CLAIMS

What is claimed is:

1. A method of expressing at least one α galactosidase A (α-Gal A) protein in a cell, the method comprising administering an expression construct comprising a mutated WPRE sequence, optionally a mut6 mutated WRPE sequence, and a GLA transgene encoding at least one α-Gal A protein to the cell such that the α-Gal A protein is expressed in the cell.

2. The method of claim 1, wherein the expression construct comprises a wild-type GLA sequence or a codon optimized GLA sequence.

3. The method of claim 1 or 2, wherein the expression construct further comprises one or more of the following: an enhancer, a promoter, an intron, a sequence encoding a signal peptide and/or a polyadenylation signal, wherein the mutated WPRE sequence, optionally the mut6 mutated WRPE sequence, and the GLA transgene encoding at least one α-Gal A protein is located between the signal peptide and the sequence encoding the polyadenylation signal.

4. The method of claim 3, wherein the expression construct comprises the sequence of SEQ ID No: 9.

5. The method of any one of claims 1-4, wherein the cell is in a subject with Fabry's disease.

6. The method of any one of claims 1-5, wherein the cell is in a male subject.

7. The method of any one of claims 1-6, wherein the expression construct is administered in a pharmaceutically acceptable carrier.

8. The method of claim 7, wherein the pharmaceutically acceptable carrier comprises phosphate buffered saline containing $CaCl_2$, $Mg\ Cl_2$, NaCl, sucrose and Kolliphor (Poloxamer) P 188.

9. The method of any one of claims 1-8, wherein the expression construct sequence comprises the sequence as shown in Table 1 and wherein the expression construct is delivered to the cell by an AAV viral vector.

10. The method of claim 9, wherein the AAV viral vector serotype is AAV2/6.

11. The method of any one of claims 5-10, wherein the expression construct is administered to the subject at a dose of between about 5.0E+12 and 1.0E+14 vector genomes per kilogram (vg/kg).

12. The method of any one of claims 5-11, wherein the expression construct is administered to the liver of the subject.

13. The method of any one of claims 5-12, wherein the expression vector is administered to the subject by intravenous infusion.

14. The method of any one of claims 5-13, wherein only one dose of the expression construct is administered to the subject.

15. The method of any one of claims 5-14, wherein the subject is administered an immunosuppressant prior to and/or during administration of the expression construct.

16. The method of claim 15, wherein the immunosuppressant comprises prednisone.

17. The method of any one of claims 1-16, wherein expression of the at least one α galactosidase A (α-Gal A) protein is sustained for at least 3 months, at least 9 months, or at least 12 months.

18. The method of any one of claims 5-17, wherein the α-Gal A protein expressed from the transgene decreases the amount of glycospingolipids in the subject by between at least about 2-fold to about 9-fold as compared to untreated subjects.

19. The method of any one of claims 5-18, wherein the α-Gal A protein expressed from the transgene decreases the amount of glycospingolipids in the subject by at least about 80%.

20. The method of any one of claims 5-19, wherein the α-Gal A protein expressed from the transgene decreases the amount of glycospingolipids in one or more of the subject's plasma, liver, heart, kidney, or spleen.

21. The method of any one of claims 5-20, wherein the expression construct manufactured in a HEK293 cell system provides GLA levels in the subject at about 21-fold higher as compared to GLA levels in subjects administered the expression construct manufactured in a Sf9 cell system.

22. The method of any one of claims 5-21, wherein α-Gal A protein activity in a subject is between about 100-fold higher to 1,500-fold higher than physiologial normal/wild type.

23. The method of any one of claims 5-22, wherein the α-Gal A protein expressed from the transgene is active in kidneys, liver and heart of the subject.

24. The method of any one of claims 1-23, wherein the GLA transgene is maintained extra-chromosomally and not integrated into a genome of the cell.

25. The method of any one of claims 1-23, further comprising administering one or more nucleases that cleave an endogenous albumin gene in a

liver cell in a subject such that the transgene is integrated into and expressed from the albumin gene.

26. A genetically modified cell comprising an exogenous GLA transgene, made by the method of any one of claims 1-4.

27. The genetically modified cell of claim 26, wherein the cell is a stem cell or a precursor cell.

28. The genetically modified cell of claim 27, wherein the cell is a liver or muscle cell.

29. The genetically modified cell of any one of claims 26-28, wherein the GLA transgene is maintained extra-chromosomally and not integrated into the genome of the cell.

30. The genetically modified cell of any one of claims 26-28, wherein the GLA transgene is integrated into the genome of the cell.

31. A method of preventing, inhibiting or treating Fabry disease or one or more symptoms associated with Fabry disease, the method comprising, administering an expression construct to a subject in need thereof, the expression construct comprising a mutated WPRE sequence, optionally a mut6 mutated WRPE sequence, and a GLA transgene encoding at least one α-Gal A protein.

32. The method of claim 31, wherein the symptoms comprise one or more of Gb3 levels above normal, lyso-Gb3 levels above normal, renal disease, cardiac disease, acroparesthesia, angiokeratomas, GI tract pain, corneal and lenticular opacities, or cerebrovascular disease.

33. The method of claim 31 or 32, wherein the subject is male and wherein the subject has α-Gal A enzyme activity of less than about 5%.

34. The method of claim 31, wherein the expression construct comprises a wild-type GLA sequence or a codon optimized GLA sequence.

35. The method of claim 31, 33, or 34 wherein the expression construct further comprises one or more of the following: an enhancer, a promoter, an intron, a sequence encoding a signal peptide and/or a polyadenylation signal, wherein the mutated WPRE sequence, optionally the mut6 mutated WRPE sequence, and the GLA transgene encoding at least one α-Gal A protein is located between the signal peptide and the sequence encoding the polyadenylation signal.

36. The method of claim 31, wherein the expression construct is administered in a pharmaceutically acceptable carrier.

37. The method of claim 36, wherein the pharmaceutically acceptable carrier comprises phosphate buffered saline containing $CaCl_2$, $Mg\ Cl_2$, NaCl, sucrose and Kolliphor (Poloxamer) P 188.

38. The method of claim 31, wherein the expression construct sequence comprises the sequence as shown in Table 1 and wherein the expression construct is delivered to cells of the subject by an AAV viral vector.

39. The method of claim 38, wherein the AAV viral vector serotype is AAV2/6.

40. The method of any one of claims 31-39, wherein the expression construct is administered to the subject at a dose of between about 5.0E+12 and 1.0E+14 vector genomes per kilogram (vg/kg).

41. The method of any one of claims 31-40, wherein the expression construct is administered to the liver of the subject.

42. The method of any one of claims 31-41, wherein the expression vector is administered to the subject by intravenous infusion.

43. The method of any one of claims 31-42, wherein only one dose of the expression construct is administered to the subject.

44. The method of any one of claims 31-43, wherein the subject is administered an immunosuppressant prior to and/or during administration of the expression construct.

45. The method of claim 44, wherein the immunosuppressant comprises prednisone.

46. The method of any one of claims 31-45, wherein expression of the at least one α galactosidase A (α-Gal A) protein is sustained for at least 3 months, at least 9 months, or at least 12 months.

47. The method of any one of claims 31-46, wherein the α-Gal A protein expressed from the transgene decreases the amount of glycospingolipids in the subject by between at least about 3-fold to about 9-fold as compared to untreated subjects.

48. The method of any one of claims 31-46, wherein the α-Gal A protein expressed from the transgene decreases the amount of glycospingolipids in the subject by at least about 80%.

49. The method of any one of claims 31-48, wherein the α-Gal A protein expressed from the transgene decreases the amount of glycospingolipids in one or more of the subject's plasma, liver, heart, kidney, or spleen.

50. The method of any one of claims 31-49, wherein the expression construct is manufactured in a HEK293 cell system and wherein the GLA levels in the subject are 21-fold higher as compared to GLA levels in subjects administered the expression construct manufactured in a Sf9 cell system.

51. The method of any one of claims 31-50, wherein α-Gal A protein activity in a subject is between about 100-fold higher to 1,500-fold higher than physiological normal.

52. The method of any one of claims 31-51, wherein the α-Gal A protein expressed from the transgene is active in kidneys, liver and heart of the subject.

53. The method of any one of claims 31-52, wherein the GLA transgene is maintained extra-chromosomally and not integrated into a genome of the subject's cell.

54. The method of any one of claims 31-52, further comprising administering one or more nucleases that cleave an endogenous albumin gene in a liver cell in a subject such that the transgene is integrated into and expressed from the albumin gene.

55. A composition comprising an expression construct, the expression construct comprising a mutated WPRE sequence, optionally a mut6 mutated WRPE sequence, and a GLA transgene encoding the at least one α-Gal A protein for the treatment of Fabry's disease.

56. The composition of claim 55, further comprising a pharmaceutically acceptable carrier.

57. The composition of claim 56, wherein the pharmaceutically acceptable carrier comprises $CaCl_2$, $Mg\ Cl_2$, NaCl, sucrose and Kolliphor (Poloxamer) P 188.

58. The composition of claim 55, wherein the expression construct comprises a wild-type GLA sequence or a codon optimized GLA sequence.

59. The composition of any one of claims 55-58, wherein the expression construct further comprises one or more of the following: an enhancer, a promoter, an intron, a sequence encoding a signal peptide and/or a polyadenylation signal, wherein

the mutated WPRE sequence, optionally the mut6 mutated WRPE sequence, and the GLA transgene encoding at least one α-Gal A protein is located between the signal peptide and the sequence encoding the polyadenylation signal.

60. The composition of any one of claims 55-59, wherein the expression construct sequence comprises the sequence as shown in Table 1 and wherein the expression construct is delivered to a cell by an AAV viral vector.

61. The composition of any one of claims 55-60, wherein the AAV viral vector serotype is AAV2/6.

62. The composition of claim 60 or 61, wherein the expression construct comprises between about 5.0E+12 and 1.0E+14 vector genomes per subject kilogram (vg/kg).

63. The composition of claim 59, wherein the expression construct comprises the sequence of SEQ ID No: 9.

64. A method of producing an α-Gal A protein for the treatment of Fabry disease, the method comprising expressing the α-Gal A protein in an isolated cell according to the method of any one of claims 1-4, and isolating the α-Gal A protein produced by the cell.

65. A delivery vector comprising a mutated WRPE sequence, optionally a mut6 WPRE sequence and a GLA transgene for use in the method of claim 1.

66. The vector of claim 65, wherein the delivery vector is a viral vector or a lipid nanoparticle (LNP).

67. The vector of claim 66, wherein the viral vector comprises an AAV2/6 and wherein the viral vector delivers the expression construct to at least 50%, at least 60%, at least 70%, or at least 80% of cells.

68. Use of an expression construct, an AAV vector and/or genetically modified cell of any of the preceding claims for the treatment of Fabry's disease.

69. The method of claim 3, wherein the enhancer comprises SEQ ID No: 2, the promotor comprises SEQ ID No: 3, the intron comprises SEQ ID No: 4, the GLA transgene comprises SEQ ID No: 5, the mutated WPRE sequence comprises SEQ ID No: 6, and the polyadenylation signal comprises SEQ ID No. 7.

70. The composition of claim 59, wherein the enhancer comprises SEQ ID No: 2, the promotor comprises SEQ ID No: 3, the intron comprises SEQ ID No: 4, the GLA transgene comprises SEQ ID No: 5, the mutated WPRE sequence comprises SEQ ID No: 6, and the polyadenylation signal comprises SEQ ID No. 7.