CLAIMS

1. A modified β-Glucocerebrosidase (GCase) polypeptide which comprises at least one mutation, wherein the at least one mutation provides increased stability, wherein the at least one mutation comprises two mutations, and wherein each of the two mutations is a substitution with cysteine which enables the formation of a disulphide bridge.

~~2. The modified GCase polypeptide of claim 1, wherein the modified GCase polypeptide retains at least 80%, or at least 85% activity when measured after 120 mins of incubation at pH 7.4 and 37 degrees Celsius.~~

2~~3~~. The modified GCase polypeptide of claim 1 ~~or 2~~, wherein the mutation is at a position corresponding to a position selected from the group consisting of 351, 380, ~~272, 262, 313, 404,~~ 407, 482, 484, ~~490,~~ 494, 503, and 534 of SEQ ID NO: 1, optionally wherein the at least one mutation comprises:

(i) a mutation at a position corresponding to position 351 of SEQ ID NO: 1; and

(ii) a mutation at a position corresponding to position 380 of SEQ ID NO: 1.

3~~4~~. The modified GCase polypeptide of ~~any one of the preceding~~ claim~~s~~ 1 or 2, wherein the at least one mutation:

(i) provides higher effective activity; and/or

(ii) provides increased thermostability; and/or

(iii) provides structural stabilisation at physiological pH; and/or

(iv) provides a longer half-life.

4~~5~~. The modified GCase polypeptide of any one of the preceding claims, wherein the modified GCase polypeptide has higher effective activity relative to a reference GCase polypeptide, optionally wherein:

(i) the reference GCase polypeptide is a wild-type GCase polypeptide, optionally the polypeptide of SEQ ID NO: 3, or the polypeptide of SEQ ID NO: 4 or 5; and/or

(ii) the effective activity of the modified GCase polypeptide is at least 1.2 fold, at least 1.5 fold, at least 2 fold, at least 2.5 fold, at least 3 fold, at least 3.5

fold, at least 4 fold, at least 4.5 fold, at least 5 fold, at least 5.5. fold, at least 6 fold, at least 6.5 fold, at least 7 fold, at least 7.5 fold, at least 8 fold, at least 10 fold, at least 15 fold, at least 20 fold, at least 35 fold, at least 40 fold, at least 45 fold, or at least 50 fold higher than the effective activity of the reference GCase polypeptide.

56. The modified GCase polypeptide of any one of the preceding claims, wherein:

(i) the at least one mutation provides increased stability and the increased stability is at pH 7.4; and/or

(ii) the at least one mutation provides increased stability and the increased stability is measured after 120 minutes of incubation at pH 7.4 and 37 degrees Celsius; and/or

(iii) the at least one mutation provides structural stabilisation at pH 7.4; and/or

(iv) the modified GCase polypeptide is more structurally stable at physiological pH relative to a reference GCase polypeptide, optionally wherein the pH is pH 7.4, further optionally wherein the reference GCase polypeptide is a wild-type GCase polypeptide or the polypeptide of SEQ ID NO: 4 or 5; and/or

(v) the modified GCase polypeptide has increased stability relative to a reference GCase polypeptide, optionally wherein the increased stability is increased stability at pH 7.4, further optionally wherein the reference GCase polypeptide is a wild-type GCase polypeptide or the polypeptide of SEQ ID NO: 4 or 5; and/or

(vi) the modified GCase polypeptide retains at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, or at least 85% activity when measured after at least 10 minutes, at least 30 minutes, at least 60 minutes, at least 120 minutes, at least 1 day, at least 2 days, at least 3 days, at least 4 days, at least 5 days, at least 6 days, or at least 7 days of incubation at pH 7.4 and 37 degrees Celsius; and/or

(vii) the modified GCase polypeptide retains activity which is at least 1.1 fold, at least 1.2 fold, at least 1.3 fold, at least 1.4 fold, at least 1.5 fold, at least 1.8 fold, at least 2 fold, at least 2.5 fold, at least 2.8 fold, or at least 3 fold higher than the activity of a reference GCase polypeptide when measured after 120 minutes of incubation at pH 7.4 and 37 degrees Celsius, optionally

wherein the reference GCase polypeptide is a wild-type GCase polypeptide or the polypeptide of SEQ ID NO: 4 or 5.

67. The modified GCase polypeptide of any one of the preceding claims, wherein:

(i) the modified GCase polypeptide retains at least 70%, at least 75%, at least 80%, or at least 85% activity when measured after 120 mins of incubation at pH 7.4 and 37 degrees Celsius; and/or

(ii) the modified GCase polypeptide retains at least 40%, at least 45%, at least 50%, at least 55%, or at least 60% activity when measured after 72 hours of incubation at pH 7.4 and 37 degrees Celsius, optionally wherein the incubation is in PBS; and/or

(iii) the modified GCase polypeptide retains at least 15%, or at least 20% activity when measured after 7 days of incubation at pH 7.4 and 37 degrees Celsius, optionally wherein the incubation is in serum or plasma; and/or

(iv) the modified GCase polypeptide retains at least 40% activity when measured after 7 days of incubation at pH 7.4 and 37 degrees Celsius, optionally wherein the incubation is in serum or plasma; and/or

(v) the modified GCase polypeptide retains at least 60% activity when measured after 7 days of incubation at pH 7.4 and 37 degrees Celsius; and/or

(vi) the modified GCase polypeptide retains at least 80% activity when measured after 7 days of incubation at pH 7.4 and 37 degrees Celsius; and/or

(vii) the activity, effective activity, stability, and/or half-life is determined using a fluorometric assay.

78. The modified GCase polypeptide of any one of the preceding claims having a longer half-life relative to a reference GCase polypeptide, optionally wherein the modified GCase polypeptide has a half-life of at least 1.2 fold, at least 1.5 fold, at least 2 fold, at least 3 fold, at least 4 fold, at least 5 fold, or at least 10 fold longer than the half-life of the reference GCase polypeptide.

89. The modified GCase polypeptide of claim 78, wherein:

a. the longer half-life is longer half-life at pH 7.4 or pH 5.6; and/or

b. the modified GCase polypeptide has a half-life at pH5.6 of at least 20 fold longer than the half-life of the reference GCase polypeptide; and/or
c. the longer half-life is longer half-life in serum or plasma; and/or
d. the half-life is determined using a fluorometric assay; and/or
e. the reference GCase polypeptide is (a) a wild type GCase polypeptide, (b) the polypeptide of SEQ ID NO: 3, or (c) the polypeptide of SEQ ID NO: 4 or 5.

9~~10~~. The modified GCase polypeptide of any one of the preceding claims, wherein:

a. the at least one mutation comprises a mutation at a position corresponding to position 272 of SEQ ID NO: 1, optionally wherein the mutation at a position corresponding to position 272 of SEQ ID NO: 1 is a substitution with glutamine, further optionally E272Q; and/or
b. the at least one mutation comprises a mutation at a position corresponding to position 262 of SEQ ID NO: 1, optionally wherein the mutation at a position corresponding to position 262 of SEQ ID NO: 1 is a substitution with asparagine or tyrosine; and/or
c. the at least one mutation comprises a mutation at a position corresponding to position 262 of SEQ ID NO: 1, wherein the mutation at a position corresponding to position 262 of SEQ ID NO: 1 is a substitution with asparagine, optionally H262N; and/or
d. the at least one mutation comprises a mutation at a position corresponding to position 313 of SEQ ID NO: 1, optionally wherein the mutation at a position corresponding to position 313 of SEQ ID NO: 1 is a substitution with asparagine, further optionally H313N; and/or
e. the at least one mutation comprises a mutation at a position corresponding to position 404 of SEQ ID NO: 1, optionally wherein the mutation at a position corresponding to position 404 of SEQ ID NO: 1 is a substitution with lysine, further optionally H404K; and/or
f. the at least one mutation comprises a mutation at a position corresponding to position 490 of SEQ ID NO: 1, optionally wherein the mutation at a position corresponding to position 490 of SEQ ID NO: 1 is a substitution with lysine, further optionally H490K; and/or

g. the at least one mutation comprises a mutation at a position corresponding to position 534 of SEQ ID NO: 1, optionally wherein the mutation at a position corresponding to position 534 of SEQ ID NO: 1 is a substitution with asparagine, further optionally R534N.

10~~11~~. The modified GCase polypeptide of any one of the preceding claims, wherein:

(a) the at least one mutation comprises:

(i) a mutation at a position corresponding to position 482 of SEQ ID NO: 1; and

(ii) a mutation at a position corresponding to position 503 of SEQ ID NO: 1,

optionally wherein

(i) the mutation at a position corresponding to position 482 of SEQ ID NO: 1 is a substitution with cysteine, optionally an aspartic acid to cysteine mutation; and

(ii) the mutation at a position corresponding to position 503 of SEQ ID NO: 1 is a substitution with cysteine, optionally a serine to cysteine mutation; and/or

(b) the at least one mutation comprises:

(i) a mutation at a position corresponding to position 494 of SEQ ID NO: 1; and

(ii) a mutation at a position corresponding to position 534 of SEQ ID NO: 1,

optionally wherein

(i) the mutation at a position corresponding to position 494 of SEQ ID NO: 1 is a substitution with cysteine, optionally a serine to cysteine mutation; and

(ii) the mutation at a position corresponding to position 534 of SEQ ID NO: 1 is

a substitution with cysteine, optionally an arginine to cysteine mutation; and/or

(c) the at least one mutation comprises:

(i) a mutation at a position corresponding to position 351 of SEQ ID NO: 1; and

(ii) a mutation at a position corresponding to position 380 of SEQ ID NO: 1,

optionally wherein

(i) the mutation at a position corresponding to position 351 of SEQ ID NO: 1 is a substitution with cysteine, optionally a tryptophan to cysteine mutation; and

(ii) the mutation at a position corresponding to position 380 of SEQ ID NO: 1 is a substitution with cysteine, optionally an alanine to cysteine mutation; and/or

(d) the at least one mutation comprises:

(i) a mutation at a position corresponding to position 407 of SEQ ID NO: 1; and

(ii) a mutation at a position corresponding to position 484 of SEQ ID NO: 1,

optionally wherein

(i) the mutation at a position corresponding to position 407 of SEQ ID NO: 1 is a substitution with cysteine, optionally an isoleucine to cysteine mutation; and

(ii) the mutation at a position corresponding to position 484 of SEQ ID NO: 1 is a substitution with cysteine, optionally an aspartic acid to cysteine mutation; and/or

(e) the at least one mutation comprises a substitution with glutamine at a position corresponding to position 272 of SEQ ID NO: 1, and wherein the modified GCase polypeptide has increased stability compared to a reference GCase polypeptide, retaining at least 85% activity when measured after 120 mins of incubation at pH 7.4 and 37 degrees Celsius, optionally wherein the reference GCase polypeptide is a wild type GCase polypeptide; and/or

(f) the at least one mutation comprises:

(i) a tryptophan to cysteine mutation at a position corresponding to position 351 of SEQ ID NO: 1; and

(ii) an alanine to cysteine mutation at a position corresponding to position 380 of SEQ ID NO: 1;

and wherein the modified GCase polypeptide has increased stability compared to a reference GCase polypeptide, retaining at least 85% activity when measured after 120 mins of incubation at pH 7.4 and 37 degrees Celsius, optionally wherein the reference GCase polypeptide is a wild type GCase polypeptide; and/or

(g) the at least one mutation comprises:

(i) a substitution with glutamine at a position corresponding to position 272 of SEQ ID NO: 1, optionally E272Q; and

(ii) a tryptophan to cysteine mutation at a position corresponding to position 351 of SEQ ID NO: 1; and

(iii) an alanine to cysteine mutation at a position corresponding to position 380 of SEQ ID NO: 1; and/or

(h) the at least one mutation comprises:

(i) a substitution with glutamine at a position corresponding to position 272 of SEQ ID NO: 1, optionally E272Q; and

(ii) a tryptophan to cysteine mutation at a position corresponding to position 351 of SEQ ID NO: 1; and

(iii) an alanine to cysteine mutation at a position corresponding to position 380 of SEQ ID NO: 1;

and wherein the modified GCase polypeptide has increased stability compared to a reference GCase polypeptide, retaining at least 85% activity when measured after 120 mins of incubation at pH 7.4 and 37 degrees Celsius, optionally wherein the reference GCase polypeptide is a wild type GCase polypeptide; and/or

(i) the modified GCase polypeptide has higher effective activity and/or increased stability compared to a reference GCase polypeptide, optionally wherein the reference GCase polypeptide is selected from any one of SEQ ID NOs: 1 to 5.

11~~12~~. The modified GCase polypeptide of any one of the preceding claims, wherein:

(i) the modified GCase polypeptide comprises an amino acid sequence at least 80%, at least 90%, at least 95%, at least 98%, or at least 99% identical to a fragment of SEQ ID NO: 1 or SEQ ID NO: 2 of at least 200, at least 250, at

least 300, at least 400, between 300 and 497, between 400 and 497, or between 450 and 497 amino acids; or

(ii) the modified GCase polypeptide comprises an amino acid sequence at least 80%, at least 90%, at least 95%, at least 98%, or at least 99% identical to SEQ ID NO: 1 or SEQ ID NO: 2; or

(iii) the modified GCase polypeptide comprises an amino acid sequence at least 98% identical to a fragment of between 400 and 536 amino acids of SEQ ID NO: 1; or

(iv) the modified GCase polypeptide comprises an amino acid sequence at least 98% identical to a fragment of between 400 and 497 amino acids of SEQ ID NO: 2; or

(v) the modified GCase polypeptide comprises an amino acid sequence at least 98% identical to SEQ ID NO: 1; or

(vi) the modified GCase polypeptide comprises an amino acid sequence at least 98% identical to SEQ ID NO: 2; or

(vii) the modified GCase polypeptide comprises an amino acid sequence that is identical to SEQ ID NO: 1 or SEQ ID NO: 2, except that the modified GCase polypeptide comprises the at least one mutation defined in any one of the preceding claims; or

(viii) the modified GCase polypeptide comprises an amino acid sequence that is identical to SEQ ID NO: 1 or SEQ ID NO: 2, except that the modified GCase polypeptide comprises a mutation at a position corresponding to position 272 of SEQ ID NO: 1; or

(ix) the modified GCase polypeptide comprises an amino acid sequence that is identical to SEQ ID NO: 1 or SEQ ID NO: 2, except that the modified GCase polypeptide comprises a substitution with cysteine at a position corresponding to position 351 of SEQ ID NO: 1 and at a position corresponding to position 380 of SEQ ID NO: 1; or

(x) the modified GCase polypeptide comprises an amino acid sequence that is identical to SEQ ID NO: 1 or SEQ ID NO: 2, except that the modified GCase polypeptide comprises a substitution with cysteine at a position corresponding to position 351 of SEQ ID NO: 1 and at a position corresponding to position 380 of SEQ ID NO: 1, and the modified GCase polypeptide comprises a mutation at a position corresponding to position 272 of SEQ ID NO: 1.

12~~13~~. A polynucleotide comprising a modified glucocerebrosidase (GBA) nucleotide sequence, wherein the modified GBA nucleotide sequence encodes the modified GCase polypeptide of any one of the preceding claims.

13~~14~~. The polynucleotide of claim 12~~13~~, wherein:

(i) the modified GBA nucleotide sequence comprises a sequence that is at least 80%, at least 90%, at least 95%, at least 98%, at least 99%, at least 99.5%, at least 99.8%, or 100% identical to a fragment of at least 750, at least 850, at least 950, at least 1000, at least 1200, at least 1400, or at least 1494 nucleotides of any one of SEQ ID NOs: 1~~4~~6 to 29~~.~~; or

~~15. The polynucleotide of claim 13 or 14, wherein:~~

~~(i) the modified GBA nucleotide sequence comprises a sequence that is at least 80%, at least 90%, at least 95%, at least 98%, at least 99%, at least 99.5%, at least 99.8%, or 100% identical to a nucleotide sequence of SEQ ID NO: 6 or SEQ ID NO: 10; or~~

(ii) the modified GBA nucleotide sequence comprises a sequence that is at least 80%, at least 90%, at least 95%, at least 98%, at least 99%, at least 99.5%, at least 99.8%, or 100% identical to a nucleotide sequence of SEQ ID NO: 14 or SEQ ID NO: 18; or

(iii) the modified GBA nucleotide sequence comprises a sequence that is at least 80%, at least 90%, at least 95%, at least 98%, at least 99%, at least 99.5%, at least 99.8%, or 100% identical to a nucleotide sequence of SEQ ID NO: 22 or SEQ ID NO: 26.

~~16. The polynucleotide of any one of claims 13 to 15, wherein:~~

~~(i) the polynucleotide further comprises a transcription regulatory element, optionally wherein the transcription regulatory element comprises an A1AT promoter or a fragment of an A1AT promoter; and/or~~

~~(ii) the polynucleotide comprises a promoter that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, at least 99.5%, at least 99.8%, or 100% identical to SEQ ID NO: 30 or SEQ ID NO: 31; and/or~~

~~(iii) the polynucleotide further comprises a transcription regulatory element, and wherein the transcription regulatory element comprises an enhancer,~~

~~optionally wherein the enhancer is an HCR enhancer or a fragment of an HCR enhancer; and/or~~

~~(iv) the polynucleotide comprises an enhancer that is at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, at least 99.5%, at least 99.8%, or 100% identical to SEQ ID NO: 32 or SEQ ID NO: 33; and/or~~

~~(v) the polynucleotide further comprises a transcription regulatory element, and wherein the transcription regulatory element is at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, at least 99.5%, at least 99.8%, or 100% identical to SEQ ID NO: 34; and/or~~

~~(vi) the polynucleotide further comprises a transcription regulatory element, and wherein the transcription regulatory element is at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, at least 99.5%, at least 99.8%, or 100% identical to SEQ ID NO: 35.~~

14~~17~~. A viral particle comprising a recombinant genome comprising the polynucleotide of ~~any one of~~ claim~~s~~ 12 or 13~~13 to 16~~.

15~~18~~. The viral particle of claim 14~~17~~:

(i) which is an AAV, adenoviral, or lentiviral viral particle, optionally wherein the viral particle is an AAV viral particle; and/or

(ii) wherein the viral particle comprises a liver-tropic or CNS-tropic capsid, optionally wherein the liver-tropic capsid comprises a sequence at least 98%, at least 99%, or at least 99.5% identical to a fragment of at least 600, at least 650, at least 700, between 600 and 736, between 650 and 736, or between 700 and 736 amino acids of SEQ ID NO: 36, 37 or 38; and/or

(iii) wherein the viral particle further comprises:

a) AAV2 ITRs;
b) a polyA sequence; and/or
c) an intron; and/or

(iv) wherein the recombinant genome is single-stranded; and/or

(v) wherein the effective activity of the modified GCase polypeptide is at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 100% of the effective activity of the GCase polypeptide encoded by the sequence of SEQ ID NO: 14 or 18.

16~~19~~. The viral particle of claim 14~~17~~ or 15~~18~~, wherein:

(i) following transduction into a host cell, the effective activity of the modified GCase polypeptide is the same or higher compared to the effective activity of a reference GCase polypeptide following transduction into a host cell of an otherwise identical viral particle comprising a GBA nucleotide sequence encoding the reference GCase polypeptide; and/or

(ii) following transduction into a host cell, the effective activity of the modified GCase polypeptide is higher compared to the effective activity of a reference GCase polypeptide following transduction into a host cell of an otherwise identical viral particle comprising a GBA nucleotide sequence encoding the reference GCase polypeptide; and/or

(iii) following administration of the viral particle, the effective activity of the modified GCase polypeptide is the same or higher in plasma, white blood cells, the spleen, bone marrow, lung tissue, and/or any other Gaucher Disease-affected organ, compared to the effective activity of a reference GCase polypeptide following administration of an otherwise identical viral particle comprising a GBA nucleotide sequence encoding the reference GCase polypeptide; and/or

(iv) following administration of the viral particle, the effective activity of the modified GCase polypeptide is the same or higher in liver compared to the effective activity of a reference GCase polypeptide following administration of an otherwise identical viral particle comprising a GBA nucleotide sequence encoding the reference GCase polypeptide; and/or

(v) following administration of the viral particle, the effective activity of the modified GCase polypeptide is higher in plasma, white blood cells, the spleen, bone marrow, lung tissue, and/or any other Gaucher Disease-affected organ, compared to the effective activity of a reference GCase polypeptide following administration of an otherwise identical viral particle

comprising a GBA nucleotide sequence encoding the reference GCase polypeptide; and/or

(vi) following administration of the viral particle, the effective activity of the modified GCase polypeptide is higher in liver compared to the effective activity of a reference GCase polypeptide following administration of an otherwise identical viral particle comprising a GBA nucleotide sequence encoding the reference GCase polypeptide; and/or

(vii) following administration of the viral particle, the effective activity of the modified GCase polypeptide is the same or higher in liver, white blood cells, the spleen, bone marrow, lung tissue, and/or any other Gaucher Disease-affected organ, compared to the effective activity of a GCase enzyme replacement therapy; and/or

(viii) following administration of the viral particle, the level of hexosylsphingosine and/or hexosylceramide is lower in plasma, liver, the spleen, bone marrow, lung tissue, and/or any other Gaucher Disease-affected organ, compared to the level of hexosylsphingosine and/or hexosylceramide

a. after administration of a GCase enzyme replacement therapy; or

b. in a subject having Gaucher Disease; or

c. following administration of an otherwise identical viral particle comprising a GBA nucleotide sequence encoding a reference GCase polypeptide; and/or

(ix) following administration of the viral particle, the number of storage cells or the level of activated macrophages is lower in the liver or in lung tissue compared to the number of storage cells or the level of activated macrophages after administration of a GCase enzyme replacement therapy~~.~~;

wherein:

(i) the reference GCase polypeptide comprises the polypeptide of SEQ ID NO: 41 or 44, optionally wherein the GBA nucleotide sequence encoding the reference GCase polypeptide comprises the nucleotide sequence of SEQ ID NO: 6 or 10; or

(ii) the reference GCase polypeptide comprises the polypeptide of SEQ ID NO: 43 or 46, optionally wherein the GBA nucleotide sequence encoding the reference GCase polypeptide comprises the nucleotide sequence of SEQ ID NO: 22 or 26; or

(iii) the reference GCase polypeptide comprises the polypeptide of SEQ ID NO: 42 or 45, optionally wherein the GBA nucleotide sequence encoding the reference GCase polypeptide comprises the nucleotide sequence of SEQ ID NO: 14 or 18; or

(iv) the reference GCase polypeptide comprises the polypeptide of SEQ ID NO: 1 or 2, optionally, wherein the GBA nucleotide sequence encoding the reference GCase polypeptide comprises the nucleotide sequence of SEQ ID NO: 59 or 60.

17~~20~~. The viral particle of claim 16~~19~~, wherein the effective activity of the modified GCase polypeptide is at least 2x, at least 3x, at least 4x, at least 5x, at least 7x, or at least 10x, at least 15x, at least 20x, at least 25x, at least 30x, at least 35x, or at least 40x higher compared to the effective activity of the reference GCase polypeptide.

~~21. The viral particle of claim 19 or claim 20, wherein:~~

~~(i) the reference GCase polypeptide comprises the polypeptide of SEQ ID NO: 41 or 44, optionally wherein the GBA nucleotide sequence encoding the reference GCase polypeptide comprises the nucleotide sequence of SEQ ID NO: 6 or 10; or~~

~~(ii) the reference GCase polypeptide comprises the polypeptide of SEQ ID NO: 43 or 46, optionally wherein the GBA nucleotide sequence encoding the reference GCase polypeptide comprises the nucleotide sequence of SEQ ID NO: 22 or 26; or~~

~~(iii) the reference GCase polypeptide comprises the polypeptide of SEQ ID NO: 42 or 45, optionally wherein the GBA nucleotide sequence encoding the reference GCase polypeptide comprises the nucleotide sequence of SEQ ID NO: 14 or 18; or~~

~~(iv) the reference GCase polypeptide comprises the polypeptide of SEQ ID NO: 1 or 2, optionally, wherein the GBA nucleotide sequence encoding the reference GCase polypeptide comprises the nucleotide sequence of SEQ ID NO: 59 or 60.~~

18~~22~~. A composition comprising the modified GCase polypeptide, polynucleotide, or viral particle of any one of the preceding claims and a pharmaceutically acceptable excipient.

19~~23~~. The modified GCase polypeptide, polynucleotide, viral particle, or composition of any one of the preceding claims for use in ~~a method of treatment~~ therapy~~, optionally wherein the method of treatment comprises administering an effective amount of the modified GCase polypeptide, polynucleotide, viral particle or composition of any one of claims 1 to 22 to a patient~~.

20. Use of the modified GCase polypeptide, polynucleotide, viral particle or composition of any one of claims 1 to 18 in the manufacture of a medicament for:

~~24. The modified GCase polypeptide, polynucleotide, viral particle or composition for use of claim 23, wherein:~~

(i) ~~the method of treatment is a method of~~ treating a disease associated with GCase deficiency; or

(ii) ~~the method of treatment is a method of~~ treating Parkinson's disease; or

(iii) ~~the method of treatment is a method of~~ treating Gaucher disease, optionally wherein the Gaucher disease is Gaucher disease type I, II or III; or

(iv) ~~the method of treatment is a method of~~ treating Gaucher disease and wherein the patient has antibodies or inhibitors to a recombinant GCase with which the patient has previously been treated as part of an enzyme replacement therapy, optionally wherein the Gaucher disease is Gaucher disease type I, II or III.

~~25. The modified GCase polypeptide, polynucleotide, viral particle, or composition of any one of claims 1 to 22 for use in treating a disease, wherein the modified GCase polypeptide or the encoded modified GCase polypeptide has increased stability at pH 7.4 compared to a GCase polypeptide encoded by a GBA nucleotide sequence of SEQ ID NO: 40.~~

~~26. The modified GCase polypeptide, polynucleotide, viral particle, or composition for use of claim 25, wherein:~~

~~- the modified GCase polypeptide or encoded modified GCase polypeptide~~

~~retains activity which is at least 1.2 times, at least 1.5 times, at least 1.8 times, or at least 2 times higher than the activity retained by the GCase polypeptide encoded by the GBA nucleotide sequence of SEQ ID NO: 40 when measured after 120 minutes of incubation at pH 7.4 and 37 degrees Celsius; and/or~~

~~– the modified GCase polypeptide or encoded modified GCase polypeptide retains activity which is at least 3 times, at least 5 times, at least 7 times, at least 10 times or at least 15 times higher than the activity retained by the GCase polypeptide encoded by the GBA nucleotide sequence of SEQ ID NO: 40 when measured after 120 minutes of incubation at pH 7.4 and 37 degrees Celsius.~~

~~27. The modified GCase polypeptide, polynucleotide, viral particle, or composition of any one of claims 1 to 22 for use in treating a disease, wherein treating the disease comprises administering a lower dose of the modified GCase polypeptide, polynucleotide, viral particle or composition compared to the dose required to be administered of an equivalent polypeptide, polynucleotide, viral particle or composition comprising or encoding a GCase polypeptide encoded by a GBA nucleotide sequence of SEQ ID NO: 40.~~