compared to polynucleotides comprising a wild type GCase encoding polynucleotides. Such a gene therapy approach would avoid the need for frequent and lifelong intravenous injections of GCase.

Summary

The present application demonstrates that specific modifications to a GBA nucleotide sequence encoding for GCase can help to improve the expression level and the activity of the expressed GCase polypeptide *in vitro* and/or *in vivo*. For example, the present application demonstrates that using a codon-optimised GBA nucleotide sequence can improve the expression and/or activity of the encoded GCase protein. Such modified (*i.e.* non wild-type) and/or codon-optimised GBA nucleotide sequences may be further modified to provide further improvements in the expression and/or activity of the encoded GCase protein. Further modifications may include providing further modifications in the GBA nucleotide sequence such as the removal of CpG motifs, and/or the use of particular gene regulatory elements comprising specific promoter and/or enhancer sequences. It is believed that such improvements to a GBA nucleotide sequence can improve the efficacy of such a nucleotide in the treatment of GD.

These modifications provide a GBA nucleotide sequence which is expressed highly, for example in the liver, and which encodes a GCase polypeptide or fragment thereof. As demonstrated in the Examples, the polynucleotides of the disclosure express GCase activity at higher levels than wild type GBA.

In one aspect, the present disclosure refers to a polynucleotide comprising a GBA nucleotide sequence, wherein the GBA nucleotide sequence encodes a β-Glucocerebrosidase (GCase) protein or fragment thereof and wherein at least a portion of the GBA nucleotide sequence is not wild type, optionally wherein the portion of the GBA nucleotide sequence that is not wild type is codon-optimised, more optionally wherein the GBA nucleotide sequence encodes a GCase protein or a fragment thereof and comprises a sequence that is: (i) 100% identical to a nucleotide sequence of any one of SEQ ID NO: 1-8; (ii) at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, at least 99.8%, or at least 100% identical to a nucleotide sequence of any one of SEQ ID NO: 1-8; and/or (iii) a variant of any one of SEQ ID NO: 1-8 encoding a GCase protein having GCase activity, wherein the variant is identical to SEQ ID NO: 1-8 respectively except that it comprises nucleotide substitutions such that the GCase protein has 1, up to 2, up to 3, up to 4, up to 5, up to 6, up to 7, up to 8, up to 9, or up to 10 amino acid substitutions relative to the wild type GCase amino acid sequence of SEQ ID NO: 25.

In another aspect, the present disclosure refers to a viral particle comprising a recombinant genome comprising the polynucleotide as disclosed herein.

In another aspect, the present disclosure refers to a composition comprising the polynucleotide or viral particle as disclosed herein and a pharmaceutically acceptable excipient.

In another aspect, the present disclosure refers to the polynucleotide, viral particle or composition of any one of the preceding claims for use in therapy.

In another aspect, the present disclosure refers to the use of the polynucleotide, viral particle, or composition as disclosed herein in the manufacture of a medicament for treating a disease associated with GCase deficiency.

In another aspect, the present disclosure refers to the use of the polynucleotide, viral particle, or composition as disclosed herein in the manufacture of a medicament for treating Parkinson's disease.

In another aspect, the present disclosure refers to the use of the polynucleotide, viral particle, or composition of any one of claims 1 to 22 in the manufacture of a medicament for treating Gaucher disease, optionally wherein: (i) the Gaucher disease is Gaucher disease type I, II or III; and/or (ii) 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.

In another aspect, the present disclosure refers to the use of the polynucleotide, viral particle or composition as disclosed herein in the manufacture of a medicament for expressing the GBA nucleotide sequence and achieving a stable GCase activity in a subject.

In another aspect, the present disclosure refers to the use of the polynucleotide, viral particle or composition as disclosed herein in the manufacture of a medicament for expressing the GBA nucleotide sequence and providing greater GCase bioavailability in a subject compared to the bioavailability from GCase enzyme replacement therapy, wherein the bioavailability is measured over a period of 2 weeks from administration.

In yet another aspect, the present disclosure refers to the use of the polynucleotide, viral particle or composition as disclosed herein in the manufacture of a medicament for reducing hexosylceramide and/or hexosylsphingosine levels in a subject suffering from a

disease or condition associated with GCase deficiency, optionally wherein reducing hexosylceramide and/or hexosylsphingosine levels leads to the treatment of the disease or condition associated with GCase deficiency.

Accordingly, in a first example of the disclosure, there is provided a polynucleotide comprising a GBA nucleotide sequence, wherein the GBA nucleotide sequence encodes a β-Glucocerebrosidase (GCase) protein or fragment thereof and wherein at least a portion of the GBA nucleotide sequence is not wild type.

In a second example of the disclosure, there is provided a polynucleotide comprising a GBA nucleotide sequence, wherein the GBA nucleotide sequence encodes a GCase protein or a fragment thereof and comprises a sequence that is at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, at least 99.8%, or 100% identical to a fragment of at least 1000, at least 1200, at least 1300, less than 1494, less than 1611, between 1000 and 1494, between 1000 and 1611, between 1300 and 1494, between 1300 and 1611, or around 1494 nucleotides of SEQ ID NO: 1-8.

In a third example of the disclosure, there is provided a viral particle comprising a recombinant genome comprising the polynucleotide of the disclosure.

In a fourth example of the disclosure, there is provided a composition comprising the polynucleotide or viral particle of the disclosure and a pharmaceutically acceptable excipient.

In a fifth example of the disclosure, there is provided a method of treatment comprising administering an effective amount of the polynucleotide or viral particle of the disclosure to a patient.

In a sixth example of the disclosure, there is provided a use of the polynucleotide, viral particle or composition of the disclosure in the manufacture of a medicament for use in a method of treatment.

In a seventh example of the disclosure, there is provided the use of the polynucleotide, viral particle or composition of the disclosure in the manufacture of a medicament for achieving a stable GCase activity in a subject.

In an eighth example of the disclosure, there is provided the use of the polynucleotide, viral particle or composition of the disclosure in the manufacture of a medicament for providing greater GCase bioavailability in a subject compared to the bioavailability from

GCase enzyme replacement therapy, wherein the bioavailability is measured over a period of 2 weeks from administration.

In a ninth example of the disclosure, there is provided a method of achieving a stable GCase activity in a subject by administering to the subject the polynucleotide, viral particle or composition of the disclosure.

In a tenth example of the disclosure, there is provided a method of providing greater GCase bioavailability in a subject compared to the bioavailability from GCase enzyme replacement therapy by administering to the subject the polynucleotide, viral particle or composition of the disclosure, wherein the bioavailability is measured over a period of 2 weeks from administration.

In an eleventh example of the disclosure, there is provided a polynucleotide, viral particle or composition of the disclosure, for use in a method of expressing the GBA nucleotide sequence and achieving a stable GCase activity in a subject.

In a twelfth example of the disclosure, there is provided a polynucleotide, viral particle or composition of the disclosure, for use in a method of expressing the GBA nucleotide sequence and providing greater GCase bioavailability in a subject compared to the bioavailability from GCase enzyme replacement therapy, wherein the bioavailability is measured over a period of 2 weeks from administration.

In a thirteenth example of the disclosure, there is provided the use of the polynucleotide, viral particle or composition of the disclosure in the manufacture of a medicament for reducing the levels of hexosylceramide and/or hexosylsphingosine in a subject suffering from a disease or condition associated with GCase deficiency.

In a fourteenth example of the disclosure, there is provided a method of reducing the levels of hexosylceramide and/or hexosylsphingosine in a subject suffering from a disease or condition associated with GCase deficiency by administering to the subject the polynucleotide, viral particle or composition of the disclosure.

In a fifteenth example of the disclosure, there is provided a polynucleotide, viral particle or composition of the disclosure, for use in a method of reducing levels of hexosylceramide and/or hexosylsphingosine in a subject suffering from a disease or condition associated with GCase deficiency, optionally wherein reducing hexosylceramide and/or hexosylsphingosine levels leads to the treatment of the disease or condition associated with GCase deficiency.

Description of the Figures

Figure 1 – Schematics of the GBA cassettes from the constructs FLF-PL01, FLF-PL28, and FLF-PL64. LSP-S and LSP-L: liver specific promoters; GBAwt: wild type human GBA nucleotide sequence; GBAco: human GBA nucleotide sequence codon-optimised (except for stretch encoding signal peptide, the end of which is represented by a dotted line).

Figure 2 – Dose-dependent liver expression and secretion of human GCase into murine bloodstream following AAV2/8-FLF-PL28 injection. (A) Representative image of mouse livers stained for GCase 12-weeks post AAV2/8-PL28 injection. DAB (3,3′-Diaminobenzidine) was used to visualize GCase and haematoxylin was used as counterstain. (B) Levels of GCase as measured by activity assay in serum of mice treated with increased doses of AAV2/8-PL28. n=5, C57BL/6 mice in each treatment group. Error bars show mean ± SD.

Figure 3 – Relative GCase levels observed for each tested GBA codon-optimised construct (FLF-PL16 to FLF-PL36; '16' to '36') upon transfection onto Huh-7 cells. Each construct was tested independently in 3 to 5 experiments. Data shown here represent GCase activity relative to wild type GBA construct FLF-PL01 ('01'). Error bars represent mean ± SD.

Figure 4 – Measurement of GCase activity present in the mouse bloodstream upon injection of vectors AAV2/8- FLF-PL- 01, 21, 28, 30, 36 and 37 (see example 5 for description of constructs). (A) GCase activity levels found in mouse serum 8-weeks after

Claims

1. A polynucleotide comprising a GBA nucleotide sequence, wherein the GBA nucleotide sequence encodes a β-Glucocerebrosidase (GCase) protein or fragment thereof and wherein at least a portion of the GBA nucleotide sequence is not wild type, optionally wherein the portion of the GBA nucleotide sequence that is not wild type is codon-optimised, more optionally wherein the GBA nucleotide sequence encodes a GCase protein or a fragment thereof and comprises a sequence that is:

(i) 100% identical to a nucleotide sequence of any one of SEQ ID NO: 1-8;

(ii) at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, at least 99.8%, or at least 100% identical to a nucleotide sequence of any one of SEQ ID NO: 1-8; and/or

(iii) a variant of any one of SEQ ID NO: 1-8 encoding a GCase protein having GCase activity, wherein the variant is identical to SEQ ID NO: 1-8 respectively except that it comprises nucleotide substitutions such that the GCase protein has 1, up to 2, up to 3, up to 4, up to 5, up to 6, up to 7, up to 8, up to 9, or up to 10 amino acid substitutions relative to the wild type GCase amino acid sequence of SEQ ID NO: 25.

2. The polynucleotide of claim 1, wherein the GBA nucleotide sequence comprises a sequence that is:

(i) 100% identical to a nucleotide sequence of SEQ ID NO: 1 or SEQ ID NO: 5;

(ii) at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or at least 99.8% identical to a nucleotide sequence of SEQ ID NO: 1 or SEQ ID NO: 5; and/or

(iii) a variant of SEQ ID NO: 1 or SEQ ID NO: 5 encoding a GCase protein having GCase activity, wherein the variant is identical to SEQ ID NO: 1 or SEQ ID NO: 5 respectively except that it comprises nucleotide substitutions such that the GCase protein has 1, up to 2, up to 3, up to 4, up to 5, up to 6, up to 7, up to 8, up to 9, or up to 10 amino acid substitutions relative to the wild type GCase amino acid sequence of SEQ ID NO: 25.

3. The polynucleotide of claim 1 or 2, wherein the variant is a variant of SEQ ID NO: 1 and the variant of SEQ ID NO: 1:

(i) is identical to SEQ ID NO: 1 except that it comprises nucleotide substitutions such that the GCase protein has 1, up to 2, up to 3, up to 4, up to 5, up to 6, up to 7, up to 8, up to 9, or up to 10 amino acid substitutions relative to the wild type GCase amino acid sequence of SEQ ID NO: 25;

(ii) has 1, up to 2, up to 3, up to 4, up to 5, up to 6, up to 7, up to 8, up to 9, up to 10, up to 20, or up to 30 nucleotide substitutions relative to the sequence of SEQ ID NO: 1;

(iii) has 1, up to 2, up to 3, up to 4, up to 5, or up to 6 nucleotide substitutions relative to the sequence of SEQ ID NO: 1;

(iv) has up to 4 nucleotide substitutions relative to the sequence of SEQ ID NO: 1 and/or encodes a GCase protein having up to 3 amino acid substitutions relative to the wild type amino acid GCase sequence of SEQ ID NO: 25;

(v) has up to 3 nucleotide substitutions relative to the sequence of SEQ ID NO: 1 and/or encodes a GCase protein having up to 2 amino acid substitutions relative to the wild type GCase amino acid sequence of SEQ ID NO: 25; and/or

(vi) has 1 nucleotide substitution relative to the sequence of SEQ ID NO: 1 and/or encodes a GCase protein having up to 1 amino acid substitution relative to the wild type GCase amino acid sequence of SEQ ID NO: 25.

4. The polynucleotide of claim 1 or 2, wherein the variant is a variant of SEQ ID NO: 5 and the variant of SEQ ID NO: 5:

(i) is identical to SEQ ID NO: 5 except that it comprises nucleotide substitutions such that the GCase protein has 1, up to 2, up to 3, up to 4, up to 5, up to 6, up to 7, up to 8, up to 9, or up to 10 amino acid substitutions relative to the wild type GCase amino acid sequence of SEQ ID NO: 25;

(ii) has 1, up to 2, up to 3, up to 4, up to 5, up to 6, up to 7, up to 8, up to 9, up to 10, up to 20, or up to 30 nucleotide substitutions relative to the sequence of SEQ ID NO: 5;

(iii) has 1, up to 2, up to 3, up to 4, up to 5, or up to 6 nucleotide substitutions relative to the sequence of SEQ ID NO: 5;

(iv) has up to 4 nucleotide substitutions relative to the sequence of SEQ ID NO: 5 and/or encodes a GCase protein having up to 3 amino acid substitutions relative to the wild type amino acid GCase sequence of SEQ ID NO: 25;

(v) has up to 3 nucleotide substitutions relative to the sequence of SEQ ID NO: 5 and/or encodes a GCase protein having up to 2 amino acid substitutions relative to the wild type GCase amino acid sequence of SEQ ID NO: 25; and/or

(vi) has 1 nucleotide substitution relative to the sequence of SEQ ID NO: 5 and/or encodes a GCase protein having up to 1 amino acid substitution relative to the wild type GCase amino acid sequence of SEQ ID NO: 25.

5. The polynucleotide of any one of the preceding claims, wherein the GBA nucleotide sequence encodes a GCase protein having:

(i) up to 3 amino acid substitutions relative to the wild type GCase amino acid sequence of SEQ ID NO: 25;

(ii) up to 2 amino acid substitutions relative to the wild type GCase amino acid sequence of SEQ ID NO: 25; and/or

(iii) up to 1 amino acid substitution relative to the wild type GCase amino acid sequence of SEQ ID NO: 25.

6. A polynucleotide comprising a GBA nucleotide sequence, wherein the GBA nucleotide sequence encodes a GCase protein or a fragment thereof and comprises a sequence that is at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, at least 99.8%, or 100% identical to a fragment of at least 1000, at least 1200, at least 1300, less than 1494, less than 1611, between 1000 and 1494, between 1000 and 1611, between 1300 and 1494, between 1300 and 1611, around 1494, or around 1611 nucleotides of any one of SEQ ID NO: 1-8.

7. The polynucleotide of any one of the preceding claims, wherein at least a portion of the GBA nucleotide sequence is codon-optimised.

8. The polynucleotide of claim 7, wherein:

(a) the at least a portion of the GBA nucleotide sequence that is codon-optimised is codon-optimised for expression in human liver cells;

(b) the portion of the GBA nucleotide sequence that is codon-optimised is a contiguous portion;

(c) the GBA nucleotide sequence is codon-optimised for expression in human liver cells;

(d) the portion of the GBA nucleotide sequence that is codon-optimised is at least 1000, at least 1200, at least 1300, less than 1494, between 1000 and 1494, between 1300 and 1494, or around 1494 nucleotides in length;

(e) the portion of the GBA nucleotide sequence that is codon-optimised corresponds to a mature GCase protein;

(f) the portion of the GBA nucleotide sequence that is codon-optimised does not encode all or a portion of a signal peptide;

(g) the GBA nucleotide sequence or the portion of the GBA nucleotide sequence that is codon-optimised comprises a reduced number of CpGs compared to a corresponding portion of a wild type GBA nucleotide sequence; optionally wherein the GBA nucleotide sequence or the portion of the GBA nucleotide sequence that is codon-optimised comprises less than 40, less than 20, less than 18, less than 10, or less than 5 CpGs, more optionally wherein the GBA nucleotide sequence or the portion of the GBA nucleotide sequence that is codon-optimised comprises less than 5, less than 4, less than 3, or less than 2 CpGs per 100 nucleotides, more optionally wherein the GBA nucleotide sequence or the portion of the GBA nucleotide sequence that is codon-optimised is CpG-free, preferably wherein the wild type GBA nucleotide sequence is SEQ ID NO: 9;

(h) the portion of the GBA nucleotide sequence that is codon-optimised 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 a fragment of at least 1000, at least 1200, at least 1300, less than 1494, between 1000 and 1494, between 1300 and 1494, or around 1494 nucleotides of any one of SEQ ID NO: 1-4; and/or

(i) the portion of the GBA nucleotide sequence that is codon-optimised is at least 99%, at least 99.5%, at least 99.8%, or 100% identical to SEQ ID NO: 1.

9. The polynucleotide of any one of the preceding claims, wherein the GBA nucleotide sequence comprises a portion that is not codon-optimised, optionally wherein:

(a) the portion that is not codon-optimised encodes all or a portion of a GCase signal peptide;

(b) the portion that is not codon-optimised is at least 80, at least 90, at least 100, at least 110, less than 200, less than 170, less than 140, or around 117 nucleotides; and/or

(c) the portion that is not codon-optimised comprises 1 or more CpGs.

10. The polynucleotide of any one of the preceding claims, wherein the polynucleotide further comprises a transcription regulatory element, optionally wherein the transcription regulatory element comprises a liver-specific promoter and/or an enhancer.

11. The polynucleotide of claim 10, wherein the transcription regulatory element comprises an A1AT promoter or a fragment of an A1AT promoter, optionally wherein:

(a) the A1AT promoter or the fragment of an A1AT promoter is at least 100, at least 120, at least 150, at least 180, less than 255, between 100 and 255, between 150 and 225, between 150 and 300, or between 180 and 255 nucleotides in length, more optionally wherein the fragment of an A1AT promoter is between 180 and 255 nucleotides in length;

(b) the A1AT promoter or the fragment of an A1AT promoter is at least 200, at least 250, at least 300, less than 500, between 200 and 500, between 250 and 500, between 350 and 450, or around 418 nucleotides in length, more optionally wherein the fragment of an A1AT promoter is between 350 and 450 nucleotides in length; and/or

(c) 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: 15 or SEQ ID NO: 12.

12. The polynucleotide of claims 10 or 11, wherein the enhancer is an HCR enhancer or a fragment of an HCR enhancer, optionally wherein:

(a) the HCR enhancer or the fragment of an HCR enhancer is a fragment of at least 80, at least 90, at least 100, less than 192, between 80 and 192, between 90 and 192, between 100 and 250, or between 117 and 192 nucleotides in length, more optionally wherein the fragment of an HCR enhancer is between 117 and 192 nucleotides in length;

(b) the HCR enhancer or the fragment of an HCR enhancer is a fragment of at least 150, at least 190, at least 230, less than 400, between 150 and 400, between 190 and 370, between 230 and 340, between 250 and 340, or around 321 nucleotides in length, more optionally wherein the fragment of an HCR enhancer is between 250 and 340 nucleotides in length;

(c) 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: 16 or SEQ ID NO: 11.

13. The polynucleotide of claim 10, 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: 14 or 10.

14. The polynucleotide of any one of the preceding claims, wherein the GCase encoded by the GBA nucleotide sequence is expressed in human liver cells at higher levels compared to a GCase encoded by a wild type GBA nucleotide sequence in an otherwise identical reference polynucleotide, optionally wherein the GCase encoded by the GBA nucleotide sequence is expressed in human liver cells at least 1.1x, at least 1.2x, at least 1.3x, at least 1.4x, or at least 1.5x higher compared to a GCase encoded by a wild type GBA nucleotide sequence in an otherwise identical reference

polynucleotide, more optionally wherein the reference polynucleotide comprises a wild type GBA nucleotide sequence of SEQ ID NO: 9, optionally wherein the reference polynucleotide comprises a promoter of SEQ ID NO: 13.

15. A viral particle comprising a recombinant genome comprising the polynucleotide of any one of the preceding claims.

16. The viral particle of claim 15, which is an AAV, adenoviral, or lentiviral viral particle, optionally which is an AAV viral particle.

17. The viral particle of claim 15 or 16, wherein the viral particle comprises a liver-tropic or CNS-tropic capsid.

18. The viral particle of claim 17, wherein the liver-tropic capsid comprises a sequence at least 98%, at least 99%, at least 99.5% 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: 19, 20 or 24, optionally wherein the liver-tropic capsid comprises a sequence at least 99% identical to SEQ ID NO: 19 or 20.

19. The viral particle of claim 17, wherein the CNS-tropic capsid comprises a sequence at least 98%, at least 99%, at least 99.5% 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: 21, optionally wherein the CNS-tropic capsid comprises a sequence at least 99% identical to SEQ ID NO: 21.

20. The viral particle of any one of claims 15 to 19, wherein the recombinant genome further comprises:

a) AAV2 ITRs;

b) a poly A sequence; and/or

c) an intron;

optionally wherein the recombinant genome is single-stranded.

21. The viral particle of any one of claims 15 to 20, wherein on transduction into Huh-7 cells, the viral particle expresses GCase or a fragment thereof such that the GCase activity in the transduced cell is greater than the activity of GCase or a fragment thereof in a cell transduced with an otherwise identical viral particle comprising a GBA nucleotide sequence of SEQ ID NO: 9, optionally wherein on transduction into Huh-7 cells, the viral particle expresses GCase or a fragment thereof such that the GCase activity in the transduced cell is at least 2x, at least 3x, at least 4x, at least 5x, at least 10x, or at least 20x greater than the activity of GCase or a fragment thereof in a cell transduced with an otherwise identical viral particle comprising a GBA nucleotide sequence of SEQ ID NO: 9, more optionally wherein the activity is measured using a fluorometric substrate which is specific for GCase.

22. A composition comprising the polynucleotide or viral particle of any one of the preceding claims and a pharmaceutically acceptable excipient.

23. The polynucleotide, viral particle or composition of any one of the preceding claims for use in therapy.

24. The polynucleotide, viral particle or composition for use of claim 23, wherein the polynucleotide, composition or viral particle of any one of claims 1 to 22 is to be administered to a patient.

25. Use of the polynucleotide, viral particle, or composition of any one of claims 1 to 22 in the manufacture of a medicament for treating a disease associated with GCase deficiency.

26. Use of the polynucleotide, viral particle, or composition of any one of claims 1 to 22 in the manufacture of a medicament for treating Parkinson's disease.

27. Use of the polynucleotide, viral particle, or composition of any one of claims 1 to 22 in the manufacture of a medicament for treating Gaucher disease, optionally wherein:

(i) the Gaucher disease is Gaucher disease type I, II or III; and/or
(ii) 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.

28. Use of the polynucleotide, viral particle or composition of any one of claims 1 to 22 in the manufacture of a medicament for expressing the GBA nucleotide sequence and achieving a stable GCase activity in a subject.

29. Use of the polynucleotide, viral particle or composition of any one of claims 1 to 22 in the manufacture of a medicament for expressing the GBA nucleotide sequence and providing greater GCase bioavailability in a subject compared to the bioavailability from GCase enzyme replacement therapy, wherein the bioavailability is measured over a period of 2 weeks from administration.

30. The use of claim 28 or 29, wherein achieving a stable GCase activity and/or providing greater GCase bioavailability leads to the treatment of a disease in the subject.

31. The use of any of claims 28 to 30, wherein:
(i) the GCase activity and/or bioavailability is measured using a fluorometric substrate which is specific for GCase;
(ii) the GCase activity is measured in the serum or plasma of the subject;
(iii) the GCase activity is measured in the macrophages of the subject;
(iv) the GCase activity is stable at a level of at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, or at least 9 µmol/h/ml in the subject;
(v) the GCase activity is stable at a level of at least 3 µmol/h/ml in the subject;
(vii) the GCase activity is stable at a level of at least 5 µmol/h/ml in the subject;
(vii) the GCase activity is stable at a level of at least 9 µmol/h/ml in the subject;
(viii) wherein an effective dose of the polynucleotide, viral particle or composition is to be administered to the subject;

(ix) the stable GCase activity is a GCase activity of at least 10%, at least 20%, at least 30%, at least 40%, or at least 50% relative to the GCase activity of a healthy subject;

(x) the stable GCase activity is a GCase activity of between 10% and 100%, between 20% and 90%, between 30% and 70%, between 40% and 70%, or between 50% and 70% relative to the GCase activity of a healthy subject;

(xi) the stable GCase activity is stable for at least 5 weeks from administration;

(xii) the stable GCase activity is stable for at least 10 weeks from administration;

(xiii) the stable GCase activity is stable for at least 15 weeks from administration;

(xiv) the stable GCase activity is stable for at least 20 weeks from administration;

(xv) the stable GCase activity is stable for at least 25 weeks from administration;

(xvi) the stable GCase activity is stable for at least 30 weeks from administration;

(xvii) the stable GCase activity is stable for at least 35 weeks from administration;

(xviii) the stable GCase activity is stable for at least 40 weeks after administration;

(xix) a greater GCase activity is achieved in the liver, spleen, and/or bone marrow of the subject at least 5, at least 10, at least 15, at least 20, at least 25, at least 30, or at least 35 weeks after administration when compared to the activity measured in a subject administered an effective dose of a GCase enzyme replacement therapy, when measured in the same assay at the same time point after administration; and/or

(xx) a greater GCase bioavailability is achieved in the liver spleen and/or bone marrow subject over a period of at least 5, at least 10, at least 15, at least 20, at least 25, at least 30, or at least 35 weeks after administration when compared to the bioavailability measured in a subject administered an effective dose of a GCase enzyme replacement therapy, when measured in the same assay at the same time point after administration.

32. The use of any of claims 30 to 31, wherein the disease is Gaucher disease, optionally wherein the Gaucher disease is Gaucher disease type I, II or III.

33. Use of the polynucleotide, viral particle or composition of any one of claims 1 to 22 in the manufacture of a medicament for reducing hexosylceramide and/or

hexosylsphingosine levels in a subject suffering from a disease or condition associated with GCase deficiency, optionally wherein reducing hexosylceramide and/or hexosylsphingosine levels leads to the treatment of the disease or condition associated with GCase deficiency.

34. The use of claim 33, wherein:
(i) the hexosylceramide and/or hexosylsphingosine levels are reduced by 2 times or more, 3 times or more, 4 times or more, 5 times or more, 6 times or more, 2 to 3 times, 2 to 4 times, 2 to 5 times, 2 to 6 times, or 3 to 5 times when compared to the hexosylceramide and/or hexosylsphingosine levels at the time of administration of the polynucleotide, viral particle or composition of any one of claims 1 to 22;
(ii) the reduction in hexosylceramide and/or hexosylsphingosine levels is greater than the reduction achieved in a subject administered an effective dose of a GCase enzyme replacement therapy, optionally when the hexosylceramide and/or hexosylsphingosine levels are measured at least 6 weeks, at least 8 weeks, at least 10 weeks or at least 12 weeks after administration;
(iii) the hexosylceramide and/or hexosylsphingosine levels are measured in the macrophages of the subject;
(iv) the hexosylceramide and/or hexosylsphingosine levels are measured in the spleen of the subject;
(v) the hexosylceramide and/or hexosylsphingosine levels are measured in the liver of the subject;
(vi) the hexosylceramide and/or hexosylsphingosine levels are measured in the serum of the subject;
(vii) the hexosylceramide and/or hexosylsphingosine levels are measured by mass spectrometry; and/or
(viii) the disease is Gaucher disease, optionally wherein the Gaucher disease is Gaucher disease type I, II or III.

35. The polynucleotide, viral particle, or composition for use, or use of any one of claims 23 to 34, 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.