7 (2.8)	10	+2.8 (3.9)	8
	Lower Body Max score 40	31.0 (3.0)	10	+0.9 (1.8)	9	+2.2 (2.4)	10	+2.3 (2.9)	8
	Total Body Max score 80	66.4 (8.1)	10	+2.8 (3.4)	10	+4.0 (3.1)	10	+4.5 (2.8)	8

Fig. 26A

Manual Muscle Test Score in Cohort 2: ERT-Switch Nonambulatory Patients

Increases were observed in manual muscle strength in ERT-switch nonambulatory
patients at Months 6 and 9

	Body Area	Baseline		Change From Baseline			
				Month 6		Month 9	
		mean (SD)	n	mean (SD)	n	mean (SD)	n
Cohort 2 ERT-Switch Nonambulatory	Upper Body Max score 40	13.3 (12.2)	3	+5.7 (2.1)	3	+2.3 (5.5)	3

Fig. 26B

Manual Muscle Test Score in Cohort 3: ERT-Naïve Patients

Increases were observed in manual muscle strength
in ERT-naïve patients at Months 6, 9, and 12

	Body Area	Baseline		Change From Baseline					
				Month 6		Month 9		Month 12	
		mean (SD)	n	mean (SD)	n	mean (SD)	n	mean (SD)	n
Cohort 3 ERT-Naïve	Upper Body Max score 40	36.8 (3.4)	5	-0.2 (1.3)	5	+0.8 (1.9)	5	-0.5 (0.7)	2
	Lower Body Max score 40	29.0 (2.0)	4	+0.5 (1.9)	4	+1.3 (3.6)	4	+4.5 (0.7)	2
	Total Body Max score 80	66.9 (3.7)	5	-0.7 (3.2)	5	+1.3 (3.1)	5	+4.0 (1.4)	2

Fig. 26C

Pulmonary Function Tests: FVC, MIP, MEP

	Assessment	Baseline, Mean (SD)	Change From Baseline to Month 6, Mean (SD)	Change From Baseline to Month 9, Mean (SD)	Change From Baseline to Month 12, Mean (SD)
Cohort 1 ERT-switch		n=9	n=9	n=9	n=7
	FVC, % predicted ^a	52.6 (14.7)	-1.3 (4.1)	-1.7 (3.9)	-3.1 (4.8)
	MIP	35.7 (11.0)	+0.3 (4.6)	-0.6 (3.0)	+0.3 (3.6)
	MEP	72.6 (32.6)	+16.1 (42.1)	+23.7 (38.1)	+36.8 (45.7)
Cohort 2 ERT-Naïve		n=5	n=5	n=5	n=2
	FVC, % predicted	53.4 (20.3)	+4.2 (5.6)	+6.2 (5.3)	+6.0 (7.1)
	MIP	32.6 (18.5)	+11.0 (5.0)	+12.0 (10.3)	-0.5 (9.2)
	MEP	60.6 (8.3)	-0.4 (12.4)	+7.2 (15.3)	-2.0 (9.9)

MEP=maximal expiratory pressure; MIP=maximal inspiratory pressure.

^aFVC not available for 1 patient.

MIP and MEP were measured in centimeters of water.

Fig. 27

Fatigue Severity Scale

	Baseline, Mean (SD)	Change From Baseline to Month 6, Mean (SD)	Change From Baseline to Month 9, Mean (SD)	Change From Baseline to Month 12, Mean (SD)
Cohort 1 Ambulatory ERT-Switch	n=10 53.5 (7.7)	n=10 -8.0 (10.7)	n=10 -6.8 (6.8)	n=8 -7.8 (6.0)
Cohort 2 Nonambulatory ERT-Switch	n=4 54.0 (8.5)	n=2 -3.5 (7.8)	n=2 -6.5 (5.0)	— —
Cohort 3 ERT Naïve	n=5 39.2 (12.7)	n=5 -5.2 (11.7)	n=5 -7.8 (7.5)	n=2 -1.5 (2.1)

Fatigue Severity Scale consists of 9 questions, each scored on a scale from 1 to 7. The total score ranges from 9 to 63, with higher values representing higher level of fatigue due to the disease condition. The normative value in the healthy population is ~21.⁴

Fig. 28

Mean Percentage Change From Baseline in Markers of Muscle Damage in Cohort 1 Patients

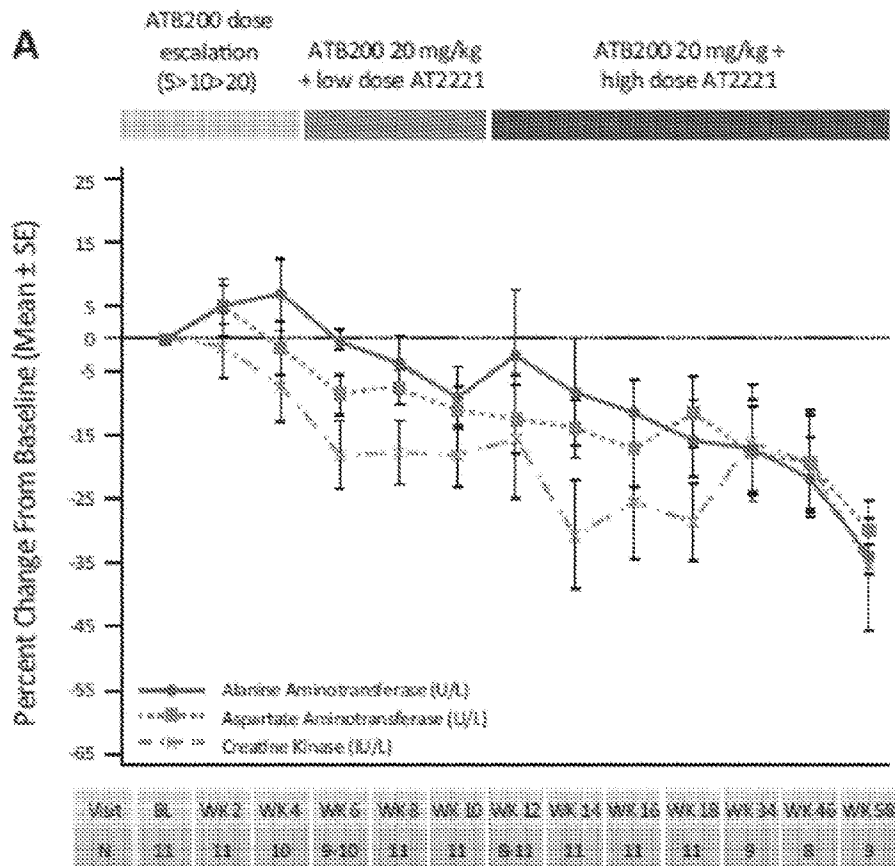


Fig. 29A

Mean Percentage Change From Baseline in Markers of Muscle Damage in Cohort 2 Patients

B

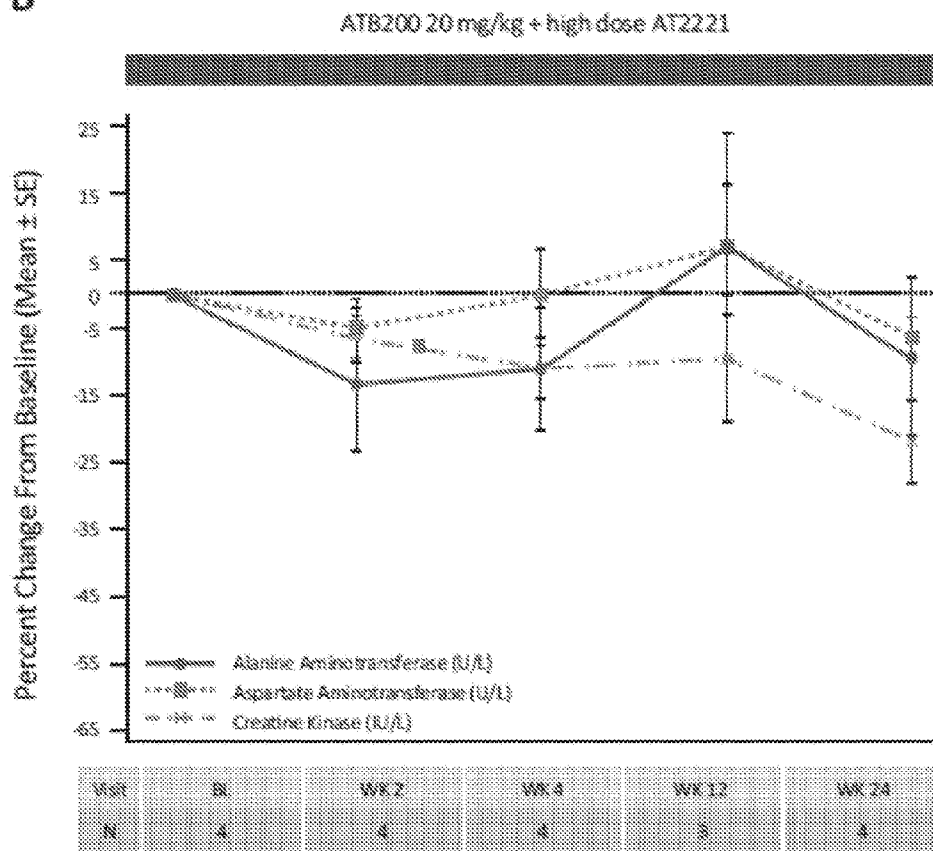


Fig. 29B

Mean Percentage Change From Baseline in Markers of Muscle Damage in Cohort 3 Patients

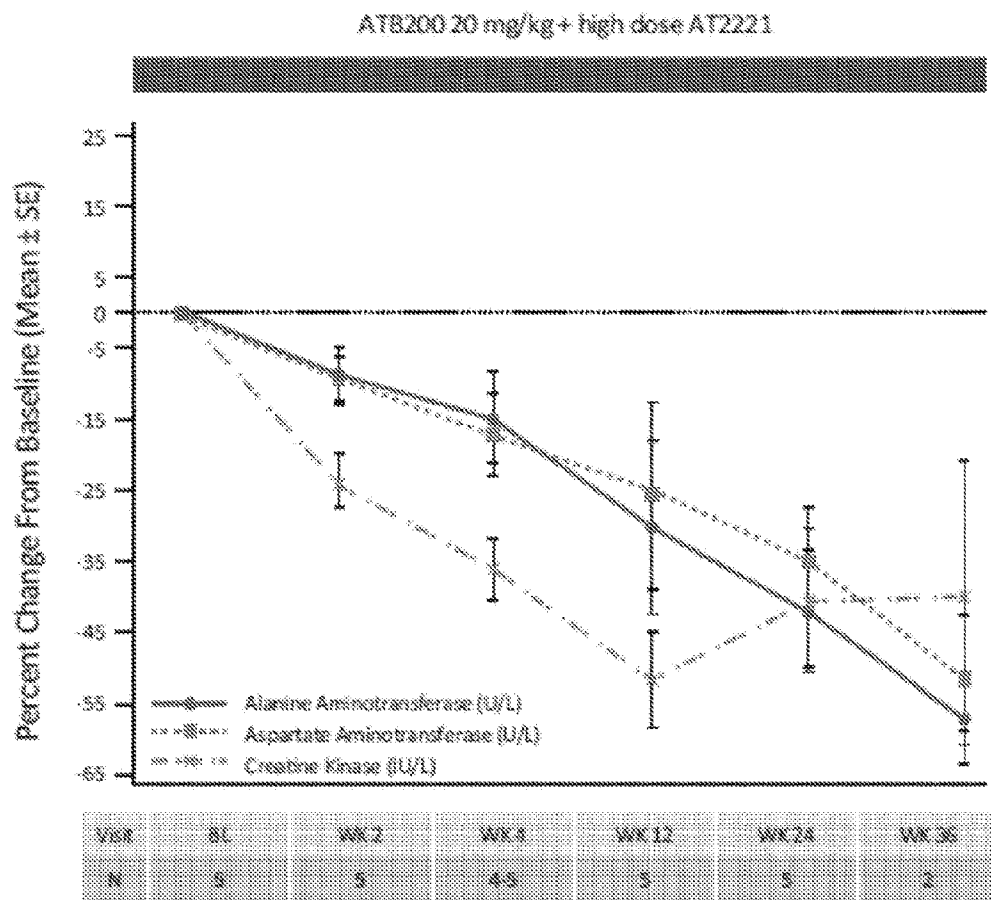
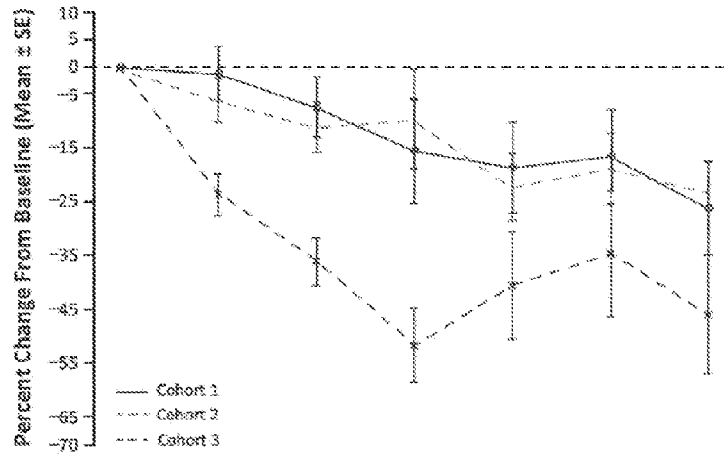


Fig. 29C

Mean Percentage Change From Baseline in Markers of
 (A) Muscle Damage (Creatine Kinase) and
 (B) Glycogen Accumulation (Urine Hexose Tetrasaccharide)

A



B

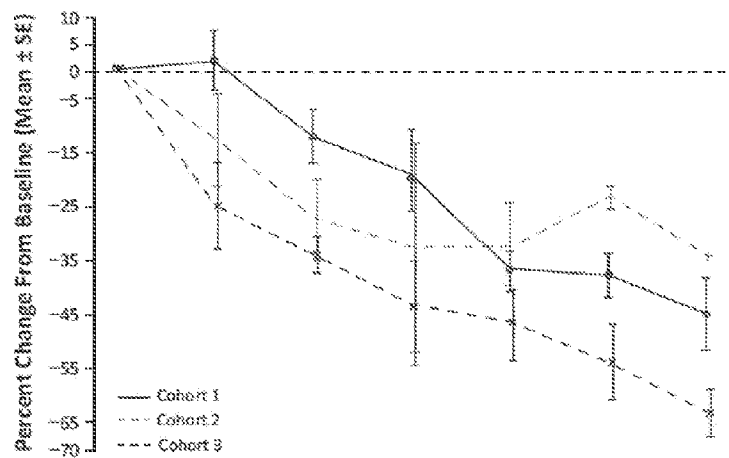


Fig. 29D

Safety Summary (N=20)^a

Safety data for ATB200/ATZ221 show that AEs have been generally mild and transient with very low rates of IARs (<1%) after 550+ total infusions across all cohorts

- AEs were generally mild and transient
 - The most common treatment-emergent AEs were abdominal pain^b (8/20), diarrhea (8/20), nasopharyngitis (6/20), nausea (5/20), headache (5/20), and upper respiratory tract infection (5/20)
- 3 incidents of IARs in 550+ infusions, which were controlled by standard premedication
 - 1 IAR event in a nonambulatory ERT-switch patient (skin discoloration)
 - 2 IAR events in a ERT-naïve patient (localized pruritus; erythema and burning sensation)
- Longest duration of treatment is 20+ months

AE, adverse event; IAR, infusion-associated reaction.

^aReported through interim data analysis (maximum 20+ months).

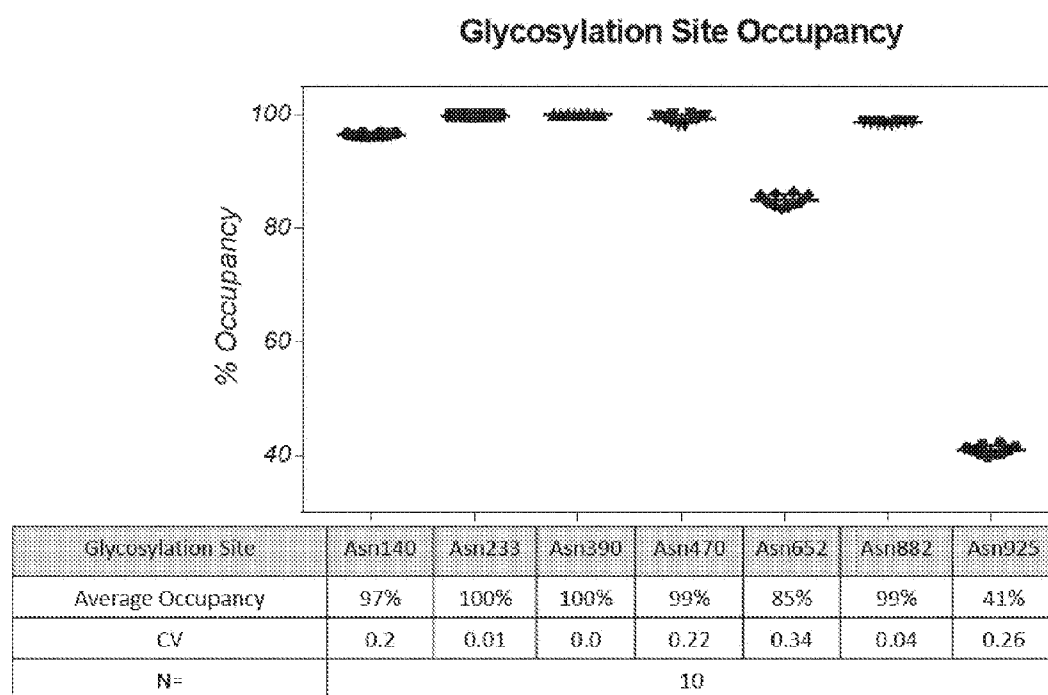
^bIncludes upper and lower abdominal pain.

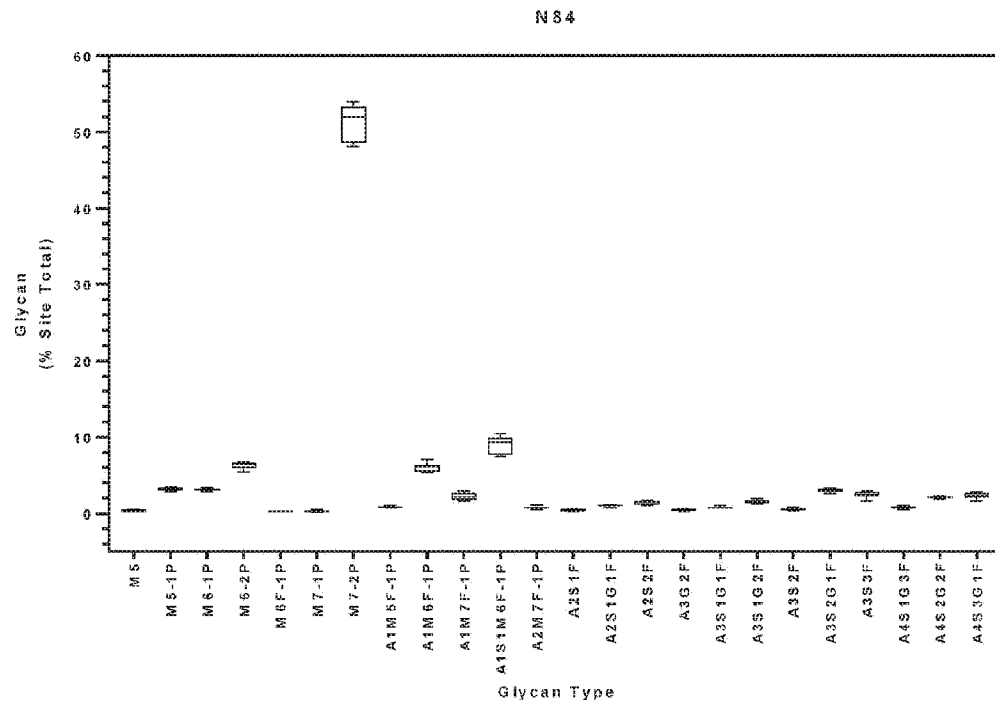
Fig. 30

Conclusions

- Muscle function
 - 6MWT distance continued to improve in ERT-switch ambulatory and ERT-naïve patients out to Month 12
 - Other motor function tests were consistent with 6MWT results in both cohorts
 - Increases were observed in manual muscle strength in all cohorts
 - Increases in elbow and shoulder muscle strength in nonambulatory ERT-switch patients at Months 6 and 9
- Pulmonary function
 - FVC, MIP, and MEP were generally stable in ERT-switch patients
 - FVC, MIP, and MEP generally increased in ERT-naïve patients
- Fatigue Severity Scale
 - Improvement in fatigue score observed in all cohorts
- Biomarkers and safety
 - CK and Hex4 levels decreased in all cohorts
 - ATB200/AT2221 was generally well tolerated

Fig. 31

**Fig. 32A**

**Fig. 32B**

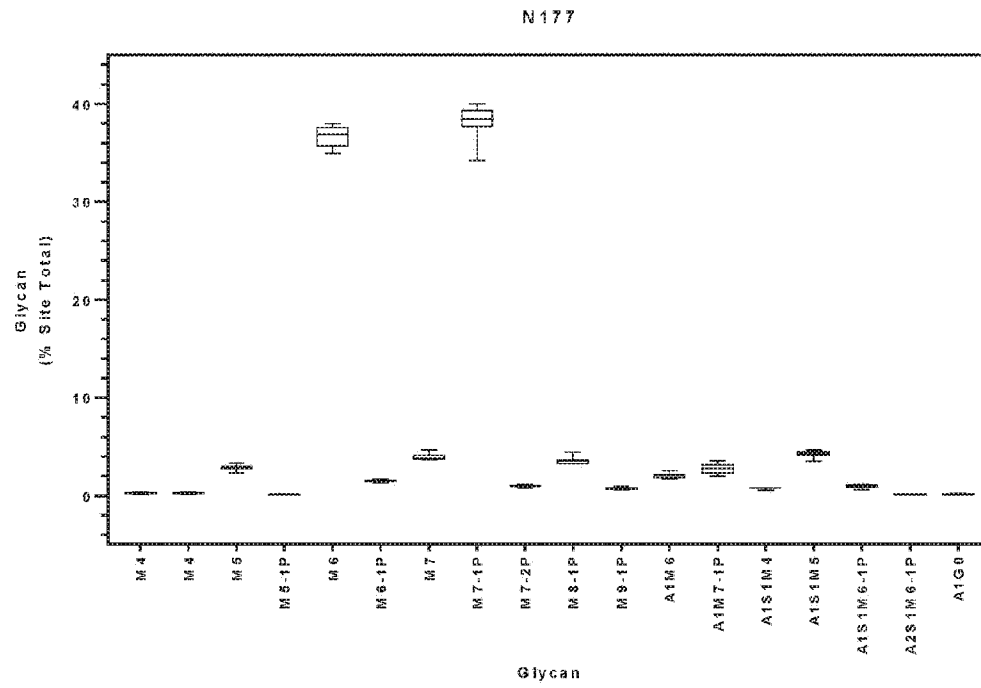


Fig. 32C

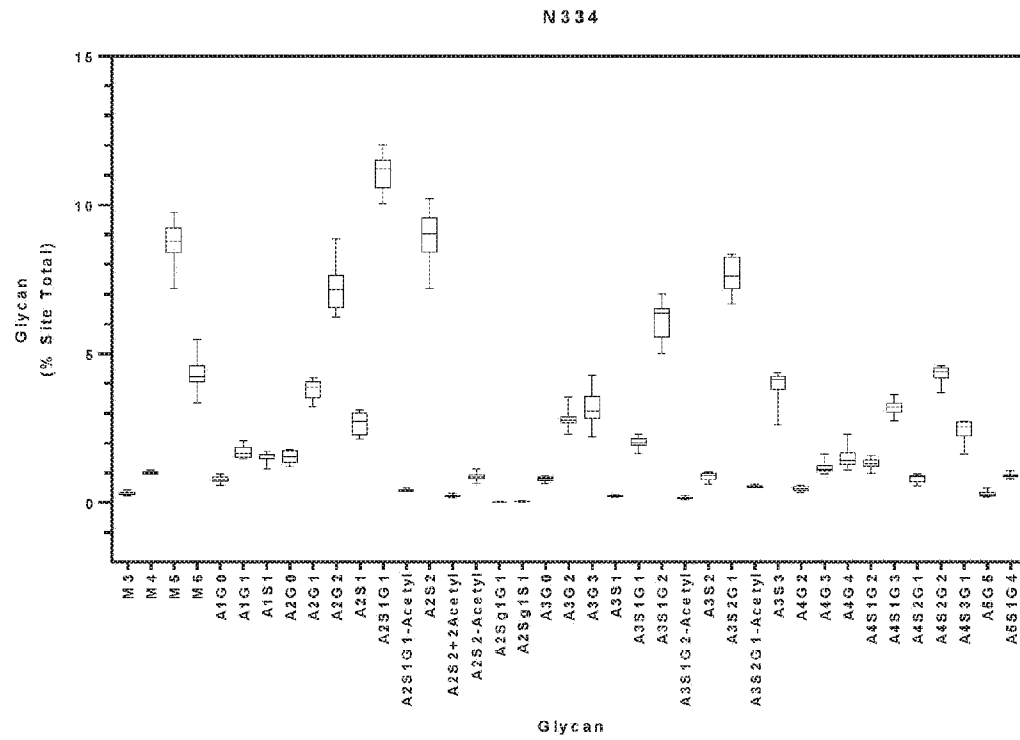
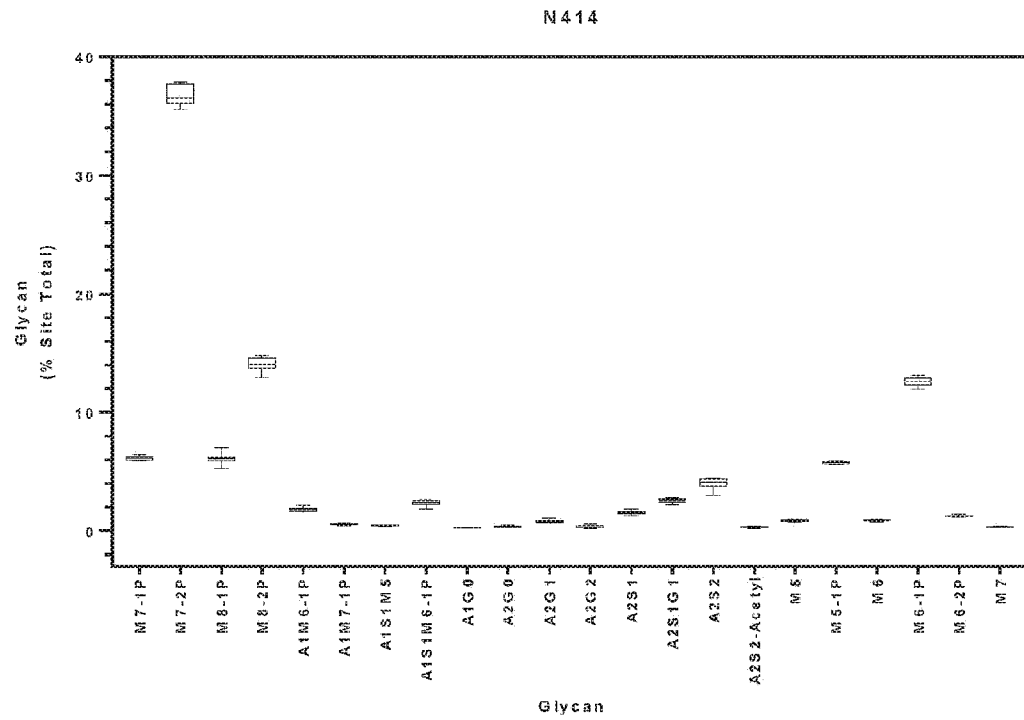
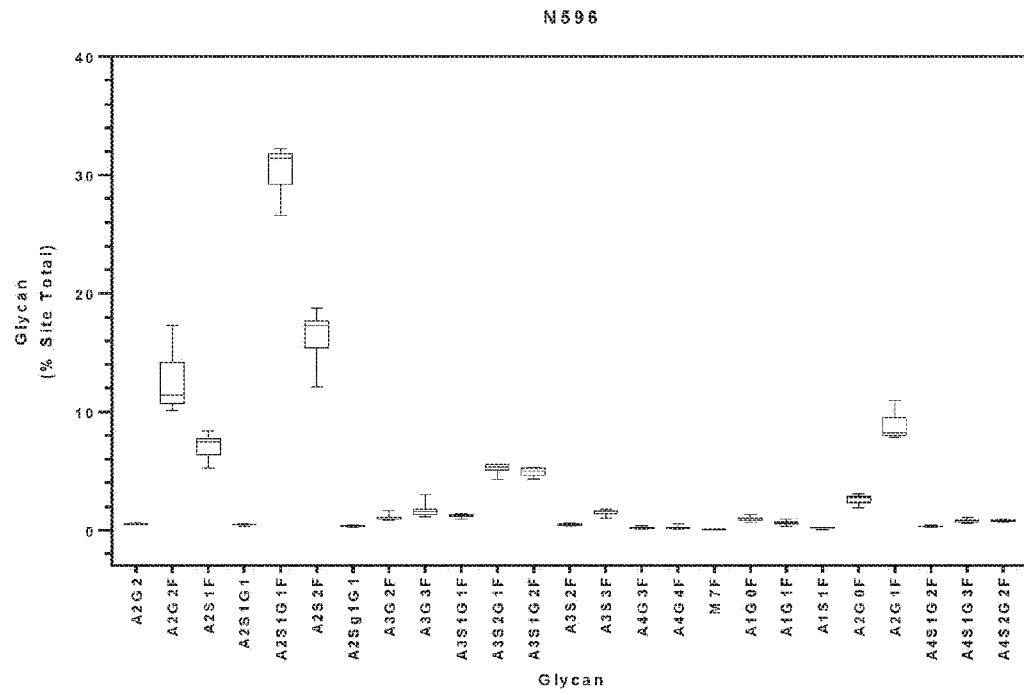


Fig. 32D

**Fig. 32E**

**Fig. 32F**

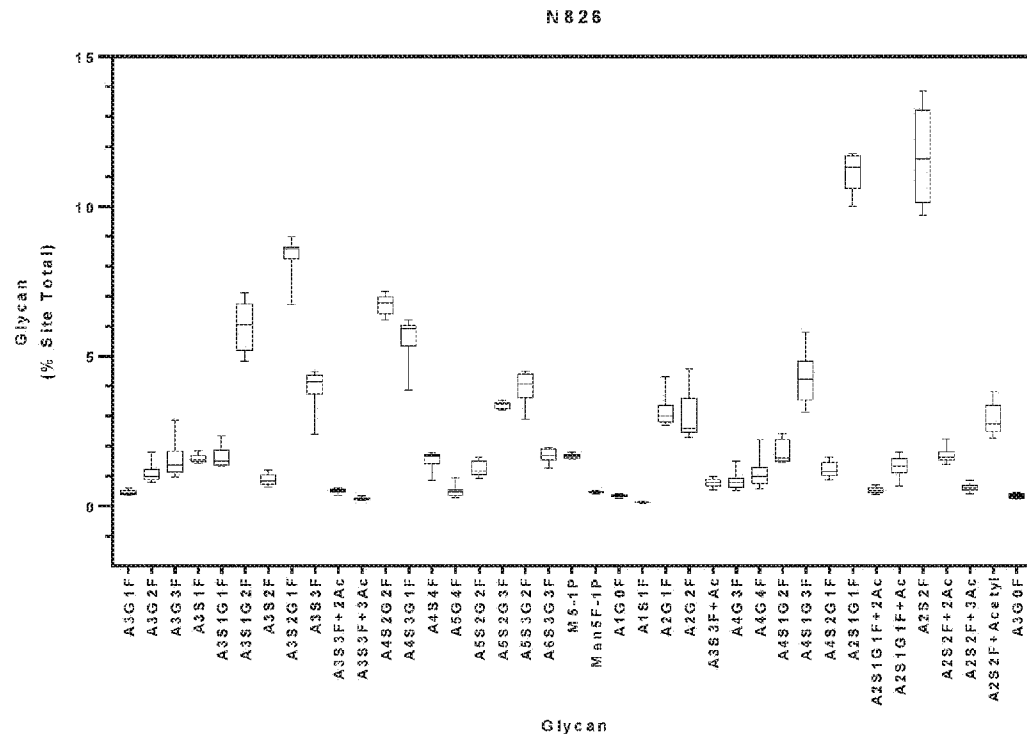


Fig. 32G

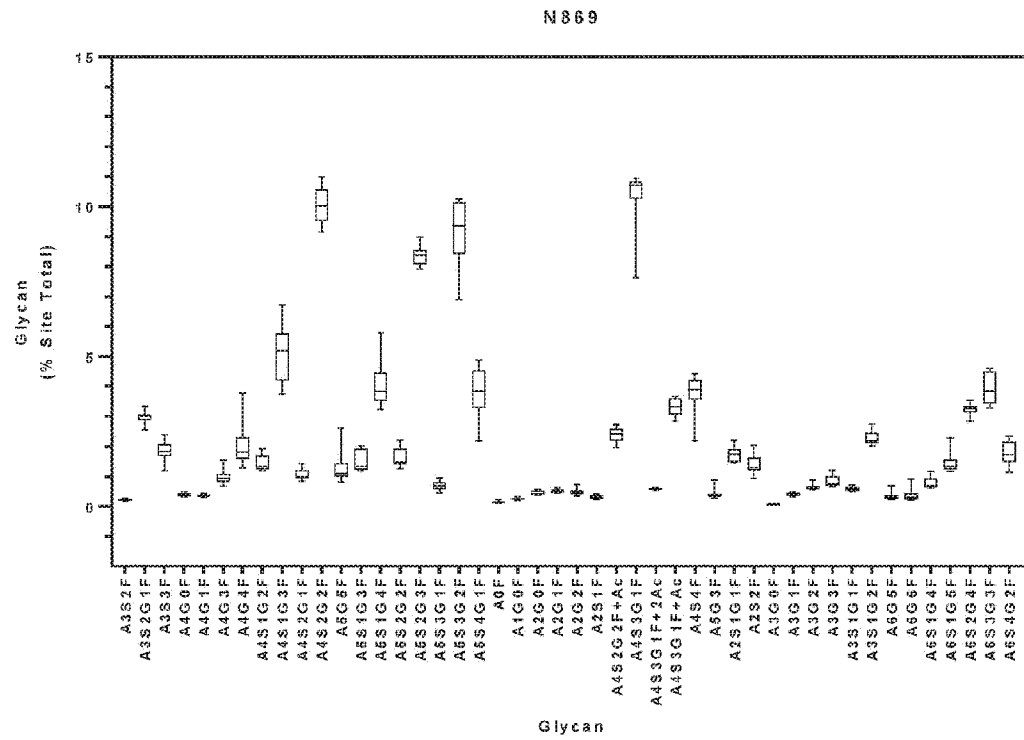


Fig. 32H

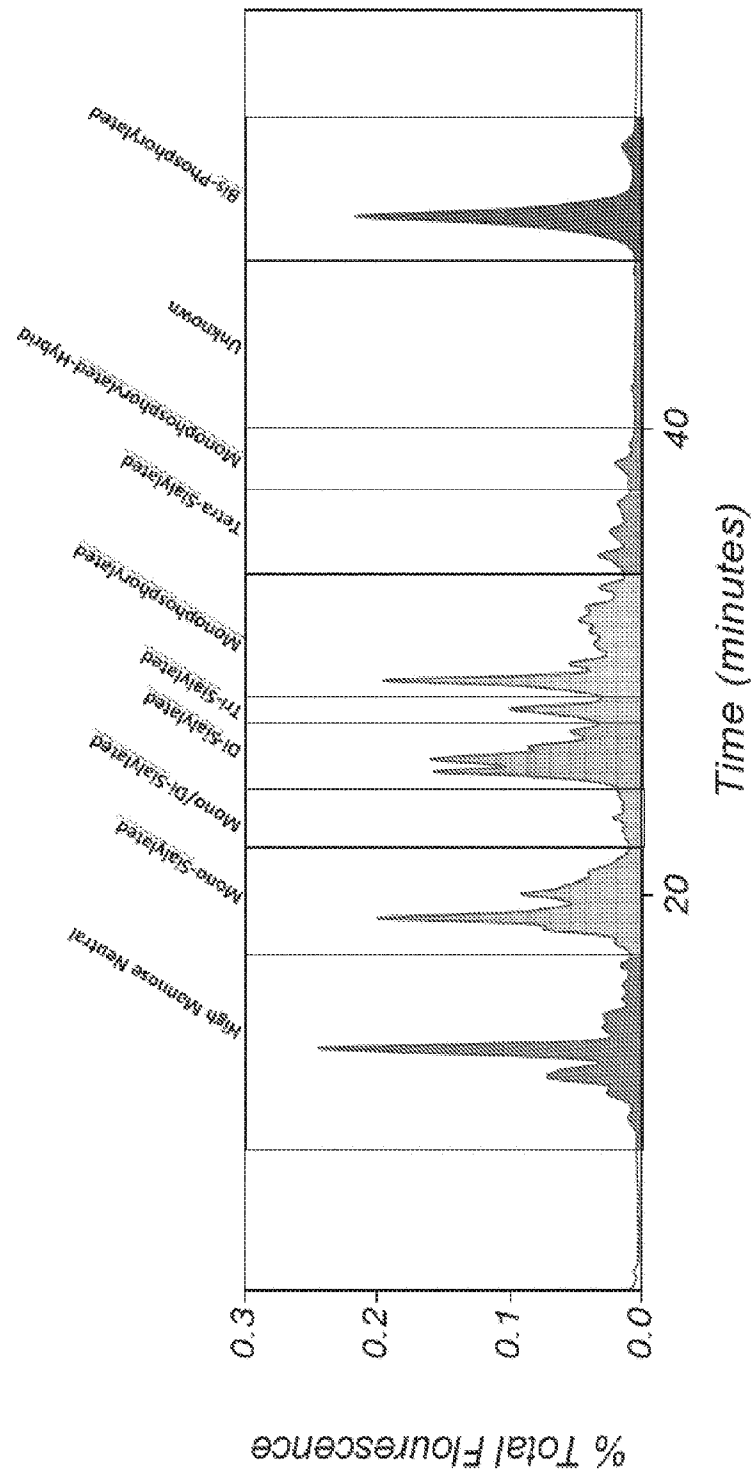


Fig. 33A

Site Specific Glycan Summary														
	N84		N177		N334		N414		N596		N826		N869	
	Average	Stdev	Average	Stdev	Average	Stdev	Average	Stdev	Average	Stdev	Average	Stdev	Average	Stdev
Phosphorylated Glycans	83.2%	5.4%	48.9%	3.0%	ND		87.4%	3.1%	ND		2.1%	0.1%	ND	
Mono-Phosphorylated	25.6%	2.8%	48.0%	2.9%	ND		35.3%	1.6%	ND		2.1%	0.1%	ND	
Bis-phosphorylated	57.6%	2.6%	1.0%	0.1%	ND		52.0%	1.5%	ND		ND		ND	
Sialylated Glycans	25.0%	3.3%	6.2%	0.6%	54.6%	4.9%	11.2%	1.2%	70.4%		85.4%	10.5%	86.7%	10.8%
Fully Sialylated	13.7%	2.0%	6.2%	0.6%	19.3%	2.3%	8.6%	1.0%	26.0%		26.6%	4.0%	7.6%	1.4%
High Mannose	64.9%	3.1%	88.9%	4.1%	14.4%	1.4%	84.7%	2.8%	0.1%	0.0%	2.1%	0.1%	ND	
Hybrid	18.7%	2.3%	10.9%	1.4%	ND		5.2%	0.6%	ND		ND		ND	
Complex	16.4%	2.4%	0.2%	0.0%	85.6%	9.0%	10.1%	1.2%	99.9%	11.8%	97.9%	13.9%	100.0%	14.3%
Number of Glycans	24		17		39		22		25		39		46	
MSP (mol/mol Site)	1.36		0.50				1.38				0.02			
mono Glycan (mol/mol site)	0.25		0.48				0.35				0.02			
bis Glycan (mol/mol site)	0.56		0.01				0.52							
Sialic Acid (mol/mol Site)	0.32	0.04	0.06	0.01	0.97	0.06	0.074	0.004	0.86	0.07	4.2	0.1	0.86	0.07
Site Occupancy	96.5%	0.2%	99.9%	0.0%	100.0%	0.0%	99.3%	0.7%	84.9%	1.1%	98.6%	0.1%	41.0%	0.8%

ND = Not Detected

ND = Not Detected

Fig. 33B

SEKVENSLISTE

Sekvenslisten er udeladt af skriftet og kan hentes fra det Europæiske Patent Register.

The Sequence Listing was omitted from the document and can be downloaded from the European Patent Register.

