## ~~CLAIMS~~

~~1. A nucleic acid encoding human α-galactosidase A (AGA) protein codon optimized for expression in humans, the nucleic acid comprising the nucleotide sequence set forth as SEQ ID NO: 1 or comprising a nucleotide sequence at least 95% identical thereto, wherein the nucleic acid is expressed at a greater level compared with the level of expression of the wild type GLA nucleotide sequence of SEQ ID NO: 3 in an otherwise identical cell.~~

~~2. The nucleic acid according to claim 1, wherein the nucleotide sequence has a codon adaptation index of at least about 0.80, at least about 0.83, at least about 0.85, at least about 0.88 at least about 0.90, at least about .92 at least about 0.93, or from about 0.80 to about 0.99, or from about 0.85 to about 0.99, or from about .88 to about 0.99 or from about 0.90 to about 0.99 or from about 0.93 to about 0.99.~~

~~3. The nucleic acid according to claim 1, comprising the nucleotide sequence set forth as SEQ ID NO: 1.~~

~~4. An expression cassette comprising the nucleic acid according to any one of claims 1 to 3 and an expression control sequence operably linked and heterologous to the nucleic acid sequence.~~

~~5. The expression cassette of claim 4, wherein the expression control sequence is a constitutive promoter.~~

~~6. The expression cassette of claim 4, wherein the expression control sequence is a promoter that directs preferential expression of the nucleic acid in a muscle cell.~~

~~7. A vector comprising the nucleic acid according to any one of claims 1 to 3 or an expression cassette according to any one of claims 4 to 6.~~

~~8. The vector of claim 7, wherein the vector is a recombinant adeno-associated (rAAV) vector.~~

~~9. The vector of claim 8, wherein the rAAV vector comprises an AAV capsid of serotype 1, 2, 6 or 8 or a variant thereof.~~

~~10. The vector of claim 9, wherein the rAAV vector comprises an AAV2 capsid or variant thereof.~~

~~11. The vector of claim 10, wherein the AAV2 capsid variant comprises the sequence of SEQ ID NO:4.~~

~~12. The vector according to claim 11, wherein the rAAV vector comprises a nucleic acid comprising from 5' to 3': (a) an AAV2 terminal repeat (b) a CAG promoter (c) a nucleic acid according to claim 3 (d) a polyadenylation sequence and (e) an AAV2 terminal repeat.~~

~~13. The vector according to claim 12, wherein the rAAV comprises a nucleotide sequence comprising or consisting of the sequence set forth as SEQ ID NO:6 or a sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98% or at least 99% identical thereto.~~

~~14. A host cell comprising the nucleic acid according to any one of claims 1 to 3 or an expression cassette according to any one of claims 4 to 6 or a vector according to any one of claims 7 to 13.~~

~~15. The host cell according to claim 14, wherein the host cell is a mammalian cell.~~

~~16. The host cell of claim 14 or 15, wherein the host cell is a CHO cell, an HEK293T cell, an HEK293 cell, a HeLa cell, a BHK21 cell or a Vero cell and/or wherein the host cell is grown in a suspension or adherent culture and/or wherein the host cell is a cardiac or skeletal muscle cell.~~

17. Use of a nucleic acid according to any one of claims 1-3 or a vector according to any one of claims 7-13 in the manufacture of a medicament for treating Fabry disease, preferably wherein the nucleic acid or vector is for administration in an amount effective to reduce the level of globotriaosylceramide (Gb3) in a subject.

18. Use of an infectious rAAV comprising (i) a capsid comprising a capsid protein having the sequence of SEQ ID NO:4 and (ii) a nucleic acid comprising the nucleotide sequence of SEQ ID NO:1 operably linked to a CAG promoter in the manufacture of a medicament for treating Fabry disease, preferably wherein the nucleic acid comprises or consists of the nucleotide sequence set forth a SEQ ID NO:6 or a sequence at least 80% identical thereto, optionally, wherein the rAAV is administered at one or more doses of about $10^{12}$ to about $10^{14}$ vector genomes (vg)/kg, or about 1 x$10^{13}$, 3 x $10^{13}$ or 5 x $10^{13}$ vg/kg, preferably wherein the rAAV is for administration by a single intravenous injection.

19. Use according to claim 17 or 18, wherein the nucleic acid or vector is formulated for administration by intravenous and/or intramuscular injection.

20. Use of a nucleic acid according to any one of claims 1-3 or a vector according to any one of claims 7-13 in the manufacture of a medicament for treating a disease or condition mediated by a decreased level of AGA in a mammal.

21. Use of a nucleic acid according to any one of claims 1-3 or a vector according to any one of claims 7-13 in the manufacture of a medicament for increasing the level of AGA in a mammal.

22. A pharmaceutical composition comprising a nucleic acid having a nucleotide sequence at least 90%, at least 95% or at least 98% identical to the nucleotide sequence of SEQ ID NO:1, optionally operably linked to an expression control sequence, and at least one pharmaceutically acceptable excipient.

~~23. The pharmaceutical composition according to claim 22, comprising a nucleic acid comprising the nucleotide sequence of SEQ ID NO:1 operably linked to a CAG promoter and at least one pharmaceutically acceptable excipient.~~

~~24. A pharmaceutical composition comprising at least one pharmaceutically acceptable excipient and an infectious rAAV comprising (i) a nucleic acid comprising a nucleotide sequence of SEQ ID NO:1 operably linked to a CAG promoter and (ii) a capsid comprising a capsid protein having the amino acid sequence of SEQ ID NO:4.~~

~~25. A pharmaceutical composition according to claim 24, wherein the nucleic acid comprises or consists of the nucleotide sequence set forth as SEQ ID NO:6 or a sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98% or at least 99% identical thereto.~~

## Claims

1. A recombinant adeno-associated virus (rAAV) comprising: (a) a variant AAV capsid protein comprising a heterologous peptide insertion with a length of 7 to 20 amino acids covalently inserted in the GH-loop of the capsid protein relative to a corresponding parental AAV capsid protein, wherein the peptide insertion comprises the amino acid sequence NKTTNKD and (ii) a heterologous nucleic acid comprising a nucleotide sequence encoding human α-galactosidase A (AGA) protein, wherein said nucleic acid is codon optimized for expression in human cells, wherein said nucleic acid comprises a nucleotide sequence having at least 90% sequence identity to the nucleotide sequence set forth in SEQ ID NO: 1.

2. The rAAV according to claim 1, wherein the insertion peptide has from 1 to 3 spacer amino acids ($Y_1$-$Y_3$) at the amino and/or carboxyl terminus of amino acid sequence NKTTNKD.

3. The rAAV according to claim 1 or 2, wherein the insertion peptide is LANKTTNKDA.

4. The rAAV according to any one of claims 1 to 3, wherein the variant capsid protein comprises a V708I amino acid substitution relative to VP1 of AAV2 (SEQ ID NO:2) or the corresponding position in the capsid protein of another AAV serotype and wherein the variant capsid protein comprises an amino acid sequence at least 90% identical to the entire length of the amino acid sequence set forth as SEQ ID NO:4.

5. The rAAV according to claim 4, wherein the variant capsid protein comprises an amino acid sequence at least 95% identical to the entire length of the amino acid sequence set forth as SEQ ID NO:4.

6. The rAAV according to any one of claims 1 to 5, wherein the insertion site is located between two adjacent amino acids at a position between amino acids 570 and 611 of VP1 of AAV2, or the corresponding position in the capsid protein of another AAV serotype.

7. The rAAV according to any one of claims 1 to 6, wherein the insertion site is located between amino acids corresponding to amino acids 587 and 588 of VP1 of AAV2 or

between amino acids corresponding to amino acids 588 and 589 of VP1 of AAV2 or the corresponding position in the capsid protein of another AAV serotype.

8. The rAAV according to any one of claims 1 to 7, wherein said rAAV vector comprises a heterologous nucleic acid comprising a nucleotide sequence having at least 90% sequence identity to the nucleotide sequence set forth in SEQ ID NO: 1.

9. The rAAV according to any one of claims 1 to 8, wherein said rAAV vector comprises a heterologous nucleic acid comprising a nucleotide sequence having at least 95% sequence identity to the nucleotide sequence set forth in SEQ ID NO: 1.

10. The rAAV according to any one of claims 1 to 9, wherein the rAAV vector comprises a nucleic acid comprising from 5' to 3': (a) an AAV2 terminal repeat (b) a CAG promoter (c) the nucleic acid set forth in SEQ ID NO: 1, (d) a polyadenylation sequence and (e) an AAV2 terminal repeat.

11. A host cell comprising the rAAV according to any one of claims 1 to 10.

12. The host cell of claim 11, wherein the host cell is a CHO cell, an HEK293T cell, an HEK293 cell, a HeLa cell, a BHK21 cell or a Vero cell and/or wherein the host cell is grown in a suspension or adherent culture and/or wherein the host cell is a cardiac or skeletal muscle cell.

13. A pharmaceutical composition comprising the rAAV according to any one of claims 1 to 10, and at least one pharmaceutically acceptable excipient.

14. The pharmaceutical composition of claim 13 for use in therapy.

15. Use of the pharmaceutical composition of claim 13 in the manufacture of a medicament for the treatment of Fabry disease.

16. The use of claim 15, wherein said pharmaceutical composition is to be administered by intravenous and/or intramuscular injection.

17. The use of claim 16, wherein the use comprises a single intravenous administration of at least $1\times10^{8}$ vg/kg of the rAAV vector.

18. The use of claim 16 or 17, wherein the use comprises a single intravenous administration of $1\times10^{12}$ vg/kg to $1\times10^{14}$ vg/kg of the rAAV vector.

19. The use of the rAAV according to any one of claims 1 to 10 in the manufacture of a medicament for the treatment of Fabry disease.

20. The use according to claim 19, wherein the medicament is to be administered by intravenous and/or intramuscular injection.