~~**We claim:**~~CLAIMS

1. ~~A method of treating Pompe disease in a subject in need thereof,~~ composition comprising ~~administering to the subject~~ a population of recombinant human acid α-glucosidase (rhGAA) molecules for use in the treatment of Pompe disease in a subject in need thereof, wherein the rhGAA molecules are produced from Chinese hamster ovary (CHO) cells;

~~wherein~~ the rhGAA molecules comprise seven potential N-glycosylation sites;

~~wherein~~ the rhGAA molecules on average comprise 3-4 mannose-6-phosphate (M6P) residues;

~~wherein~~ the rhGAA molecules on average comprise at least about 0.5 mol bis-mannose-6-phosphate (bis-M6P) per mol of rhGAA at the first potential N-glycosylation site as determined using liquid chromatography-~~tandom~~tandem mass spectrometry (LC-MS/MS); ~~and~~

~~wherein the population of rhGAA is administered at a dosage capable of reversing disease progression in the subject.~~

~~2. The method of claim 1, wherein reversing disease progression includes reducing lysosomal size in a muscle of the subject.~~

~~3. The method of claim 1 or claim 2, wherein reversing disease progression includes resolving autophagic buildup in a muscle of the subject.~~

~~4. The method of claim 3, wherein fewer than 65% of muscle fibers analyzed in the subject have autophagic buildup after treatment.~~

~~5. The method of any one of claims 1-4, wherein the subject is an ERT-switch patient.~~

~~6. The method of claim 5, wherein the ERT-switch patient had previously been treated with alglucosidase alfa for at least two years.~~

~~7. The method of any one of claim 1-6, wherein at least 36% of muscle fibers analyzed in the subject have normal or near-normal appearance after treatment.~~

8. A method of treating Pompe disease in a subject in need thereof, comprising administering to the subject a population of recombinant human acid α-glucosidase (rhGAA) molecules from Chinese hamster ovary (CHO) cells;

wherein the rhGAA molecules comprise seven potential N-glycosylation sites;

wherein the rhGAA molecules on average comprise 3-4 mannose-6-phosphate (M6P) residues;

wherein the rhGAA molecules on average comprise at least about 0.5 mol bis-mannose-6-phosphate (bis-M6P) per mol of rhGAA at the first potential N-glycosylation site as determined using LC-MS/MS; and

wherein the glycogen content in a muscle of the subject after treatment is reduced faster than when alglucosidase alfa is administered at the same dosage.

9. The method of claim 8, wherein the glycogen content in a muscle of the subject after treatment is reduced at a rate that is at least about 1.25, 1.5, 1.75, 2.0, or 3.0 times faster than the rate when alglucosidase alfa is administered at the same dosage.

10. The method of claim 8 or claim 9, wherein the glycogen content in a muscle of the subject after treatment is reduced more effectively than when alglucosidase alfa is administered at the same dosage, when assessed after one, two, three, four, five, or six administrations.

11. The method of claim 10, wherein the glycogen content in a muscle of the subject after treatment is reduced at least about 10%, 20%, 30%, 50%, 75%, or 90% more effectively than when alglucosidase alfa is administered at the same dosage.

12. The method of claim 10 or claim 11, wherein the glycogen content is assessed after six administrations.

13. The method of any one of claims 8-12, wherein the subject exhibits reduced levels of urine hexose tetrasaccharide after treatment.

14. The method of claim 13, wherein the levels of urine hexose tetrasaccharide at six months after treatment are reduced at least 30% compared to baseline.

15. The method of claim 13, wherein the subject is an ambulatory ERT-switch patient or a nonambulatory ERT-switch patient, and wherein the subject's levels of urine hexose tetrasaccharide at six months after treatment are reduced at least 35% compared to baseline.

16. The method of claim 13, wherein the subject in an ambulatory ERT-naïve patient and the subject's levels of urine hexose tetrasaccharide at six months after treatment are reduced at least 45% compared to baseline.

17. A method of treating Pompe disease in a subject in need thereof, comprising administering to the subject a population of recombinant human acid α-glucosidase (rhGAA) molecules from Chinese hamster ovary (CHO) cells;

wherein the rhGAA molecules comprise seven potential N-glycosylation sites;

wherein the rhGAA molecules on average comprise 3-4 mannose-6-phosphate (M6P) residues;

wherein the rhGAA molecules on average comprise at least about 0.5 mol bis-mannose-6-phosphate (bis-M6P) per mol of rhGAA at the first potential N-glycosylation site as determined using LC-MS/MS; and

wherein the population of rhGAA is administered at a dosage capable of improving motor function in the subject.

18. The method of claim 17, wherein the improved motor function in the subject is measured by at least one motor function test selected from the group consisting of a six-minute walk test (6MWT), a timed up-and-go test, a four-stair climb test, a ten-meter walk test, a gowers test, a gait-stair-gower-chair (GSGC) test, and combinations thereof.

19. The method of claim 18, wherein, compared to baseline, the subject's 6MWT distance at six months after treatment is increased at least 20 meters, the subject's timed up-and-go test time at six months after treatment is decreased at least 1 second, the subject's four-stair climb test time at six months after treatment is decreased at least 0.6 seconds, the subject's ten-meter walk test time at six months after treatment is decreased at least 0.7 seconds, the subject's gowers test time at six months after treatment is decreased at least 1 second, or the subject's GSGC score at six months after treatment is decreased by at least 1.

20. The method of claim 18, wherein the subject is an ambulatory ERT-switch patient and wherein, compared to baseline, the subject's 6MWT distance at six months after treatment is increased at least 20 meters, the subject's timed up-and-go test time at six months after treatment is decreased at least 1.5 seconds, the subject's four-stair climb test time at six months after treatment is decreased at least 0.6 seconds, or the subject's gowers test time at six months after treatment is decreased at least 1 second.

21. The method of claim 18, wherein the subject is an ambulatory ERT-naive patient and wherein, compared to baseline, the subject's 6MWT distance at six months after treatment is increased at least 40 meters, the subject's timed up and go test time at six months after treatment is decreased at least 1 second, the subject's four-stair climb test time at six months after treatment is decreased at least 0.6 seconds, the subject's ten-meter walk test time at six months after treatment is decreased at least 0.7 seconds, or the subject's GSGC score at six months after treatment is decreased by at least 1.

22. The method of claim 18, wherein the subject previously received alglucosidase alfa enzyme replacement therapy, wherein the subject exhibits an improvement in at least one motor function test after treatment with the population of rhGAA compared to the subject's motor function test result after the previous alglucosidase alfa enzyme replacement therapy.

23. A method of treating Pompe disease in a subject in need thereof, comprising administering to the subject a population of recombinant human acid α-glucosidase (rhGAA) molecules from Chinese hamster ovary (CHO) cells;

wherein the rhGAA molecules comprise seven potential N-glycosylation sites;

wherein the rhGAA molecules on average comprise 3-4 mannose-6-phosphate (M6P) residues;

wherein the rhGAA molecules on average comprise about at least 0.5 mol bis-mannose-6-phosphate (bis-M6P) per mol of rhGAA at the first potential N-glycosylation site as determined using LC-MS/MS; and

wherein the population of rhGAA is administered at a dosage capable of improving upper body strength in the subject.

24. The method of claim 23, wherein the improved upper body strength in the subject is measured by a manual muscle strength score.

25. The method of claim 24, wherein the subject is an ambulatory ERT-switch patient and at six-months after treatment exhibits an improvement in upper body manual muscle strength score of at least 1 compared to baseline.

26. The method of claim 24, wherein the subject is a nonambulatory ERT-switch patient and at six-months after treatment exhibits an improvement in upper body manual muscle strength score of at least 5.5 compared to baseline.

27. The method of claim 23, wherein the improved upper body strength in the subject is improved upper extremity strength, wherein upper extremity strength is measured by quantitative muscle testing or manual muscle testing of at least one upper extremity muscle group selected from the group consisting of shoulder adduction, shoulder abduction, elbow flexion, and elbow extension.

28. The method of claim 27, wherein the subject is a nonambulatory ERT-switch patient and wherein, compared to baseline, the subject's shoulder adduction at six months after treatment is improved by at least 8 pounds of force, the subject's shoulder abduction at six months after treatment is improved by at least 1 pound of force, the subject's elbow flexion at six months after treatment is improved by at least 2 pounds of force, or the subject's elbow extension at six months after treatment is improved by at least 5 pounds of force.

29. The method of claim 23, wherein the subject is ambulatory and further exhibits improved lower body strength and/or total body strength after treatment.

30. The method of claim 23, wherein the subject previously received alglucosidase alfa enzyme replacement therapy, wherein the subject exhibits an improvement in upper body strength after treatment with the population of rhGAA compared to the subject's upper body strength after the previous alglucosidase alfa enzyme replacement therapy.

31. A method of treating Pompe disease in a subject in need thereof, comprising administering to the subject a population of recombinant human acid α-glucosidase (rhGAA) molecules from Chinese hamster ovary (CHO) cells;

wherein the rhGAA molecules comprise seven potential N-glycosylation sites;

wherein the rhGAA molecules on average comprise 3-4 mannose-6-phosphate (M6P) residues;

wherein the rhGAA molecules on average comprise about at least 0.5 mol bis-mannose-6-phosphate (bis-M6P) per mol of rhGAA at the first potential N-glycosylation site as determined using LC-MS/MS; and

wherein the population of rhGAA is administered at a dosage capable of improving pulmonary function in the subject.

32. The method of claim 31, wherein the improved pulmonary function in the subject is measured by at least one pulmonary function test selected from the group consisting of an upright forced vital capacity

(FVC) test, a maximal expiratory pressure (MEP) test, a maximal inspiratory pressure (MIP) test, and combinations thereof.

33. The method of claim 32, wherein, compared to baseline, the subject's FVC at six months after treatment is improved by at least 4%, the subject's MEP at six months after treatment is improved by at least 16 cmH_2O, or the subject's MIP at six months after treatment is improved by at least 0.3 cmH_2O.

34. The method of claim 32, wherein the subject is an ambulatory ERT-switch patient and wherein, compared to baseline, the subject's MEP at six months after treatment is improved by at least 16 cmH_2O.

35. The method of claim 32, wherein the subject is an ambulatory ERT-naïve patient and wherein, compared to baseline, the subject's FVC at six months after treatment is improved by at least 4% or the subject's MIP at six months after treatment is improved by at least 11 cmH_2O.

36. The method of claim 32, wherein the subject previously received alglucosidase alfa enzyme replacement therapy, wherein the subject exhibits an improvement in at least one pulmonary function test after treatment with the population of rhGAA compared to the subject's pulmonary function test result after the previous alglucosidase alfa enzyme replacement therapy.

37. A method of treating Pompe disease in a subject in need thereof, comprising administering to the subject a population of recombinant human acid α-glucosidase (rhGAA) molecules from Chinese hamster ovary (CHO) cells;

wherein the rhGAA molecules comprise seven potential N-glycosylation sites;

wherein the rhGAA molecules on average comprise 3-4 mannose-6-phosphate (M6P) residues;

wherein the rhGAA molecules on average comprise about at least 0.5 mol bis-mannose-6-phosphate (bis-M6P) per mol of rhGAA at the first potential N-glycosylation site as determined using LC-MS/MS; and

wherein the population of rhGAA is administered at a dosage capable of reducing fatigue in the subject, as measured according to a fatigue severity scale (FSS) score.

38. The method of claim 37, wherein the subject's FSS score at six months after treatment is decreased by at least 3.5 compared to baseline.

39. The method of claim 38, wherein the subject is a nonambulatory ERT-switch patient.

40. The method of claim 37 or claim 38, wherein the subject is an ambulatory ERT-switch patient and wherein the subject's FSS score at six months after treatment is decreased by at least 8 compared to baseline.

41. The method of claim 37 or claim 38, wherein the subject is an ambulatory ERT-naïve patient and wherein the subject's FSS score at six months after treatment is decreased by at least 5 compared to baseline.

42. The method of claim 37, wherein the subject previously received alglucosidase alfa enzyme replacement therapy, wherein the subject has a lower FSS score after treatment with the population of rhGAA compared to the subject's FSS score after the previous alglucosidase alfa enzyme replacement therapy.

43. A method of treating Pompe disease in a subject in need thereof, comprising administering to the subject a population of recombinant human acid α-glucosidase (rhGAA) molecules from Chinese hamster ovary (CHO) cells;

wherein the rhGAA molecules comprise seven potential N-glycosylation sites;

wherein the rhGAA molecules on average comprise 3-4 mannose-6-phosphate (M6P) residues;

wherein the rhGAA molecules on average comprise about at least 0.5 mol bis-mannose-6-phosphate (bis-M6P) per mol of rhGAA at the first potential N-glycosylation site as determined using LC-MS/MS; and

wherein the population of rhGAA is administered at a dosage capable of reducing the levels of at least one muscle injury biomarker selected from the group consisting of creatine kinase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and combinations thereof.

44. The method of claim 43, wherein the at least one muscle injury biomarker is creatine kinase.

45. The method of claim 43, wherein, compared to baseline, the subject's creatine kinase levels at six months after treatment are reduced at least 15%, the subject's ALT levels at six months after treatment are reduced at least 5%, or the subject's AST levels at six months after treatment are reduced at least 5%.

46. The method of claim 43, wherein the subject is an ambulatory ERT-switch patient and wherein, compared to baseline, the subject's creatine kinase levels at six months after treatment are reduced at least

~~15%, the subject's ALT levels at six months after treatment are reduced at least 15%, or the subject's AST levels at six months after treatment are reduced at least 10%.~~

~~47. The method of claim 43, wherein the subject is a nonambulatory ERT-switch patient and wherein, compared to baseline, the subject's creatine kinase levels at six months after treatment are reduced at least 20%, the subject's ALT levels at six months after treatment are reduced at least 5%, or the subject's AST levels at six months after treatment are reduced at least 5%.~~

~~48. The method of claim 43, wherein the subject is an ambulatory ERT-naïve patient and wherein, compared to baseline, the subject's creatine kinase levels at six months after treatment are reduced at least 35%, the subject's ALT levels at six months after treatment are reduced at least 35%, or the subject's AST levels at six months after treatment are reduced at least 30%.~~

~~49. The method of any one of claims 1-48, wherein the population of rhGAA molecules is administered at a dose of about 1 mg/kg to about 100 mg/kg.~~

~~50. The method of any one of claims 1-49, wherein the population of rhGAA molecules is administered at a dose of about 20 mg/kg.~~

~~51. The method of any one of claims 1-50, wherein the population of rhGAA molecules is administered bimonthly, monthly, bi-weekly, weekly, twice weekly, or daily.~~

~~52. The method of claim 51, wherein the population of rhGAA molecules is administered bi-weekly.~~

~~53. The method of any one of claims 1-52, wherein the population of rhGAA molecules is administered intravenously.~~

~~54. The method of any one of claims 1-53,~~ wherein the population of rhGAA molecules is administered concurrently or sequentially with a pharmacological chaperone~~.~~, and wherein the pharmacological chaperone is miglustat or a pharmaceutically acceptable salt thereof;

~~55. The method of claim 54, wherein the pharmacological chaperone is miglustat or a pharmaceutically acceptable salt thereof.~~

~~56. The method of claim 55, wherein the miglustat or pharmaceutically acceptable salt thereof is administered orally.~~

~~57. The method of claim 55 or claim 56, wherein the miglustat or pharmaceutically acceptable salt thereof is administered at a dose of about 200 mg to about 600 mg.~~

~~58. The method of claim 57, wherein the miglustat or pharmaceutically acceptable salt thereof is administered at a dose of about 260 mg.~~

~~59. The method of claim 57,~~ wherein the population of rhGAA molecules is administered intravenously at a dose of about ~~5 mg/kg to about~~ 20 mg/kg and the miglustat or pharmaceutically acceptable salt thereof is administered orally at a dose of about ~~233~~260 mg ~~to about 500 mg.~~; and wherein

~~60. The method of claim 56, wherein the miglustat or pharmaceutically acceptable salt thereof is administered orally at a dose of about 50 mg to about 200 mg.~~

~~61. The method of claim 56, wherein the population of rhGAA molecules is administered intravenously at a dose of about 20 mg/kg and the miglustat or pharmaceutically acceptable salt thereof is administered orally at a dose of about 260 mg.~~

~~62. The method of any one of claims 55-61~~(i) the subject is an ERT-switch patient, and, compared to baseline, the subject's pulmonary function, as measured by an upright forced vital capacity (FVC) test, is stable or improved at six months after treatment; and/or

(ii) the subject is an ambulatory ERT-switch patient and, compared to baseline, the subject's creatine kinase levels at six months after treatment are reduced at least 15%; and/or

(iii) the subject is a nonambulatory ERT-switch patient and, compared to baseline, the subject's creatine kinase levels at six months after treatment are reduced at least 20%; and/or

(iv) the subject is an ERT-switch patient, and, compared to baseline, the subject's levels of urine hexose tetrasaccharide at six months after treatment are reduced at least 35%.

2. The composition for use of claim 1, wherein the miglustat or pharmaceutically acceptable salt thereof is administered prior to administration of the rhGAA.

~~63.~~

3. The ~~method~~composition for use of claim ~~62, wherein the miglustat~~1 or ~~pharmaceutically acceptable salt thereof is administered about one hour prior to administration of the rhGAA.~~

~~64. The method of~~ claim ~~62 or claim 63, wherein the subject fasts for at least two hours before and at least two hours after the administration of miglustat or a pharmaceutically acceptable salt thereof.~~

~~65. The method of any one of claims 1-64~~2, wherein the rhGAA molecules comprise an amino acid sequence at least 95% identical to SEQ ID NO:~~-~~1 or SEQ ID NO:~~-~~5.

~~66.~~

4. The ~~method~~composition for use of any one of claims 1~~-65, wherein the rhGAA molecules comprise an amino acid sequence identical to SEQ ID NO: 1 or SEQ ID NO: 5.~~

~~67. The method of any one of claims 1-66, wherein at least 30% of the rhGAA molecules comprise one or more N-glycan units bearing one mannose-6-phosphate residue (mono-M6P) or bis-M6P, as determined using LC-MS/MS.~~

~~68. The method of any one of claims 1-67~~ to 3, wherein the rhGAA molecules comprise on average from about 0.5 mol to about 7.0 mol of mono-M6P or bis-M6P per mol of rhGAA, as determined using LC-MS/MS.

~~69.~~

5. The ~~method~~composition for use of any one of claims 1~~-68~~ to 4, wherein the rhGAA molecules comprise on average at least 2.5 mol M6P per mol of rhGAA and at least 4 mol sialic acid per mol of rhGAA, as determined using LC-MS/MS.

~~70.~~

6. The ~~method~~composition for use of any one of claims 1~~-69~~ to 5, wherein, per mol of rhGAA, the rhGAA molecules comprise on average:

~~———~~(a) about 0.4 to about 0.6 mol mono-M6P at the second potential N-glycosylation site;

~~———~~(b) about 0.4 to about 0.6 mol bis-M6P at the fourth potential N-glycosylation site; and

~~———~~(c) about 0.3 to about 0.4 mol mono-M6P at the fourth potential N-glycosylation site;

wherein (a)-(c) are determined using LC-MS/MS.

~~71.~~ 7. The ~~method~~composition for use of ~~claim 70~~any one of claims 1to 6, wherein, per mol of rhGAA, the rhGAA molecules further comprise about 4 mol to about 7.3 mol sialic acid; and

~~wherein, per mol of rhGAA, the rhGAA molecules comprise on average:~~

~~(a) about 0.9 to about 1.2 mol sialic acid at the third potential N-glycosylation site;~~

~~(b) about 0.8 to about 0.9 mol sialic acid at the fifth potential N-glycosylation site; and~~

~~(c) about 1.5 to about 4.2 mol sialic acid at the sixth potential N-glycosylation site;~~

wherein (a)-(c) are determined using LC-MS/MS.

8. ~~72.~~ The ~~method~~composition for use of any one of claims 1~~-71~~ to 7, wherein the population of rhGAA molecules is formulated in a pharmaceutical composition~~.~~

~~73. The method of claim 72,~~, and wherein the pharmaceutical composition further comprises at least one buffer selected from the group consisting of a citrate, a phosphate, and a combination thereof, and at least one excipient selected from the group consisting of mannitol, polysorbate 80, and a combination thereof; wherein the pharmaceutical composition has a pH of about 5.0 to about 7.0.

~~74.~~

9. The ~~method of claim 73, wherein the pharmaceutical~~ composition ~~has a pH~~for use of any one of ~~about 5.0~~claims 1 to ~~about 6.0.~~

~~75. The method of claim 73 or claim 74, wherein the pharmaceutical composition further comprises water, an acidifying agent, an alkalizing agent, or a combination thereof.~~

~~76. The method of claim 73~~8, wherein, in the pharmaceutical composition, the population of rhGAA molecules is present at a concentration of about 5-50 mg/mL, the at least one buffer is a

sodium citrate buffer present at a concentration of about 10-100 mM, the at least one excipient is mannitol present at a concentration of about 10-50 mg/mL and polysorbate 80 present at a concentration of about 0.1-1 mg/mL, and the pharmaceutical composition further comprises water and optionally comprises an acidifying agent and/or alkalizing agent; wherein the pharmaceutical composition has a pH of about 6.0.

~~77. The method of claim 76, wherein, in the pharmaceutical composition, the population of rhGAA molecules is present at a concentration of about 15 mg/mL, the sodium citrate buffer is present at a concentration of about 25 mM, the mannitol is present at a concentration of about 20 mg/mL, and the polysorbate 80 is present at a concentration of about 0.5 mg/mL.~~