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DESCRIPTION

Field of the Invention

[0001] The present invention relates to polynucleotides comprising a GBA nucleotide sequence encoding β-Glucocerebrosidase (GCase), viral particles comprising the polynucleotides and treatments utilising the polynucleotides.

Background to the Invention

[0002] Gaucher disease (GD) is an autosomal recessive lipid storage disease characterised by the deposition of glucocerebroside in cells of the macrophage-monocyte system. GD is caused by mutations in the housekeeping GBA gene that impairs activity and/or production of the enzyme β-Glucocerebrosidase (GCase).

[0003] There are three major types of GD which are characterised by the specific mutations which have been identified, and each type can display differing clinical symptoms. Type 1 GD has little or no involvement with the central nervous system but is mainly characterised by visceral manifestations such as enlarged spleen and liver, low blood cell counts, bleeding problems and bone disease. For the past 20 years, enzyme replacement therapy has emerged as the standard of care for type 1 GD. In addition to its high cost (-$200,000 or ~ £150,000/patient/year), enzyme replacement therapy treatment in GD generally requires one or more injections every other week for life. This leads to a high proportion of GD patients displaying high levels of treatment burden.

[0004] Accordingly, there is a need to provide an effective therapy vector for the treatment of GD, *i.e.* one that allows for a high level of GCase expression.

[0005] The present application relates to a gene therapy approach for treating GD, involving administering a viral particle comprising a GBA polynucleotide encoding GCase. The polynucleotides and viral particles described herein can provide higher GCase expression 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.

[0006] WO 2019/009979 describes the use of lentiviral vectors in the treatment of lysosomal storage disorders.

Summary of the Invention

[0007] 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.

[0008] 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 invention express GCase activity at higher levels than wild type GBA.

[0009] The present invention is defined in the claims. In particular, the present invention provides a polynucleotide comprising a GBA nucleotide sequence, wherein the GBA nucleotide sequence encodes a β-Glucocerebrosidase (GCase) protein and wherein the GBA nucleotide sequence comprises a sequence that is:

1. (i) 100% identical to SEQ ID NO: 1 or SEQ ID NO: 5;
2. (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 SEQ ID NO: 1 or SEQ ID NO: 5, wherein the GCase encoded by the GBA nucleotide sequence has GCase activity and 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; and/or
3. (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, further wherein the GCase encoded by the GBA nucleotide 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.

[0010] Any aspects, embodiments and examples of the present disclosure which do not fall under the scope of the appended claims do not form part of the invention and are merely provided for illustrative purposes.

[0011] Described herein is 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.

[0012] Also described herein is 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.

[0013] In one aspect of the invention, there is provided a viral particle comprising a recombinant genome comprising the polynucleotide of the invention.

[0014] In another aspect of the invention, there is provided a composition comprising the polynucleotide or viral particle of the invention and a pharmaceutically acceptable excipient.

[0015] Also described herein is a method of treatment comprising administering an effective amount of the polynucleotide or viral particle of the invention to a patient.

[0016] Also described herein is a use of the polynucleotide, viral particle or composition of the invention in the manufacture of a medicament for use in a method of treatment.

[0017] Also described herein is the use of the polynucleotide, viral particle or composition of the invention in the manufacture of a medicament for achieving a stable GCase activity in a subj ect.

[0018] Also described herein is the use of the polynucleotide, viral particle or composition of the invention 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.

[0019] Also described herein is a method of achieving a stable GCase activity in a subject by administering to the subject the polynucleotide, viral particle or composition of the invention.

[0020] Also described herein is 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 invention, wherein the bioavailability is measured over a period of 2 weeks from administration.

[0021] In another aspect of the invention, there is provided a polynucleotide, viral particle or composition of the invention, for use in a method of expressing the GBA nucleotide sequence and achieving a stable GCase activity in a subject.

[0022] In another aspect of the invention, there is provided a polynucleotide, viral particle or composition of the invention, for use in a method of treatment wherein the method comprises 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.

[0023] Also described herein is the use of the polynucleotide, viral particle or composition of the invention 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.

[0024] Also described herein is 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 invention.

[0025] In another aspect of the invention, there is provided a polynucleotide, viral particle or composition of the invention, 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

[0026]

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 injection of tested GBA constructs. (B) GCase activity levels observed in mouse serum at 4-, 8-, 12-, and 36-weeks post-injection of constructs FLF-PL01 and FLF-PL28. Error bars represent mean ± SD, n = 5-8 animals per experimental group. * $p \leq 0.05$; ** $p \leq 0.001$ (one-way ANOVA).

Figure 5 - Uptake levels of GCase in spleen and bone marrow tissue following AAV2/8-FLF-PL28 injection in wild type mice. Representative image of spleen and bone marrow tissue stained for GBA is shown for naive or AAV2/8-PL28 treatment mice at 4-weeks post-injection. DAB (3,3'-Diaminobenzidine) was used to visualize GBA and haematoxylin was used as counterstain.

Figure 6 - Levels of co-localization of human GCase with canonical murine macrophage marker F4/80 observed in spleen upon injection of wild type mice with AAV2/8-FLF-PL28. Representative immunofluorescence image of spleen tissue stained for GBA and F4/80 antibodies. DAPI (Blue) was used to visualise nuclei.

Figure 7 - Levels of GCase activity found in mouse bloodstream 4 weeks post-injection of AAV2/8- FLF-PL28 and FLF-PL64. GCase activity was determined for mouse serum collected 4-weeks post-injection at the dose of 2×10^{12} vg/kg. Error bars represents mean ± SD. N = 5, C57BL/6 mice in each treatment group.

Figure 8 - Uptake levels observed in spleen, bone marrow and lung following AAV2/8-FLF-PL28 and FLF-PL64 injection observed in mice 5-weeks post treatment.

Figure 9 - Sequence listing.

Figure 10 - Levels of GCase secretion by human-derived cell lines following transduction with AAV-FLF-PL64. Cells were transduced at a MOI of 1×10^{5} vg/cell with the vector AAV-FLF-PL64. (A) Levels of active GBA were determined fluorometrically with 4MU-Glc as the substrate. (B) The level of transduction for each cell line was obtained by qPCR using primers specific for the polyA sequence. Blank values for each cell line were subtracted to obtain a value for the level of active GCase. Error bars represent mean ± SD of duplicate wells.

Figure 11: (A) Enzyme replacement therapy (VPRIV ® (60 U/kg)) pharmacokinetics and half-life calculation in wild type mice. One-phase decay model equation: Y0 is the Y-value when X (time) is zero. Plateau is the Y-value at infinite times. K is the rate constant. Tau is the time constant. Half-life is in the time units of the X-axis. Span is the difference between Y0 and Plateau. (B) Comparison between serum pharmacokinetic profile of GCase activity after a single injection of enzyme replacement therapy (VPRIV (60 U/kg), solid black) and gene therapy with FLF-PL64, following administration in wild type mice.

Figure 12: GCase immunostaining in murine liver, spleen and bone following administration of VPRIV or FLF-PL64. DAB (3,3'-Diaminobenzidine) was used to visualise GCase and haematoxylin was used as counterstain. FLF-PL64 samples were obtained at five weeks post-injection, while VPRIV treated samples were collected at the time labelled. Each image

represents n=5, C57BL/6 mice for each treatment group. All pictures are at the same magnification.

Figure 13: Increase in GCase activity observed in $gba^{9v/null}$ mouse liver (a), white blood cells (b), spleen (c), and bone marrow (d) upon administration of velaglucerase alfa (VPRIV ®) (labelled ERT) or AAV-GBA (AAV-FLF-PL64). ERT samples were collected 1-2 hours post the last injection corresponding to peak of uptake in tissues. AAV-GBA (AAV-FLF-PL64) samples were collected 12 weeks post-injection and corresponding to steady state levels of uptake. GCase activity is represented as a percentage of the activity measured in wild-type healthy mice (at 20 weeks of age). All mice were treated at age of 8-week pre-overt symptomatology. ERT at dose 60 U/kg and administered by injection every two weeks; AAV-FLF-PL64 injected at dose of 2×10^{12} vg/kg. n=10. **** $P \leq 0.0001$

Figure 14: AAV-GBA (AAV-FLF-PL64) gene therapy reduces activated macrophages and inflammation in the liver of $gba^{9v/null}$ mice. Upper panel: H&E stained liver sections showing a representative image from each group. Storage cells are identified by circles. Lower left panel: graph showing comparison between storage cells counted in AAV-FLF-PL64 and ERT treated groups compared to vehicle control groups. Lower right panel: graph showing CD68positive cells counted in AAV-FLF-PL64 and ERT treated groups compared to vehicle control groups following staining with anti-CD68 antibody. AAV-GBA (AAV-FLF-PL64) injected at dose of 2×10^{12} vg/kg; samples collected 12-weeks p.i. ERT at dose 60 U/kg and administered by injection every two weeks. ERT samples were collected 1-2 hours post the last injection. Mean ± SEM, (n=10),** $P\leq0.005$,**** $P\leq 0.0005$

Figure 15: AAV-GBA (AAV-FLF-PL64) gene therapy shows better substrate clearance than velaglucerase alfa (VPRIV ®, labelled ERT) in $gba^{9v/null}$ mice. LC/MS analysis of hexosylceramide and hexosylsphingosine levels in liver, spleen, and bone marrow in AAV-FLF-PL64 and ERT treated groups. Levels were standardised to the levels measured in the vehicle control group. AAV-GBA (AAV-FLF-PL64) injected at dose of 2×10^{12} vg/kg; samples collected 12-weeks p.i.; ERT at dose 60 U/kg and administered by injection every two weeks. ERT samples were collected 1-2 hours post the last injection. Mean ±SEM, (n=10),** $P\leq0.005$, **** $P\leq 0.0005$

Detailed Description

General definitions

[0027] Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by a person skilled in the art to which this invention

belongs.

[0028] In general, the term "*comprising*" is intended to mean including but not limited to. For example, the phrase "*a polynucleotide comprising a GBA nucleotide sequence* " should be interpreted to mean that the polynucleotide has a GBA nucleotide sequence, but the polynucleotide may contain additional nucleotides.

[0029] In some embodiments of the invention, the word "*comprising*" is replaced with the phrase "*consisting of*". The term "*consisting of*" is intended to be limiting. For example, the phrase "*a polynucleotide consisting of a GBA nucleotide sequence*" should be understood to mean that the polynucleotide has a GBA nucleotide sequence and no additional nucleotides.

[0030] As used herein, "*between*" when referring to two endpoints to define a range of values should be taken to mean "*between and including*". Thus, a range defined as "*between 5 and 10*" includes all values greater than 5 and less than 10, as well as the discrete values 5 and 10 themselves.

[0031] The terms "*protein*" and "*polypeptide*" are used interchangeably herein, and are intended to refer to a polymeric chain of amino acids of any length.

[0032] For the purpose of this invention, in order to determine the percent identity of two sequences (such as two polynucleotide or two polypeptide sequences), the sequences are aligned for optimal comparison purposes (*e.g.*, gaps can be introduced in a first sequence for optimal alignment with a second sequence). The nucleotide or amino acid residues at each position are then compared. When a position in the first sequence is occupied by the same nucleotide or amino acid residue as the corresponding position in the second sequence, then the nucleotides or amino acids are identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences (*i.e.*, % identity = number of identical positions /total number of positions in the reference sequence × 100).

[0033] Typically the sequence comparison is carried out over the length of the reference sequence. For example, if the user wished to determine whether a given ("test") sequence is 95% identical to SEQ ID NO: 1, SEQ ID NO: 1 would be the reference sequence. For example, to assess whether a sequence is at least 80% identical to SEQ ID NO: 1 (an example of a reference sequence), the skilled person would carry out an alignment over the length of SEQ ID NO: 1, and identify how many positions in the test sequence were identical to those of SEQ ID NO: 1. If at least 80% of the positions are identical, the test sequence is at least 80% identical to SEQ ID NO: 1. If the sequence is shorter than SEQ ID NO: 1, the gaps or missing positions should be considered to be non-identical positions.

[0034] The skilled person is aware of different computer programs that are available to determine the homology or identity between two sequences. For instance, a comparison of sequences and determination of percent identity between two sequences can be accomplished

using a mathematical algorithm. In an embodiment, the percent identity between two amino acid or nucleic acid sequences is determined using the Needleman and Wunsch (1970) algorithm which has been incorporated into the GAP program in the Accelrys GCG software package (available at http://www.accelrys.com/products/gcg/), using either a Blosum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6.

[0035] For the purposes of the present invention, the term *"fragment"* refers to a contiguous portion of a sequence. For example, a fragment of SEQ ID NO: 1 of 50 nucleotides refers to 50 contiguous nucleotides of SEQ ID NO: 1.

A polynucleotide

[0036] In one aspect, the present invention provides 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.

[0037] The polynucleotide may further comprise one or more of the following features. The GBA nucleotide sequence, or portion of GBA nucleotide sequence that is not wild type, may be codon-optimised. The polynucleotide may (additionally) comprise a portion that is not codon-optimised. The polynucleotide may comprise an intron or a fragment of an intron.

[0038] The term "*polynucleotide*" refers to a polymeric form of nucleotides of any length, deoxyribonucleotides, ribonucleotides, or analogs thereof. For example, the polynucleotide may comprise DNA (deoxyribonucleotides) or RNA (ribonucleotides). The polynucleotide may consist of DNA. The polynucleotide may be mRNA. Since the polynucleotide may comprise RNA or DNA, all references to T (thymine) nucleotides may be replaced with U (uracil).

A GBA nucleotide sequence encoding GCase

[0039] In one aspect, the polynucleotide provided herein comprises a GBA nucleotide sequence. The GBA nucleotide sequence typically encodes the β-Glucocerebrosidase (GCase) protein or fragment thereof.

[0040] The term *"sequence that encodes"* refers to a nucleotide sequence comprising an open reading frame comprising codons that encode the encoded polypeptide. For example, a nucleotide sequence that encodes a GCase protein or fragment thereof comprises codons that encode the amino acid sequence of a GCase protein or fragment thereof. An example of a GBA nucleotide sequence that encodes a wild type GCase protein is provided in SEQ ID NO: 9.

[0041] A GBA nucleotide sequence may be interrupted by non-coding nucleotides (*e.g.* an intron), but only nucleotides that encode the polypeptide should be considered to be part of the GBA nucleotide sequence. For example, a GBA nucleotide sequence that encodes a GCase protein will comprise any codons that encode an amino acid forming part of the GCase protein that is expressed from that coding sequence, irrespective of whether those codons are contiguous in sequence or separated by one or more non-coding nucleotides. In other words, a GBA polynucleotide which contains stretches of coding nucleotides interrupted by a stretch of non-coding nucleotides will be considered to comprise a "*GBA nucleotide sequence*" consisting of the non-contiguous coding stretches immediately juxtaposed (*i.e.* minus the non-coding stretch). However, herein, the stop codon will be considered to be part of the full length coding sequence.

[0042] A GBA nucleotide sequence encoding GCase and/or a GCase coding sequence as described herein may also include codons for a signal peptide. It is well known that some proteins, particularly those which are exported to different tissues, are expressed with a signal peptide. Signal peptides can be at the N-terminus of a protein sequence (and in this case at the 5' end of a coding sequence) and many signal peptides are cleaved following cellular processing. Thus, herein, a mature protein or polypeptide (such as a mature GCase protein or polypeptide) will be considered to be the resulting protein or polypeptide after the signal peptide has been processed and removed/cleaved (and thus no longer forms part of the polypeptide sequence).

[0043] The following Table describes codons that encode each amino acid:

Amino Acid	Codon	Amino Acid	Codon	Amino Acid	Codon
Phenylalanine	TTC TTT	Proline	CCT CCC CCA CCG	Asparagine	AAT AAC
Leucine	TTA TTG CTT CTC CTA CTG	Threonine	ACT ACC ACA ACG	Lysine	AAA AAG
Isoleucine	ATT ATC ATA	Alanine	GCT GCC GCA GCG	Aspartic Acid	GAT GAC
Methionine	ATG	Tyrosine	TAT TAC	Glutamic Acid	GAA GAG

Amino Acid	Codon	Amino Acid	Codon	Amino Acid	Codon
Valine	GTT GTC GTA GTG	Histidine	CAT CAC	Cysteine	TGT TGC
Serine	TCT TCC TCA TCG AGT AGC	Glutamine	CAA CAG	Tryptophan	TGG
Arginine	CGT CGC CGA CGG AGA AGG	Glycine	GGT GGC GGA GGG		

[0044] The corresponding RNA codons will contain Us in place of the Ts in the Table above.

[0045] Described herein is 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 any one of SEQ ID NO: 1-8. Optionally, all or a portion of the GBA nucleotide sequence is codon-optimised. The GBA nucleotide sequence may comprise a sequence that is at least 98% identical to a fragment of at least 1300 nucleotides of SEQ ID NO: 1-8. The GBA nucleotide sequence may comprise a sequence that is at least 99% identical to a fragment of at least 1300 nucleotides of SEQ ID NO: 1-8.

[0046] In one example, the GBA nucleotide sequence may comprise a sequence that is at least 95%, at least 98%, at least 99%, at least 99.5%, at least 99.8%, or 100% identical to SEQ ID NO: 1. In one embodiment, the GBA nucleotide sequence comprises a sequence that is at least 98% identical to a fragment of at least 1300 nucleotides of SEQ ID NO: 1. In one embodiment, the GBA nucleotide sequence comprises a sequence that is at least 99% identical to a fragment of at least 1300 nucleotides of SEQ ID NO: 1. The GBA nucleotide sequence may comprise a sequence that is at least 95%, at least 98%, at least 99%, at least 99.5%, at least 99.8%, or 100% identical to SEQ ID NO: 5. The GBA nucleotide sequence may

comprise a sequence that is at least 98% identical SEQ ID NO: 1. The GBA nucleotide sequence may comprise a sequence that is at least 99% identical SEQ ID NO: 1. The GBA nucleotide sequence may comprise a sequence that is at least 98% identical SEQ ID NO: 5. The GBA nucleotide sequence may comprise a sequence that is at least 99% identical SEQ ID NO: 5. In one embodiment, the GBA nucleotide sequence may comprise SEQ ID NO: 1. In another embodiment, the GBA nucleotide sequence may comprise SEQ ID NO: 5.

[0047] The GBA nucleotide sequence may comprise a sequence of SEQ ID NO: 1 or a variant of SEQ ID NO: 1 encoding a GCase protein having GCase activity. In these examples, a variant of SEQ ID NO: 1 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. In these examples, the variant of SEQ ID NO: 1 may have 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. The variant of SEQ ID NO: 1 may have 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. In one example, the variant of SEQ ID NO: 1 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. In one example, the variant of SEQ ID NO: 1 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. In one example, the variant of SEQ ID NO: 1 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.

[0048] The GBA nucleotide sequence may comprise a sequence of SEQ ID NO: 5 or a variant of SEQ ID NO: 5 encoding a GCase protein having GCase activity. In these examples, a variant of SEQ ID NO: 5 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. In these examples, the variant of SEQ ID NO: 5 may have 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. The variant of SEQ ID NO: 5 may have 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. In one example, the variant of SEQ ID NO: 5 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. In one example, the variant of SEQ ID NO: 5 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. In one example, the variant of SEQ ID NO: 5 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.

GCase protein or fragment thereof

[0049] The polynucleotide comprises a GBA nucleotide sequence that encodes a GCase protein or fragment thereof.

[0050] β-glucocerebrosidase (GCase) is an enzyme with glucosylceramidase activity (EC 3.2.1.45) that hydrolyses the beta-glucosidic linkage of the chemical glucocerebroside, an intermediate in glycolipid metabolism that is abundant in cell membranes. Mutations in the GBA gene (which encodes GCase) can lead to an accumulation of glucocerebrosides in macrophages that infiltrate many vital organs, which manifests as Gaucher disease (GD). A typical wild type GCase polypeptide is encoded by SEQ ID NO: 9.

[0051] GCase (*e.g.* a GCase of SEQ ID NO: 25 encoded by SEQ ID NO: 9) is initially expressed as a precursor "*immature*" form, comprising a signal peptide (amino acid residues 1 to 39 of SEQ ID NO: 25 and codons 1 to 39 of SEQ ID NO: 9), and a mature GCase polypeptide region. After processing, the "*mature*" form of GCase lacks the signal peptide. The term "*mature GCase* " or "*mature GCase polypeptide*" refers to a GCase polypeptide that does not comprise the signal peptide, such as a GCase encoded by SEQ ID NOs: 1-4. A typical GCase signal peptide may be encoded by a nucleotide sequence of SEQ ID NO: 17 and have the polypeptide sequence of SEQ ID NO: 18.

[0052] The GCase or fragment thereof may be a variant GCase or fragment thereof, *i.e.* a GCase that does not have a sequence identical to SEQ ID NO: 25. In an embodiment, the GCase or fragment thereof that is encoded by a polypeptide of the present invention and/or by a GBA nucleotide sequence is at least 95%, at least 98%, at least 99%, at least 99.5%, at least 99.8%, or 100% identical to SEQ ID NO: 25; or at least 95%, at least 98%, at least 99%, at least 99.5%, at least 99.8%, or 100% identical to a fragment of SEQ ID NO: 25 at least 300, at least 350, at least 400, less than or equal to 536, less than or equal to 497, between 300 and 536, or between 300 and 497 amino acids in length. In an embodiment, the GCase protein or fragment thereof is at least 95%, at least 98%, at least 99%, at least 99.5%, at least 99.8%, or 100% identical to SEQ ID NO: 25; or at least 95%, at least 98%, at least 99%, at least 99.5%, at least 99.8%, or 100% identical to a fragment of SEQ ID NO: 25 about 497 amino acids in length. The GCase protein or fragment thereof may have a sequence of SEQ ID NO: 25. Preferably the GCase protein or a fragment thereof does not comprise the signal peptide of SEQ ID NO: 18. Preferably the GCase protein or fragment thereof is functional. A functional GCase protein or fragment is one which carries out hydrolysis of glucocerebroside.

[0053] The GBA nucleotide sequence may encode a GCase protein having 1, up to 2, up to 3, up to 4, or up to 5 amino acid substitutions relative to the wild type GCase amino acid sequence of SEQ ID NO: 25. In such examples, the GBA nucleotide sequence may encode a

GCase protein having up to 3 amino acid substitutions relative to the wild type GCase amino acid sequence of SEQ ID NO: 25. The GBA nucleotide sequence may encode a GCase protein having up to 2 amino acid substitutions relative to the wild type GCase amino acid sequence of SEQ ID NO: 25. The GBA nucleotide sequence may encode a variant GCase protein having up to 1 amino acid substitution relative to the wild type GCase amino acid sequence of SEQ ID NO: 25.

[0054] It is within the abilities of the person skilled in the art to determine whether a GCase protein or fragment encoded by a GBA nucleotide sequence is functional. The skilled person merely needs to express the GCase nucleotide sequence, and test whether the expressed protein is active. For example, the skilled person could prepare a viral particle of the invention comprising a GBA nucleotide sequence linked to an operable promoter, and transduce cells with the viral particle under conditions suitable for expression of the GCase protein or fragment thereof. The activity (amount) of the expressed GCase protein or fragment thereof can be analysed using a a fluorometric, assay, such as the "*serum GBA activity assay*" described in Example 1.

[0055] For example, a suitable fluorogenic assay is as follows. β-Glucocerebrosidase (acid β-glucosidase; GCase) activity can be determined fluorometrically with 4-Methylumbelliferyl-β-D-glucopyranoside (4MU-Glc) as a substrate. Briefly, serum samples (0.5 µL, diluted 1:50) can be assayed in 50 mM Sodium Citrate, 25 mM Taurocholate, pH -5.75, 6 mM 4MU-Glc, for 30 min at 37°C. Relative fluorescence levels (RFU) may then be evaluated using excitation and emission wavelengths of 365 nm and 445 nm, respectively. GCase is expressed as nanomoles/h/ mL of serum based on a 4-Methylumbelliferone (4-MU) standard curve.

A portion of the GBA nucleotide sequence is not wild type

[0056] A portion of the GBA nucleotide sequence, for example the coding sequence that encodes GCase protein or a fragment thereof, may not be wild type. The wild type GCase-encoding GBA nucleotide sequence is represented by SEQ ID NO: 9, and a GBA nucleotide sequence that comprises a portion differing in sequence from that of SEQ ID NO: 9 comprises a portion that is not wild type.

[0057] In an embodiment, the portion of the GBA nucleotide sequence that is not wild type is codon-optimised. Codon-optimisation can improve expression of the nucleotide sequence, for example a GBA nucleotide sequence, in a particular tissue and/or in a particular organism. For example, if a nucleotide sequence is codon-optimised for expression in the human liver, the nucleotide sequence is modified to increase the number of codons that may be favoured (in the sense that such codons correspond to tRNA species which are more abundant than other tRNA species specific for the same amino acid) in the human liver. The skilled person would appreciate that codon-optimising a sequence may not entail changing every codon, not least because a "*favoured codon*" may already be present at some positions.

[0058] Such codon-optimisation may be subject to other factors. For example, it can be seen that the presence of CpGs has an adverse effect on expression and so the user may decide not to use favoured codons at positions where doing so introduces CpGs into the sequence; this will still be considered to be codon-optimisation. In an embodiment, a favoured codon that ends with a C nucleotide will not be included in the portion of the coding sequence that is codon-optimised, where the next codon in the sequence begins with a G. For example, codon CTC encodes leucine. In schemes where CTC is a favoured codon, it should not be used for encoding leucine where the next codon in the sequence begins with a G, such as codon GTT (or alternatively, the next codon - where possible - could be selected to avoid a G at the first position).

[0059] It is straightforward to determine the frequency of each codon used in a portion of a nucleotide sequence. The skilled person merely needs to enter the sequence of that portion into one of the readily-available algorithms that looks at codon usage and review the results. Alternatively, the user could simply count them.

[0060] In one embodiment, a polynucleotide of the invention comprises a GBA nucleotide sequence wherein 67% of the codons that encode histidine are CAC and 33% of the codons that encode histidine are CAT.

[0061] Optionally, the portion of the GBA nucleotide sequence that is codon-optimised is codon-optimised for expression in human liver cells. Optionally, the GBA nucleotide sequence is codon-optimised for expression in the human liver. Optionally, the portion of the GBA nucleotide sequence that is codon-optimised is a contiguous portion.

[0062] The portion that is codon-optimised can correspond to a sequence encoding part of, or an entire, GCase protein. For example, the coding sequence could be full-length (such as SEQ ID NO: 9), including the signal peptide which is not part of the mature GCase protein, and the entire coding sequence could be codon-optimised. Hence, reference herein to *"a portion of the GBA sequence is codon-optimised"* should be understood to mean *"at least a portion of the GBA sequence is codon-optimised".* Optionally, the portion of the GBA nucleotide sequence that is codon-optimised is at least 1000, at least 1200, at least 1300, less than 1600, less than 1500, between 1000 and 1600, between 1000 and 1500, between 1300 and 1500, or around 1494 nucleotides in length. Optionally, the portion of the GBA nucleotide sequence that is codon-optimised encodes (corresponds to) a mature GCase protein. For example, the GBA nucleotide sequence may encode a precursor GCase protein (*i.e.* including signal peptide), and if the portion of the GBA nucleotide sequence that is codon-optimised corresponds to the mature GCase protein, the signal peptide is not codon-optimised.

[0063] Thus in some embodiments, a portion of the GBA nucleotide sequence may not be codon-optimised, for example a portion of the coding sequence is not codon-optimised for expression in the liver. In some embodiments, 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. In some embodiments, the portion that is not codon-optimised in a

GBA nucleotide sequence is the portion which encodes the signal peptide.

[0064] As discussed above, providing a polynucleotide sequence comprising a GBA nucleotide sequence that is partially or wholly codon-optimised can ensure that the encoded polypeptide (*i.e.* a GCase polypeptide) is expressed at a high level. It will be appreciated by one skilled in the art that expression of GCase from a polynucleotide sequence, such as a GBA nucleotide sequence of the present invention, or from a viral particle of the present invention, generally requires the presence of a promoter sequence or region upstream of and/or operably linked to the polynucleotide sequence. Thus in one embodiment, the present invention provides a polynucleotide comprising a GBA nucleotide sequence, wherein the GBA nucleotide sequence encodes a GCase polypeptide which is expressed in human liver cells at high levels when the GBA nucleotide sequence is operably linked to a promoter sequence. In some embodiments, the promoter sequence may be part of a transcriptional regulatory element. In some embodiments, the promoter sequence may be a liver-specific promoter sequence. In one embodiment, the promoter sequence is a promoter having SEQ ID NO: 12. In another embodiment, the promoter sequence is a promoter having SEQ ID NO: 15.

[0065] It will also be appreciated by one skilled in the art that making comparisons between polynucleotides or vectors of the invention and reference (comparator) polynucleotides or vectors such as a reference polynucleotide or a viral particle comprising a GBA nucleotide sequence of SEQ ID NO: 9, the reference polynucleotides or vectors may be identical to the polynucleotides or vectors of the invention except that the GBA nucleotide sequences are different. In other words, the different GBA nucleotide sequences being compared may be operably linked to the same promoter sequence. In some embodiments, the different GBA nucleotide sequences being tested may be operably linked to different (specified) promoter sequences.

[0066] Thus, in one embodiment, a GCase polypeptide encoded by the GBA nucleotide sequence is expressed in human liver cells at higher levels compared to a reference wild type GBA sequence. The reference wild type GBA nucleotide sequence may be SEQ ID NO: 9. In an embodiment, a polypeptide encoded by the GBA nucleotide sequence is expressed in human liver cells at higher levels compared to a polypeptide encoded by a nucleotide sequence comprising a GBA nucleotide sequence of SEQ ID NO: 9 and a promoter element of SEQ ID NO: 13 (wherein the GBA nucleotide sequence of SEQ ID NO: 9 and the promoter element of SEQ ID NO: 13 are preferably operably linked). In an embodiment, a polypeptide encoded by the GBA nucleotide sequence is expressed in human liver cells at higher levels compared to a polypeptide encoded by a nucleotide sequence comprising a GBA nucleotide sequence of SEQ ID NO: 9 and a transcription regulatory element of SEQ ID NO: 10 (wherein the GBA nucleotide sequence of SEQ ID NO: 9 and the promoter element of SEQ ID NO: 10 are preferably operably linked). In such embodiments the GCase encoded by the GBA nucleotide sequence may be 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. In an embodiment, a GCase polypeptide encoded by the GBA nucleotide sequence is expressed in human liver cells at higher or non-statistically significant different levels compared to a polypeptide encoded by an otherwise identical

reference polynucleotide comprising a GBA nucleotide sequence of SEQ ID NO: 9 operably linked to a promoter of SEQ ID NO: 13, wherein the two polynucleotides are delivered to the cells in the same way and in the same amounts.

[0067] In one embodiment, when the polynucleotide sequence comprising a GBA nucleotide sequence is administered to a subject, or a non-human mammal such as a mouse, the GCase is present in the serum of the subject or non-human animal at higher levels (for example, at 4 or 8 or 12 weeks post-administration) compared to GCase encoded by an otherwise identical nucleotide sequence comprising a GBA nucleotide sequence of SEQ ID NO: 9 operably linked to a promoter element of SEQ ID NO: 12, 13 or 15, wherein the polynucleotides comprising the GBA nucleotide are administered in the same way and in the same amounts.

[0068] The skilled person may determine whether GCase is expressed from a given GBA nucleotide sequence (for example, a codon-optimised GBA nucleotide sequence) at higher levels compared to a reference sequence (for example, a wild type GBA nucleotide sequence, such as SEQ ID NO: 9) by transducing some cells with a viral particle comprising the GBA nucleotide sequence, and some cells with a particle comprising the reference sequence. The cells may be cultured under conditions suitable for expressing the GCase protein or fragment thereof encoded by the GBA nucleotide sequence, and the level of expressed GCase protein can be compared. The level of expressed GCase protein can be assessed using a fluorometric assay as described in the section entitled "*GCase protein or fragment thereof*", or an ELISA using a GCase-specific antibody. Suitable cells include cultured human liver cells, such as Huh-7 cells.

[0069] As discussed above, the presence of CpGs (*i.e.* CG dinucleotides) may reduce expression efficiency. This is because CpGs may be methylated, and their methylation may lead to gene silencing thereby reducing expression. Also, it is possible that high CpG content could trigger a TLR response, increasing the risk of an anti-AAV immune response. For this reason, it is preferred that the portion of the coding sequence that is codon-optimised comprises a reduced number of CpGs compared to a corresponding portion of a reference wild type GBA nucleotide sequence (such as SEQ ID NO: 9). In an embodiment, the portion of the GBA nucleotide sequence that is codon-optimised (which may be all of the GBA nucleotide sequence) comprises less than 40, less than 20, less than 10, or less than 5 CpGs. In an embodiment, the portion of the GBA nucleotide sequence that is codon-optimised (which may be all of the GBA nucleotide sequence) comprises less than 5, less than 4, less than 3, or less than 2 CpGs per 100 nts. In some embodiments, the portion of the coding sequence that is codon-optimised is CpG-free, *i.e.* contains no (0) CG dinucleotides.

[0070] In an embodiment, 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 SEQ ID NO: 1-4. In an embodiment, the portion of the coding 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 SEQ ID NO: 1-4. In an embodiment, 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 SEQ ID NO: 1. In an embodiment, the portion of the GBA nucleotide sequence that is codon-optimised is at least 99.5%, at least 99.8%, or 100% identical to a fragment of at least 1300 nucleotides of SEQ ID NO: 1. In an embodiment, the portion of the coding 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 SEQ ID NO: 1.

[0071] The present invention provides a polynucleotide comprising a GBA nucleotide sequence that encodes a GCase protein or a fragment thereof and the GBA sequence comprises a sequence that is at least 95%, at least 98%, at least 99%, at least 99.5%, at least 99.8%, or 100% identical to SEQ ID NO: 1. Optionally, the sequence that is at least 95%, at least 98%, at least 99%, at least 99.5%, or at least 99.8% identical to SEQ ID NO: 1 is codon-optimised.

Portion of the coding sequence that is not codon-optimised

[0072] In an embodiment, the GBA nucleotide sequence comprises a portion that is not codon-optimised. The portion that is not codon-optimised may be a contiguous portion.

[0073] As would be understood in the art, the portion that is not codon-optimised is therefore not modified to include a greater number of favoured codons compared to the wild type sequence. A contiguous non-codon-optimised polynucleotide sequence is a wild type sequence.

[0074] Optionally, 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. In some embodiments, the portion that is not codon-optimised in a GBA nucleotide sequence is the portion which encodes (corresponds to) all or a portion of the signal peptide. Optionally, the portion that is not codon-optimised encodes all or a portion of a GCase signal peptide. In some embodiments, the portion that is not codon-optimised in a GBA nucleotide sequence is a portion having a sequence of SEQ ID NO: 17.

The polynucleotide may further comprise a transcription regulatory element

[0075] The polynucleotide may comprise a transcription regulatory element.

[0076] In one embodiment, 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: 10. In an embodiment, the polynucleotide comprises a transcription regulatory element that is at least 98%, at least 99%, at least 99.5%, at least 99.8%, or 100% identical to SEQ ID NO: 10. Optionally, the polynucleotide comprises a transcription regulatory element at least 98% identical to SEQ ID NO: 10. Optionally, the polynucleotide comprises a transcription regulatory element of SEQ ID NO: 10. Optionally, the polynucleotide comprises a transcription regulatory element consisting of SEQ ID NO: 10.

[0077] In another embodiment, 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. In an embodiment, the polynucleotide comprises a transcription regulatory element that is at least 98%, at least 99%, at least 99.5%, at least 99.8%, or 100% identical to SEQ ID NO: 14. Optionally, the polynucleotide comprises a transcription regulatory element at least 98% identical to SEQ ID NO: 14. Optionally, the polynucleotide comprises a transcription regulatory element of SEQ ID NO: 14. Optionally, the polynucleotide comprises a transcription regulatory element consisting of SEQ ID NO: 14.

[0078] Any appropriate transcription regulatory element may be used, such as HLP2, HLP1, LP1, HCR-hAAT, ApoE-hAAT, and LSP, which are all liver-specific transcription regulatory elements. These transcription regulatory elements are described in more detail in the following references: HLP1: McIntosh J. et al., Blood 2013 Apr 25, 121(17):3335-44; LP1: Nathwani et al., Blood. 2006 April 1, 107(7): 2653-2661; HCR-hAAT: Miao et al., Mol Ther. 2000;1: 522-532; ApoE-hAAT: Okuyama et al., Human Gene Therapy, 7, 637-645 (1996); and LSP: Wang et al., Proc Natl Acad Sci USA. 1999 March 30, 96(7): 3906-3910.

[0079] The transcription regulatory element may comprise a promoter and/or an enhancer, such as the promoter element and/or enhancer element from HLP2, HLP1, LP1, HCR-hAAT, ApoE-hAAT, and LSP. Each of these transcription regulatory elements comprises a promoter, an enhancer, and optionally other nucleotides.

[0080] In an embodiment, the transcription regulatory element comprises an enhancer which is the human apolipoprotein E (ApoE) hepatic locus control region (HCR; Miao et al (2000), Molecular Therapy 1(6):522), or a fragment thereof. In an embodiment, the transcription regulatory element comprises a fragment of the HCR enhancer which 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. Optionally, the fragment of the HCR enhancer is between 100 and 250 nucleotides in length. In another embodiment, 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. Optionally, the fragment of the HCR enhancer is between 250 and 340 nucleotides in length.

[0081] Suitable HCR enhancer element fragment are described in SEQ ID NOs: 11 and 16. Optionally, the transcription regulatory element comprises an enhancer that is 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 and the enhancer comprises a polynucleotide sequence 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 SEQ ID NO: 11. Optionally, the transcription regulatory element comprises an enhancer that is between 117 and 192 nucleotides in length and the enhancer comprises a polynucleotide sequence that is at least 98%, at least 99%, at least 99.5%, at least 99.8%, or 100% identical SEQ ID NO. 11. Optionally, the transcription regulatory element 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 a fragment of at least 90, at least 100, or at least 110 nucleotides of SEQ ID NO: 11. Optionally, 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: 11. Optionally, the polynucleotide comprises an enhancer that is at least 98%, at least 99%, at least 99.5%, at least 99.8%, or 100% identical to SEQ ID NO: 11. Optionally, the polynucleotide comprises an enhancer of SEQ ID NO: 11. Optionally, the transcription regulatory element comprises a fragment of an HCR enhancer that is equal to or less than 321 nucleotides, equal to or less than 192 nucleotides or equal to or less than 117 nucleotides in length and comprises SEQ ID NO: 11.

[0082] In another embodiment, the transcription regulatory element comprises an enhancer that is 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 318 nucleotides in length and the enhancer comprises a polynucleotide sequence 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 SEQ ID NO: 16. Optionally, the transcription regulatory element comprises an enhancer that is between 250 and 340 nucleotides in length and the enhancer comprises a polynucleotide sequence that is at least 98%, at least 99%, at least 99.5%, at least 99.8%, or 100% identical SEQ ID NO: 16. Optionally, the transcription regulatory element 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 a fragment of at least 250 nucleotides of SEQ ID NO: 16. Optionally, 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. Optionally, the polynucleotide comprises an enhancer that is at least 98%, at least 99%, at least 99.5%, at least 99.8%, or 100% identical to SEQ ID NO: 16. Optionally, the polynucleotide comprises an enhancer of SEQ ID NO: 16.

[0083] In an embodiment, the transcription regulatory element comprises a promoter which is a human alpha-1 anti-trypsin promoter (A1AT; Miao et al (2000), Molecular Therapy 1(6):522), or a fragment thereof. Optionally, a fragment of an A1AT promoter which 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. Optionally, the fragment of an A1AT promoter is between 150 and 300 nucleotides in length. In another embodiment, a fragment of an A1AT promoter which at least 200, at least 250, at least 300, less than 500, between 200 and 500, between 250 and 500, or between 350 and 450

nucleotides in length. Optionally, the fragment of an A1AT promoter is between 350 and 450 nucleotides in length.

[0084] Suitable A1AT promoter fragments are described in SEQ ID NOs: 12 and 15. Optionally, the transcription regulatory element comprises a promoter that is at least 100, at least 120, at least 150, at least 180, less than 255, between 100 and 255, between 150 and 300, or between 180 and 255 nucleotides in length and the promoter comprises a polynucleotide sequence 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: 12. Optionally, the transcription regulatory element comprises a promoter that is between 180 and 255 nucleotides in length and the promoter comprises a polynucleotide sequence that is at least 98%, at least 99%, at least 99.5%, at least 99.8%, or 100% identical to SEQ ID NO: 12. Optionally, 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 a fragment of at least 100, at least 120, or at least 150 nucleotides of SEQ ID NO: 12. Optionally, 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: 12. Optionally, the polynucleotide comprises a promoter that is at least 98%, at least 99%, at least 99.5%, at least 99.8%, or 100% identical to SEQ ID NO: 12. Optionally, the polynucleotide comprises a promoter of SEQ ID NO: 12. Optionally, the transcription regulatory element comprises a fragment of an A1AT promoter that is equal to or less than 418 nucleotides, equal to or less than 255 nucleotides or equal to or less than 185 nucleotides in length and comprises SEQ ID NO: 12.

[0085] In another embodiment, the transcription regulatory element comprises a promoter that 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 and the promoter comprises a polynucleotide sequence 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. Optionally, the transcription regulatory element comprises a promoter that is between 350 and 450 nucleotides in length and the promoter comprises a polynucleotide sequence that is at least 98%, at least 99%, at least 99.5%, at least 99.8%, or 100% identical to SEQ ID NO: 15. Optionally, 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 a fragment of at least 350 nucleotides of SEQ ID NO: 15. Optionally, 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. Optionally, the polynucleotide comprises a promoter that is at least 98%, at least 99%, at least 99.5%, at least 99.8%, or 100% identical to SEQ ID NO: 15. Optionally, the polynucleotide comprises a promoter of SEQ ID NO: 15.

[0086] If the polynucleotide is intended for expression in the liver, the promoter may be a liver-specific promoter. Optionally, the promoter is a human liver-specific promoter. A "*liver-specific promoter*" is a promoter that provides a higher level of expression in liver cells compared to

other cells in general. For example, the skilled person can determine whether a promoter is a liver-specific promoter by comparing expression of the polynucleotide in liver cells (such as Huh-7 cells) with expression of the polynucleotide in cells from other tissues. If the level of expression is higher in the liver cells, compared to the cells from other tissues, the promoter is a liver-specific promoter. Optionally, the transcription regulatory element or the promoter is liver-specific if it promotes protein expression at higher levels in liver cells compared to cells from at least one other organ or tissue and the transcription regulatory element or the promoter promotes protein expression in the cells from at least one other organ or tissue at a level less than 40%, less than 30%, less than 25%, less than 15%, less than 10%, or less than 5% of the level that the transcription regulatory element or the promoter promotes protein expression in liver cells. Optionally, the cells from at least one other organ or tissue are at least one of kidney cells, pancreatic cells, breast cells, neuroblastoma cells, lung cells, and early B cells. Optionally, the cells from at least one other organ or tissue are kidney cells, pancreatic cells, breast cells, neuroblastoma cells, lung cells, and early B cells. Optionally, the cells from at least one other organ or tissue are at least one of HEK293T cells, PANC1 cells, BxPC-3 cells, MCF7 cells, 1643 cells, MRC-9 cells, and 697 cells. Optionally, the cells from at least one other organ or tissue are HEK293T cells, PANC1 cells, BxPC-3 cells, MCF7 cells, 1643 cells, MRC-9 cells, and 697 cells.

[0087] In one embodiment, the polynucleotide of the invention may provide for GCase to be specifically expressed in the liver. In such examples, the polynucleotide may promote substantially more GCase expression in liver cells than in at least one other tissue type or organ. In one example, the polynucleotide of the invention which provides for GCase to be specifically expressed in the liver comprises a liver-specific promoter.

[0088] Optionally, the polynucleotide of the invention may provide for GCase to be expressed at higher levels in liver cells compared to cells from at least one other organ or tissue and such that GCase is expressed in the one other organ or tissue at a level less than 40%, less than 30%, less than 25%, less than 15%, less than 10%, or less than 5% of the level of GCase expression in liver cells, when measured in the same assay.

[0089] Optionally, the cells from at least one other organ or tissue are at least one of kidney cells, pancreatic cells, breast cells, neuroblastoma cells, lung cells, and early B cells. Optionally, the cells from at least one other organ or tissue are kidney cells, pancreatic cells, breast cells, neuroblastoma cells, lung cells, and early B cells. Optionally, the cells from at least one other organ or tissue are at least one of HEK293T cells, PANC1 cells, BxPC-3 cells, MCF7 cells, 1643 cells, MRC-9 cells, and 697 cells. Optionally, the cells from at least one other organ or tissue are HEK293T cells, PANC1 cells, BxPC-3 cells, MCF7 cells, 1643 cells, MRC-9 cells, and 697 cells.

A viral particle comprising the polynucleotide

[0090] The invention further provides a viral particle comprising a recombinant genome

comprising polynucleotides of the invention. For the purposes of the present invention, the term "*viral particle*" refers to all or part of a virion. For example, the viral particle comprises a recombinant genome and may further comprise a capsid. The viral particle may be a gene therapy vector. Herein, the terms "*viral particle*" and "*vector*" are used interchangeably. For the purpose of the present application, a "*gene therapy*" vector is a viral particle that can be used in gene therapy, *i.e.* a viral particle that comprises all the required functional elements to express a transgene, such as a GBA nucleotide sequence, in a host cell after administration.

[0091] Suitable viral particles include a parvovirus, a retrovirus, a lentivirus or a herpes simplex virus. The parvovirus may be an adeno-associated virus (AAV). The viral particle is preferably a recombinant adeno-associated viral (AAV) vector or a lentiviral vector. More preferably, the viral particle is an AAV viral particle. The terms AAV and rAAV are used interchangeably herein, unless context obviously suggests otherwise.

[0092] The genomic organization of all known AAV serotypes is very similar. The genome of AAV is a linear, single-stranded DNA molecule that is less than about 5,000 nucleotides in length. Inverted terminal repeats (ITRs) flank the unique coding nucleotide sequences for the non-structural replication (Rep) proteins and the structural (VP) proteins. The VP proteins (VP1, -2 and -3) form the capsid. The terminal -145 nt (ITRs) are self-complementary and are organized so that an energetically stable intramolecular duplex forming a T-shaped hairpin may be formed. These hairpin structures function as an origin for viral DNA replication, serving as primers for the cellular DNA polymerase complex. Following wild type (wt) AAV infection in mammalian cells the Rep genes (*i.e.* encoding Rep78 and Rep52 proteins) are expressed from the P5 promoter and the P19 promoter, respectively, and both Rep proteins have a function in the replication of the viral genome. A splicing event in the Rep ORF results in the expression of four Rep proteins (i.e. Rep78, Rep68, Rep52 and Rep40). However, it has been shown that the unspliced mRNA, encoding Rep78 and Rep52 proteins, in mammalian cells are sufficient for AAV vector production. Also in insect cells the Rep78 and Rep52 proteins suffice for AAV vector production.

[0093] The recombinant viral genome of the invention may comprise ITRs. It is possible for an AAV vector of the invention to function with only one ITR. Thus, the viral genome comprises at least one ITR, but, more typically, two ITRs (generally with one either end of the viral genome, i.e. one at the 5' end and one at the 3' end). There may be intervening sequences between the polynucleotide of the invention and one or more of the ITRs. The polynucleotide may be incorporated into a viral particle located between two regular ITRs or located on either side of an ITR engineered with two D regions.

[0094] AAV sequences that may be used in the present invention for the production of AAV vectors can be derived from the genome of any AAV serotype. Generally, the AAV serotypes have genomic sequences of significant homology at the amino acid and the nucleic acid levels, provide an identical set of genetic functions, produce virions which are essentially physically and functionally equivalent, and replicate and assemble by practically identical mechanisms. For the genomic sequence of the various AAV serotypes and an overview of the genomic

similarities see e.g. GenBank Accession number U89790; GenBank Accession number J01901; GenBank Accession number AF043303; GenBank Accession number AF085716; Chiorini *et al*, 1997; Srivastava *et al*, 1983; Chiorini *et al*, 1999; Rutledge *et al*, 1998; and Wu *et al*, 2000. AAV serotype 1, 2, 3, 3B, 4, 5, 6, 7, 8, 9, 10, 11 or 12 may be used in the present invention. The sequences from the AAV serotypes may be mutated or engineered when being used in the production of gene therapy vectors.

[0095] Optionally, an AAV vector comprises ITR sequences which are derived from AAV1, AAV2, AAV4 and/or AAV6. Preferably the ITR sequences are AAV2 ITR sequences. Herein, the term AAVx/y refers to a viral particle that comprises genomic components such as at least ITRs from AAVx (wherein x is a AAV serotype number) and has the capsid from AAVy (wherein y is the number of the same or different serotype). For example, an AAV2/8 vector may comprise a portion of a viral genome, including the ITRs, from an AAV2 strain, and a capsid from an AAV8 strain.

[0096] In an embodiment, the viral particle is an AAV viral particle comprising a capsid. AAV capsids are generally formed from three proteins, VP1, VP2 and VP3. The amino acid sequence of VP1 comprises the sequence of VP2. The portion of VP1 which does not form part of VP2 is referred to as VP1unique or VP1U. The amino acid sequence of VP2 comprises the sequence of VP3. The portion of VP2 which does not form part of VP3 is referred to as VP2unique or VP2U. Optionally, the viral particle comprises a liver-tropic or CNS-tropic capsid. Whether a viral particle (capsid) is tropic for a particular tissue can be evaluated for example by administering such a particle expressing a marker gene such as luciferase and imaging *in vivo* at multiple time points (for example as described in Zincarelli et al (2008), Molecular Therapy, 16:1073-1080). A particle driving strong marker expression in liver or CNS tissues, respectively, especially if in contrast to lesser expression in other tissues, would be considered liver- or CNS-tropic.

[0097] In some embodiments, a liver-tropic capsid can be an AAV3- or AAV3B-derived capsid. Optionally, 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 736or between 700 and 736amino acids of SEQ ID NO: 19, 20, or 24. Optionally, the liver-tropic capsid comprises a sequence at least 99% identical to SEQ ID NO: 19. Optionally, the liver-tropic capsid comprises a sequence at least 99% identical to SEQ ID NO: 20. Optionally, the liver-tropic capsid comprises a sequence at least 99% identical to SEQ ID NO: 24. Optionally, the CNS tropic capsid comprises a sequence at least 98%, at least 99%, 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 736or between 700 and 736amino acids of SEQ ID NO: 21. Optionally, the CNS-tropic capsid comprises a sequence at least 99% identical to SEQ ID NO: 21. A viral particle of the invention may be a "*hybrid*" particle in which the viral ITRs and viral capsid are from different parvoviruses, such as different AAV serotypes. Preferably, the viral ITRs and capsid are from different serotypes of AAV, in which case such viral particles are known as transcapsidated or pseudotyped. Likewise, the parvovirus may have a "*chimeric*" capsid (*e. g.*, containing sequences from different parvoviruses, preferably different AAV

serotypes) or a "*targeted*" capsid (e. g., a directed tropism).

[0098] In some embodiments, the recombinant AAV genome comprises intact ITRs, comprising functional terminal resolution sites (TRS). Such an AAV genome may contain one or two resolvable ITRs, i.e. ITRs containing a functional TRS at which site-specific nicking can take place to create a free 3' hydroxyl group which can serve as a substrate for DNA polymerase to unwind and copy the ITR. Preferably, the recombinant genome is single-stranded (*i.e.*, it is packaged into the viral particle in a single-stranded form). Optionally, the recombinant genome is not packaged in self-complementary configuration, *i.e.* the genome does not comprise a single covalently-linked polynucleotide strand with substantial self-complementary portions that anneal in the viral particle. Alternatively, the recombinant genome may be packaged in "*monomeric duplex*" form. "*Monomeric duplexes*" are described in WO 2011/122950. The genome may be packaged as two substantially complementary but non-covalently linked polynucleotides which anneal in the viral particle.

[0099] The viral particle may further comprise a poly A sequence. The poly A sequence may be positioned downstream of the nucleotide sequence encoding a functional GCase protein. The poly A sequence may be a bovine growth hormone poly A sequence (bGHpA - SEQ ID NO: 23). The poly A sequence may be between 250 and 270 nucleotides in length.

[0100] The viral particle may further comprise an intron sequence, such as a viral intron sequence, optionally an SV40 intron sequence (SEQ ID NO: 22).

[0101] In one embodiment, the viral particle comprises a polynucleotide sequence comprising a promoter element, an intron sequence, such as an SV40 intron sequence, a GBA nucleotide sequence, and a poly A sequence, such as the bGHpA sequence. In such embodiments, the intron sequence, such as the SV40 intron sequence, may be located between the promoter element and the GBA nucleotide sequence. In such embodiments, the poly A sequence, such as the bGHpA sequence, may be located downstream of the GBA nucleotide sequence.

[0102] The viral particle of the invention optionally expresses GCase highly in host cells. For example, on transduction in Huh-7 cells, the viral particle of the present invention expresses GCase protein or a fragment thereof at a higher level compared to an otherwise identical viral particle comprising a GBA nucleotide sequence of SEQ ID NO: 9 transduced at a comparable amount into a comparable population of Huh-7 cells. Optionally, after transduction into a population of Huh-7 cells, the viral particle of the present invention expresses GCase protein at a higher level than a viral particle comprising a GBA nucleotide sequence of SEQ ID NO: 9 and a transcription regulatory element of SEQ ID NO: 10 or a promoter of SEQ ID NO: 12. Optionally, after transduction into a population of Huh-7 cells, the viral particle of the present invention expresses GCase protein at a higher level than a comparable viral particle comprising a GBA nucleotide sequence of SEQ ID NO: 9 and a transcription regulatory element of SEQ ID NO: 10 or a promoter sequence of SEQ ID NO: 12 transduced at a comparable amount into a comparable population of Huh-7 cells. Optionally, after transduction into a population of Huh-7 cells, the viral particle expresses GCase protein at comparable level

(*i.e.* a non-statistically significantly different level) to a viral particle comprising a GBA nucleotide sequence of SEQ ID NO: 9 and a promoter element of SEQ ID NO: 13 transduced at a comparable amount into a comparable population of Huh-7 cells. In such embodiments, the term *"comparable viral particle "* refers to a viral particle that is the same as an AAV viral particle of the invention, except the comparable viral particle comprises a different GBA nucleotide sequence and a different transcription regulatory element. Optionally, the comparable viral particle comprises the same transcription regulatory element as the AAV viral particle of the invention. Optionally, the activity is assessed using a chromogenic assay such as the fluorometric assay discussed above.

[0103] In one embodiment, provided is a viral particle comprising a polynucleotide sequence, the polynucleotide sequence comprising:

1. a) a GBA nucleotide sequence having at least 98% sequence identity to SEQ ID NO: 5, operably linked to:
   b) a transcriptional regulatory sequence having at least 98% sequence identity to SEQ ID NO: 14;

wherein the viral particle further comprises a capsid having at least 98% identity to SEQ ID NO: 20.

[0104] In one embodiment, provided is a viral particle comprising a polynucleotide sequence, the polynucleotide sequence comprising:

1. a) a GBA nucleotide sequence having at least 98% sequence identity to SEQ ID NO: 5, operably linked to:
   b) a transcriptional regulatory sequence having at least 98% sequence identity to SEQ ID NO: 10;

wherein the viral particle further comprises a capsid having at least 98% identity to SEQ ID NO: 20.

Compositions, methods and uses

[0105] In a further aspect of the invention, there is provided a composition comprising the polynucleotide or vector/viral particle of the invention and a pharmaceutically acceptable excipient.

[0106] The pharmaceutically acceptable excipients may comprise carriers, diluents and/or other medicinal agents, pharmaceutical agents or adjuvants, etc. Optionally, the pharmaceutically acceptable excipients comprise saline solution. Optionally, the pharmaceutically acceptable excipients comprise human serum albumin.

[0107] This invention further provides a method of expressing the GBA nucleotide sequence

and achieving a stable GCase activity in a subject and/or 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, wherein the method comprises administration of a polynucleotide, viral particle or composition of the invention to a subject.

[0108] The invention further provides a polynucleotide, vector/viral particle or composition of the invention for use in a method of treatment. Optionally the method of treatment comprises administering an effective amount of the polynucleotide or vector/viral particle of the invention to a patient.

[0109] Also described herein is a method of treatment comprising administering an effective amount of the polynucleotide or vector/viral particle of the invention to a patient.

[0110] Also described herein is a use of the polynucleotide, vector/viral particle or composition of the invention in the manufacture of a medicament for use in a method of treatment. Optionally the method of treatment comprises administering an effective amount of the polynucleotide or vector/viral particle of the invention to a patient. Optionally the method of treatment is a gene therapy. A "*gene therapy*" involves administering a vector/viral particle of the invention that is capable of expressing a transgene (such as a GBA nucleotide sequence) in the host to which it is administered.

[0111] Optionally, the method of treatment is a method of treating diseases associated with a GCase deficiency. As discussed above, GCase deficiency may lead to accumulation of glucocerebrosides in macrophages that infiltrate many vital organs which can cause a variety of diseases including synucleopathies (as discussed in WO08/144591) or Parkinson's disease. Optionally, the method of treatment is a method of treating Parkinson's disease or a synucleopathy.

[0112] Optionally, the method of treatment is a method of treating a lysosomal storage disorder such as Gaucher disease (GD), for example GD type I, type II or type III. Preferably, the lysosomal storage disorder is characterised by bruising, fatigue, anemia, low blood platelet count and enlargement of the liver and spleen. Optionally, the method of treatment is a method of treating GD, for example GD type I. In some embodiments, the patient is a patient suffering from GD, for example GD type I. Optionally the patient has antibodies or inhibitors to recombinant GCase (for example imiglucerase, velaglucerase alfa or taliglucerase alfa) with which the patient has previously been treated as part of an enzyme replacement therapy. Optionally, the polynucleotide and/or vector/viral particle is administered intravenously. Optionally, the polynucleotide and/or vector/viral particle is for administration only once (i.e. a single dose) to a patient.

[0113] When GD is "*treated*" in the above method, this means that one or more symptoms of GD type I are ameliorated. It does not mean that the symptoms of GD type I are completely remedied so that they are no longer present in the patient, although in some methods, this

may be the case. Thus, in all instances the term "*treatment*" can be replaced with the term "*amelioration*". The method of treatment may result in one or more of the symptoms of GD type I being less severe than before treatment. Optionally, relative to the situation pre-administration, the method of treatment results in an increase in the amount/concentration of circulating GCase in the blood of the patient, and/or the overall level of GCase activity detectable within a given volume of blood and/or the macrophages of the patient. In one embodiment, relative to the situation pre-administration, the method of treatment results in one or more of: an increase in haemoglobin concentration; an increase in platelet count; a decrease in spleen size; a decrease in liver size.

[0114] In addition, the methods described herein may "*prevent*" diseases such as Gaucher disease. Gaucher disease is generally associated with an accumulation of glucocerebrosidases in various tissues, and if the methods of the invention are carried out on young subjects (such as teenagers, young adults, children or babies) it should be possible to prevent Gaucher disease from establishing. Accordingly, in all instances the term "*treatment*" may be replaced with the term "*prevention*".

[0115] A "*therapeutically effective amount*" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result, such as raising the level of functional GCase in a subject (so as to lead to functional GCase production at a level sufficient to ameliorate the symptoms of GD, for example GD type I).

[0116] Optionally, the vector/viral particle is administered at a dose of less than 1×10^{11}, less than 1×10^{12}, less than 5×10^{12}, less than 2×10^{12}, less than 1.5×10^{12}, less than 3×10^{12}, less than 1×10^{13}, less than 2×10^{13}, or less than 3×10^{13} vector genomes per kg of weight of patient. Optionally, the dose of vector/viral particle that is administered is selected such that the subject expresses GCase at an level of 10%-90%, 20%-80%, 30%-70%, 25%-50%, 20%-150%, 30%-140%, 40%-130%, 50%-120%, 60%-110% or 70%-100% of the level of a healthy subject who does not suffer from GD.

[0117] Optionally, a patient administered the polynucleotide, viral particle or composition may have a GCase activity 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. Optionally, the GCase activity is measured using a fluorometric substrate which is specific for GCase. Optionally, the GCase activity is measured fluorometrically with 4-Methylumbelliferyl-β-D-glucopyranoside (4MU-Glc) as a substrate. Optionally, the GCase activity is measured in the serum, plasma, macrophages, spleen, liver and/or bone marrow of the subject.

[0118] In one embodiment, GCase activity may be determined fluorometrically with 4-Methylumbelliferyl-β-D-glucopyranoside (4MU-Glc) as a substrate as follows: (1) serum samples are collected or tissues (liver, spleen, bone marrow) are harvested and snap frozen and lysed; (2) the tissue lysate or serum/plasma sample are mixed in 50 mM Sodium Citrate, 25 mM Taurocholate, pH=5.75, 6 mM 4MU-Glc, for 30 min at 37°C; (3) the reaction is stopped

by adding one volume (100 µl) of stop solution (0.5 M Glycine, 0.3 M NaOH, pH 10.0); (4) relative fluorescence levels (RFU) are evaluated with a Spectramax I3X (Molecular devices) using excitation and emission wavelengths of 365 nm and 445 nm, respectively and fluorescence levels were then converted to nanomoles/h/mL based on a 4-Methylumbelliferone (4-MU, Sigma-Aldrich) standard curve.

[0119] Optionally, a patient administered the polynucleotide, viral particle or composition may have a greater GCase activity level 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. Optionally, a patient administered the polynucleotide, viral particle or composition may have a GCase activity level greater by 10 fold, 20 fold, 50 fold, 100 fold or 1000 fold 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.

[0120] Optionally, the dose of vector/viral particle that is administered is selected such that there is a greater GCase bioavailability to the subject when compared to the bioavailability from GCase enzyme replacement therapy. Bioavailability may be measured (*e.g.* estimated or calculated) through any known method in the art. GCase bioavailability may be measured in the serum, macrophages, spleen, liver and/or bone marrow of the subject. In one example, bioavailability may be estimated using the area under the curve ("AUC") method according to Example 8. In one example, bioavailability may be estimated by estimating the total GCase activity available in the serum, plasma, macrophages, spleen, liver and/or bone marrow of the subject. Optionally, it is calculated over a defined time period, and refers to the total activity or concentration of GCase during that time period. Optionally, the GCase activity is measured using a fluorometric substrate which is specific for GCase. Optionally, the GCase activity is measured fluorometrically with 4-Methylumbelliferyl-β-D-glucopyranoside (4MU-Glc) as a substrate. Optionally, the GCase activity is measured in the serum, plasma, macrophages, spleen, liver and/or bone marrow of the subject. Optionally the GCase activity is measured in the white blood cells of the subject. Optionally, the bioavailability is measured over a period of 2 weeks from administration. Optionally, the bioavailability is measured over a period of 5 weeks from administration. Optionally, the bioavailability is measured in serum. In one example, a greater GCase bioavailability in the subject is achieved 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.

[0121] Optionally, a patient (for example, a patient suffering from a disease or condition associated with GCase deficiency) administered the polynucleotide, viral particle or composition of the invention may have reduced hexosylceramide and/or hexosylsphingosine

levels after administration, preferably when the hexosylceramide and/or hexosylsphingosine levels are measured 6 weeks, 8 weeks, 10 weeks or 12 weeks after administration. The hexosylceramide and/or hexosylsphingosine levels may be 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 (starting) hexosylceramide and/or hexosylsphingosine levels at the time of administration of the polynucleotide, viral particle or composition of the invention. For example, after administration of the polynucleotide viral particle or composition of the invention (for example 6 weeks, 8 weeks, 10 weeks or 12 weeks after administration), the hexosylceramide and/or hexosylsphingosine levels in the patient may be 50% or less, 40% or less, 30% or less, 25% or less, 20% or less when compared to the (starting) hexosylceramide and/or hexosylsphingosine levels at the time of administration of the polynucleotide, viral particle or composition of the invention. Optionally, the patient may have increased hexosylceramide and/or hexosylsphingosine levels when compared to a healthy subject or a subject who does not have a disease or condition associated with GCase deficiency. For example, the hexosylceramide and/or hexosylsphingosine levels are measured in the spleen, liver and/or bone marrow of the patient/subject. The hexosylceramide and/or hexosylsphingosine levels may be measured in the serum and/or white blood cells (e.g. macrophages) of the patient/subject. Methods of measuring hexosylceramide and/or hexosylsphingosine levels are known in the art, and the levels of hexosylceramide and/or hexosylsphingosine are preferably measured using mass spectrometry (LC/MS analysis), for example by the method described in example 9. Optionally, the reduction of hexosylceramide and/or hexosylsphingosine levels (for example in the serum, white blood cells (e.g. macrophages), spleen, liver and/or bone marrow of the patient/subject) are greater than the reduction achieved from GCase enzyme replacement therapy, preferably when the hexosylceramide and/or hexosylsphingosine levels are measured after at least 6 weeks, at least 8 weeks, at least 10 weeks or at least 12 weeks after the start of treatment. For example levels after at least 6 weeks (e.g. at 6 weeks), at least 8 weeks (e.g. at 8 weeks), at least 10 weeks (e.g. at 10 weeks) or at least 12 weeks (e.g. at 12 weeks) from administration of the polynucleotide, viral particle or composition of the invention may be compared to levels after at least 6 weeks (e.g. at 6 weeks), at least 8 weeks (e.g. at 8 weeks), at least 10 weeks (e.g. at 10 weeks) or at least 12 weeks (e.g. at 12 weeks), respectively, from the first administration of GCase enzyme replacement therapy. As a particular example, hexosylceramide and/or hexosylsphingosine levels may be measured after at least 12 weeks (e.g. at 12 weeks) after administration of the polynucleotide, viral particle or composition of the invention and compared to the levels measured at least 12 weeks (e.g. at 12 weeks) after first administration of GCase enzyme replacement therapy. Preferably, the levels of hexosylceramide and/or hexosylsphingosine are measured in the same assay at the same time point after administration. Optionally, the GCase enzyme replacement therapy may be administered every two weeks. Optionally, the reduction of hexosylceramide levels in the subject (or patient) after administration of the polynucleotide, viral particle or composition of the invention are such that the hexosylceramide levels (for example in the serum, white blood cells (e.g. macrophages), liver and/or spleen) are no more than 200% , 150%, or 125% of the hexosylceramide levels measured in a healthy subject or a subject not suffering from a disease or condition associated with GCase deficiency. In one example, a reduction in hexosylceramide

and/or hexosylsphingosine levels may represent a reduction in glucosylceramide and/or glucosylsphingosine levels, respectively. For example, a reduction in hexosylceramide may represent a reduction in glucosylceramide. As a further example, a reduction in hexosylsphingosine levels may represent a reduction in glucosylsphingosine levels.

[0122] In one example, a reduction in hexosylceramide and/or hexosylsphingosine levels is a reduction in glucosylceramide and/or glucosylsphingosine respectively. In other words, a patient (for example, a patient suffering from a disease or condition associated with GCase deficiency) administered the polynucleotide, viral particle or composition of the invention may have reduced glucosylceramide and/or glucosylsphingosine levels after administration, preferably when the glucosylceramide and/or glucosylsphingosine levels are measured 6 weeks, 8 weeks, 10 weeks or 12 weeks after administration. The glucosylceramide and/or glucosylsphingosine levels may be 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 (starting) glucosylceramide and/or glucosylsphingosine levels at the time of administration of the polynucleotide, viral particle or composition of the invention. For example, after administration of the polynucleotide viral particle or composition of the invention (for example 6 weeks, 8 weeks, 10 weeks or 12 weeks after administration), the glucosylceramide and/or glucosylsphingosine levels in the patient may be 50% or less, 40% or less, 30% or less, 25% or less, 20% or less when compared to the (starting) glucosylceramide and/or glucosylsphingosine levels at the time of administration of the polynucleotide, viral particle or composition of the invention. Optionally, the patient may have increased glucosylceramide and/or glucosylsphingosine levels when compared to a healthy subject or a subject who does not have a disease or condition associated with GCase deficiency. For example, the glucosylceramide and/or glucosylsphingosine levels are measured in the spleen, liver and/or bone marrow of the patient/subject. The glucosylceramide and/or glucosylsphingosine levels may be measured in the serum and/or white blood cells (e.g. macrophages) of the patient/subject. Methods of measuring glucosylceramide and/or glucosylsphingosine levels are known in the art, and the levels of glucosylceramide and/or glucosylsphingosine are preferably measured using mass spectrometry (LC/MS analysis), for example by the method described in example 9. Optionally, the reduction of glucosylceramide and/or glucosylsphingosine levels (for example in the serum, white blood cells (e.g. macrophages), spleen, liver and/or bone marrow of the patient/subject) are greater than the reduction achieved from GCase enzyme replacement therapy, preferably when the glucosylceramide and/or glucosylsphingosine levels are measured after at least 6 weeks, at least 8 weeks, at least 10 weeks or at least 12 weeks after the start of treatment. For example levels after at least 6 weeks (e.g. at 6 weeks), at least 8 weeks (e.g. at 8 weeks), at least 10 weeks (e.g. at 10 weeks) or at least 12 weeks (e.g. at 12 weeks) from administration of the polynucleotide, viral particle or composition of the invention may be compared to levels after at least 6 weeks (e.g. at 6 weeks), at least 8 weeks (e.g. at 8 weeks), at least 10 weeks (e.g. at 10 weeks) or at least 12 weeks (e.g. at 12 weeks), respectively, from the first administration of GCase enzyme replacement therapy. As a particular example, glucosylceramide and/or glucosylsphingosine levels may be measured after at least 12 weeks (e.g. at 12 weeks) after administration of the polynucleotide, viral particle or composition of the invention and

compared to the levels measured at least 12 weeks (e.g. at 12 weeks) after first administration of GCase enzyme replacement therapy. Preferably, the levels of glucosylceramide and/or glucosylsphingosine are measured in the same assay at the same time point after administration. Optionally, the GCase enzyme replacement therapy may be administered every two weeks. Optionally, the reduction of glucosylceramide levels in the subject (or patient) after administration of the polynucleotide, viral particle or composition of the invention are such that the glucosylceramide levels (for example in the serum, white blood cells (e.g. macrophages), liver and/or spleen) are no more than 200% , 150%, or 125% of the glucosylceramide levels measured in a healthy subject or a subject not suffering from a disease or condition associated with GCase deficiency.

[0123] Optionally a patient (for example, a patient suffering from a disease or condition associated with GCase deficiency) administered the polynucleotide, viral particle or composition of the invention may show a reduced number of storage cells and/or activated macrophages in the liver after administration, preferably when the cells are counted after at least 6 weeks (e.g. at 6 weeks), at least 8 weeks (e.g. at 8 weeks), at least 10 weeks (e.g. at 10 weeks) or at least 12 weeks (e.g. at 12 weeks) after administration. Reduction in the number of storage cells and/or activated macrophages in the liver may be an indication of reduced inflammation and thus therapeutic benefit. The number of activated macrophages may be indicated or estimated by measuring the number of $CD68^{positive}$ cells. Identifying storage cells and $CD68^{positive}$ cells can be performed by methods known in the art, for example the methods described in example 9.

[0124] A "*GCase enzyme replacement therapy*" may refer to any therapy which comprises the administration of a GCase polypeptide to a subject. The GCase polypeptide may be wild type, such as a GCase polypeptide having the amino acid sequence of SEQ ID NO: 25. The GCase polypeptide may be administered at any suitable dose, optionally at a dose of between 40 and 100, between 50 and 80, between 60 and 70, or around 60 U/kg BW. The GCase polypeptide may be administered through any appropriate route, optionally administered through intravenous injection or subcutaneous injection.

[0125] A GCase activity level of at least X% (*e.g.* at least 20%) refers to a GCase activity level that is at least X% (e.g. 20%) of the normal GCase level range as measured from a sample of e.g. the spleen or bone marrow. The person skilled in the art would readily understand what is meant by reference to a %-of-normal GCase activity level, which is determined in routine clinical practice by *e.g.* comparison to a control sample from a healthy subject.

[0126] The term "*stable GCase activity*" or "*stable GCase activity level*" refers to a GCase activity level that maintains at or above a certain level for a continuous period of at least 5 weeks. In other words, the activity may fluctuate above said activity level but is still said to be stable as long as it remains above the stated minimum threshold. In some embodiments, the GCase activity level maintains at or above a certain level for a continuous period of at least 10, at least 15, at least 20, at least 30, at least 40, or at least 50 weeks. For example, a patient has a stable GCase activity level of at least 20% if the activity level maintains at at least 20% for a

continuous period of at least 5 weeks. In such an example, the GCase activity level may continue to be at at least 20% following the at least 5 weeks and thus maintains at at least 20% for a cumulative continuous period of at least 10, at least 15, at least 20, at least 30 or at least 40, or at least 50 weeks. A patient has a stable GCase activity level if the GCase activity level maintains at or above a certain level for a continuous period of at least 5 weeks. Optionally, a patient administered with the polynucleotide, viral particle or composition may have a stable GCase activity level of at least 20%, at least 25%, at least 30%, at least 35%, at least 40% or at least 50% relative to the GCase activity of a healthy subject. Optionally, a patient administered with the polynucleotide, viral particle or composition may have a stable GCase activity 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. Optionally, the GCase activity is measured using a fluorometric substrate which is specific for GCase. Optionally, the GCase activity is measured fluorometrically with 4-Methylumbelliferyl-β-D-glucopyranoside (4MU-Glc) as a substrate. Optionally, the GCase activity is measured in the serum, macrophages, spleen, liver and/or bone marrow of the subject.

[0127] Optionally, the GCase activity level is stable after at least 5 weeks, at least 10 weeks, at least 15 weeks, at least 20 weeks, at least 30 weeks, at least 40 weeks, or at least 50 weeks from administration of the polynucleotide, viral particle or composition. For example, where a patient has a stable GCase activity level of at least 20% after at least 5 weeks from when the patient is administered with the polynucleotide, viral particle or composition, there is a GCase activity level of at least 20% that maintains at at least 20% for a continuous period of at least 5, at least 10, at least 15, at least 20, at least 30 or at least 40, or at least 50 weeks following the initial at least 5 weeks from administration.

[0128] Optionally, the GCase activity level is at or above a certain level (*e.g.* 20%, 25%, 30%, 35%, or 40%; and/or 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) at a time point at least 5, at least 10, at least 20, at least 30, at least 40 or at least 50 weeks after administration of the polynucleotide, viral particle or composition. For example, the GCase activity level is at or above a certain level (*e.g.* 20%, 25%, 30%, 35%, or 40%; and/or 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) at a time point of around 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51 or 52 weeks after administration of the polynucleotide, viral particle or composition.

[0129] The invention will now be described with reference to the following examples, which are merely illustrative and should not in any way be construed as limiting the scope of the present invention.

Sequence Listing

[0130]

Sequence identity number	Sequence description
1	Codon-optimised GBA nucleotide sequence from FLF-PL28, without signal peptide portion
2	Codon-optimised GBA nucleotide sequence from FLF-PL21, without signal peptide portion
3	Codon-optimised GBA nucleotide sequence from FLF-PL30, without signal peptide portion
4	Codon-optimised GBA nucleotide sequence from FLF-PL36, without signal peptide portion
5	Codon-optimised GBA nucleotide sequence from FLF-PL28, with signal peptide portion
6	Codon-optimised GBA nucleotide sequence from FLF-PL21, with signal peptide portion
7	Codon-optimised GBA nucleotide sequence from FLF-PL30, with signal peptide portion
8	Codon-optimised GBA nucleotide sequence from FLF-PL36, with signal peptide portion
9	Wild type human GBA nucleotide sequence with signal peptide (from GenBank NM_000157.3)
10	LSP-S transcription regulatory element
11	HCR enhancer portion of LSP-S
12	A1AT promoter portion of LSP-S
13	CAG promoter
14	LSP-L transcription regulatory element
15	A1AT promoter portion of LSP-L
16	HCR enhancer portion of LSP-L
17	Wild type GBA nucleotide sequence corresponding to signal peptide
18	Wild type GCase polypeptide sequence of signal peptide
19	Polypeptide sequence of liver-tropic capsid
20	Polypeptide sequence of liver-tropic capsid
21	Polypeptide sequence of CNS-tropic capsid
22	Nucleotide sequence of SV40 intron
23	Nucleotide sequence of bovine growth hormone poly A sequence
24	Polypeptide sequence of liver-tropic capsid
25	Polypeptide sequence of wild type human GCase

Examples

Example 1 - Methods

[0131] Unless specified otherwise, the following general methods were followed in the examples described below.

rAAV production

[0132] AAV2/8 particles were produced by transient transfection of HEK293T cells with plasmids encoding the AAV Rep and Cap, and adenoviral helper functions, as well the recombinant genome containing the GBA construct. AAV2/8 particles were purified by aPOROS CaptureSelect affinity column and were titered by qPCR and characterised by alkaline gel analysis.

Mouse study design

[0133] AAV viral particles carrying the GBA transgene under transcriptional control of the hepatocyte-specific promoters were administered into the tail vein of wild type (C57BL/6) male mice at age of 6-8 weeks. AAV dose ranged from 6×10^{11} vg/kg to 6×10^{12} vg/kg, as herein for each study. For each experiment, an additional group of animals was left untreated to serve as a control for the effects of treatment. To assess the kinetics and durability of transgene expression, serum GCase levels were measured at various time intervals (4-, 8-, and 12-weeks) post injection. Mice were followed up to 12 weeks post AAV treatment and sacrificed for biochemical and pathological analysis.

Serum and tissue GBA activity assay

[0134] β-Glucocerebrosidase (acid β-glucosidase; GCase) activity was determined fluorometrically with 4-Methylumbelliferyl-β-D-glucopyranoside (4MU-Glc) as a substrate. Serum samples were obtained from mouse blood and stored at -80 °C. Tissues (liver, spleen, bone marrow) were harvested and snap frozen and lysed. β-Glucocerebrosidase (acid β-glucosidase, GCase) activity was determined fluorometrically with 4-Methylumbelliferyl-β-D-glucopyranoside (4MU-Glc) as a substrate. On the day of the assay, serum was diluted (0.5 µL, 1:50) and assayed in 50 mM Sodium Citrate, 25 mM Taurocholate, pH=5.75, 6 mM 4MU-Glc, for 30 min at 37°C. For tissue samples, the tissue protein lysate was assayed directly. The reaction was stopped by adding one volume (100 µl) of stop solution (0.5 M Glycine, 0.3 M

NaOH, pH 10.0). Relative fluorescence levels (RFU) were evaluated with a Spectramax I3X (Molecular devices) using excitation and emission wavelengths of 365 nm and 445 nm, respectively. Fluorescence levels were then converted to nanomoles/h/mL (serum) or nmol/h/mg of total protein (tissue) based on a 4-Methylumbelliferone (4-MU, Sigma-Aldrich) standard curve.

Vector genome copy number

[0135] To determine the number of vector genomes per liver cell post- rAAV injection, DNA was isolated from frozen liver samples using QIAGEN DNeasy Blood and Tissue Kit (QIAGEN) following manufacturers' instructions. Following DNA isolation, qPCR was performed using primer sets which bind to a region common to both LSP-S and LSP-L promoters, allowing estimation of AAV copy number.

Immunohistochemistry

[0136] Rabbit anti-human GCase (Abcam ab125065; 1:100) was used to visualize GCase in mouse tissue. The rat anti-F4/80 (Abcam ab6640; 1:100) was used to visualize mouse macrophages. The formalin-fixed mouse tissues were deparaffinized with xylene and ethanol washes, followed by antigen retrieval according to Ventana CC1 product use recommendations. Immunohistochemistry staining was performed using the Ventana Discovery XT instrument, using the Ventana DAB Map detection Kit (760-124). Sections were counterstained with haematoxylin. FITC- and Texas red-conjugated secondary antibodies were used during immunofluorescent staining. DAPI was used to visualize nuclei. The signals were visualized by confocal fluorescence microscopy (Zeiss).

Huh-7 transfection and potency assay

[0137] The day before transfection, the liver hepatocyte cell line Huh-7 was plated in a 12 well plate at the cell density of 3×10^5 cells per well. For transfection, FuGENE was used at the ratio of 4 µl per µg of plasmid and added overnight to Huh-7 cells in the presence of 10% of serum (foetal bovine serum, FBS). Transfection medium was changed, and cells were incubated for 24 hours with medium supplemented with Insulin-Transferrin-Selenium (ITS, ThermoFisher Scientific) and 25 mM Hepes buffer. Huh-7 cell transduction, was performed at defined multiplicity of infection (MOI) in the presence of serum for 24 hours, followed by a medium change and incubation for 24 hours in fresh medium. 20 µl of medium was used to measure GCase activity using 4MU-Glc as a substrate, as described above.

Statistical analysis

[0138] Statistical analysis was performed using Prism 7 (Graph Pad) software. Columns analysis was performed by one-way ANOVA. P-values and sample size are indicated in Figure descriptions.

[0139] To approximate bioavailability (AUC), a one-phase decay model equation: Y= (Y0 - Plateau)*exp(-K*X) + Plateau was used in GraphPad Prism. Y0 is the Y-value when X (time) is zero and it is expressed in the same units as Y. Plateau is the Y-value at infinite times, expressed in the same units as Y. K is the rate constant, expressed in reciprocal of the X-axis time units (i.e. if X is in minutes, then K is expressed in minutes^{-1}). Tau is the time constant, expressed in the same units as the X-axis and is computed as the reciprocal of K. Half-life is in the time units of the X-axis, computed as ln(2)/K. Span is the difference between Y0 and Plateau, expressed in the same units as the Y-values. The linear trapezoidal method was used for the AUC calculation. AUC is expressed as U*h/L where one unit is defined as the amount of enzyme required to hydrolyse 1 µmol/h of 4-methylumbelliferyl- β -D-glucopyranoside substrate at 37°C.

Example 2 - GBA constructs

[0140] In order to evaluate if a liver-directed gene therapy approach could be used to treat Gaucher disease (GD), the human full-length GBA coding sequence (as found in GenBank accession no. NM_000157.3; SEQ ID NO: 9) was cloned into a liver-specific promoter-driven adeno-associated virus (AAV) vector. In FLF-PL01 AAV construct (Fig 1A) the GBA wild type sequence (GBAwt, non-codon-optimized) is driven by a liver-specific promoter referred to herein as `LSP-S' (SEQ ID NO: 10). In order to determine a sequence optimal for expression, sequences were designed using a number of different codon optimisation strategies. In one example AAV construct (FLF-PL28) the GBA codon sequence was optimized and is driven by the same liver specific promoter LSP-S (Fig 1B). The FLF-PL64 construct contains the same GBA codon-optimized sequence as FLF-PL28 but differs in containing a longer transcription regulatory element, referred to here as `LSP-L' (SEQ ID NO: 14) instead of LSP-S (Fig 1C).

Example 3 - Analysis of wild type GBA transgene expression

[0141] In order to evaluate if the (wild type) GBA construct FLF-PL01 could lead to liver expression and subsequent secretion of β-glucocerebrosidase (GCase) into the bloodstream, FLF-PL01 was pseudotyped into AAV2/8. rAAV particles were produced and titered as described above, and characterised by alkaline gel analysis, prior to be used in mice. Eight-week-old wild type (C57BL/6) mice were treated with a single injection of AAV2/8-FLF-PL01 at a dose ranging from 6×10^{11} to 6×10^{12} vg/kg. Control (naive) mice were left untreated. Serum samples were collected at four, eight, and 12 weeks post-AAV injection and used to evaluate

levels of circulating active GCase. GCase activity was determined and immunohistochemistry staining was performed as described above. Sections were counterstained with haematoxylin.

[0142] Injection of wild-type mice with AAV2/8-FLF-PL01 resulted in an increase in expression of human GCase in the liver of treated animals (Fig 2A). An increased level of GCase expression in liver could be observed with increased vector dose, with around 12-fold increase observed at the vector dose of 6×10^{11} vg/kg group, 43-fold at the dose of 2×10^{12} vg/kg, and 57-fold at the dose of 6×10^{12} vg/kg (Fig 2B). This data shows that AAV2/8-FLF-PL01 drove expression of GCase to levels that result in significant release of GCase to the bloodstream and possible access to macrophages in GD affected tissues.

Example 4 - Analysis of *in vitro* GCase expression from codon-optimised constructs

[0143] Codon usage tables of various liver expressed sequences were used in order to generate GBA sequences codon-optimised throughout the stretch corresponding to the mature GCase protein (but not the signal peptide-encoding region). With the exception of one such codon-optimised GBA sequence (`FLF-PL36') the resulting sequences were then further manually altered to remove CpGs, cryptic splice sites, premature stop codons and unwanted amino acid substitutions. Twenty-one codon-optimised GBA sequences were created and tested for GCase expression levels upon transfection in the human liver cell line Huh-7. Huh-7 cells were plated onto a 12 well plate at the cell density of 3×10^5 per well and transfected as described above. Twenty microliters of medium was used to measure GCase activity using 4MU-Glc as a substrate. Results from this analysis allowed the identification of GBA codon-optimizations (FLF-PL21, -PL28, -PL30, and -PL36) that demonstrated increased expression of GCase (relative to wild type GBA sequence, FLF-PL01) when transfected in Huh-7 cells (Fig 3).

Example 5 - Analysis of *in vivo* GCase activity from codon-optimised constructs

[0144] The four constructs (FLF-PL21, FLF-PL28, FLF-PL30 and FLF-PL36) identified in example 4 were pseudotyped as AAV2/8 and injection into wild-type mice at the dose of 2×10^{12} vg/kg. Also included in the experiment was the non-codon-optimised construct FLF-PL01, as well as a construct (FLF-PL37) containing the same wild type GBA sequence as FLF-PL01 driven by the strong synthetic promoter CAG. Control (naïve) mice were left untreated. At time points up to 36 weeks after injection, animals were sacrificed, and serum and tissues samples were collected.

[0145] Fig 4A shows the results at 8 weeks post-injection of GCase activity found in mice injected either with the non-codon optimized GBA sequence driven by the LSP-S promoter (FLF-PL01), the codon-optimized GBA constructs (FLF-PL21, FLF-PL28, FLF-PL30 and FLF-

PL36) also driven by the LSP-S promoter, and the GBA non-codon-optimized sequence driven by CAG promoter (FLF-PL37). All four GBA codon-optimized constructs showed increased levels of GCase activity present in the bloodstream when injected in mice (Fig 4A), relative to FLF-PL01. The FLF-PL28 construct exhibited the greatest increase (about 6-fold) in GCase release to the bloodstream compared to the non-codon-optimised construct driven by the same LSP-S promoter (FLF-PL01). The elevated level of GCase driven by FLF-PL28 relative to FLF-PL01 was observed throughout the 36-week study period (Fig 4B).

[0146] Of particular note, the levels of GCase observed in liver-specific promoter-containing FLF-PL28-injected mice were as high as the GCase levels driven by the FLF-PL37 construct in which wild type GBA sequence is expressed from the ubiquitous and strong CAG promoter (Fig 4A).

[0147] At end-stage, spleen and bone marrow were collected and fixed in formalin, followed by paraffin embedding. GBA immunostaining analysis performed on paraffin sections shows that, in agreement with the circulating GCase levels, tissue uptake of GCase is increased in mice treated with the FLF-PL28 GBA codon-optimised construct compared to non-codon-optimised construct FLF-PL01 (Fig 5).

[0148] In order to evaluate levels of macrophage uptake in spleen upon liver-directed GBA expression by FLF-PL28, immunofluorescence analysis with the mouse pan-macrophage marker F4/80 and a GBA antibody was performed. The majority of F4/80 positive cells display expression of the human-specific GBA, suggesting that the majority of GCase uptake in spleen occurs in macrophages (Fig 6).

Example 6 - Analysis of promoter effect on *in vivo* GCase activity

[0149] To test if promoter engineering could further increase expression from a GBA codon-optimised sequence, the GBA construct from FLF-PL28 was placed under a liver-specific promoter (referred to herein as 'LSP-L'; SEQ ID NO: 14) to generate construct FLF-PL64 (Example 2, Fig 1C).

[0150] AAV2/8 vectors were prepared with the new construct and injected into wild type mice at the dose of 2×10^{12} vg/kg. Control (naive) mice were left untreated. After 5 weeks, animals were sacrificed, and serum and tissues were collected.

[0151] GCase activity analysis in serum shows that AAV2/8-FLF-PL64 results in an increased expression (about 2.5-fold, P=0.0001, one-way ANOVA) of GCase in the mouse bloodstream compared to mice treated with AAV2/8-FLF-PL28 (Fig 7).

[0152] Like construct FLF-PL28, FLF-PL64 allows robust uptake of GCase into GD target tissues such spleen, bone marrow and lung (Fig 8).

Example 7 - Liver expression selectivity from AAV vectors with GBA constructs

[0153] To analyse the selectivity of the LSP-L promoter for a hepatic cell line, eight human-derived cell lines from a variety of tissues were selected. Details of each cell line and its origin are summarized in the table below.

Table 1. Human-derived cell lines evaluated in this example

Cell line	Origin	Growth	Species of origin
HUH-7	Hepatocellular carcinoma (Liver)	Adherent	Human
HEK293T	Kidney	Adherent	Human
PANC-1	Pancreas (epithelioid carcinoma)	Adherent	Human
BxPC-3	Pancreas (adenocarcinoma)	Adherent	Human
MCF7	Breast (epithelial; adenocarcinoma)	Adherent	Human
1643	Neuroblastoma	Adherent	Human
MRC-9	Normal lung fibroblast (embryo)	Adherent	Human
697	B-cell leukaemia (early B-cell)	Suspension	Human

[0154] The eight human-derived cell lines as described above in Table 1 were grown in either DMEM, IMDM or RPMI media, supplemented with 10% FBS. For each cell line, 2×10^4 cells/well were transduced at a multiplicity of infection (MOI) of 1×10^5 vg/cell with AAV-FLF-PL64 (AAV with liver tropic capsid = SEQ ID NO: 20). All experiments were performed in duplicate. Cells in suspension were counted and transduced in serum-free media (300µl/well) into 48-well plates. For the adherent cell lines, media was aspirated, followed by washing with PBS (1X) and treatment with 5ml of TripLE for five minutes at 37°C, 5% CO_2, to dissociate the cells. The reaction was stopped by adding Sml of complete media. Dissociated cells were counted using a Countess™ II Automated Cell Counter (ThermoFisher) and centrifuged (250 x *g* for five minutes), followed by resuspension in complete media at a density of 2×10^5 cells/ml. These cells were plated into 96-well plates (2×10^4 cells/well) to adhere for five hours prior to transduction. Transduction mix was prepared in X-VIVO media (50 µl/well) and added to the cells. After three hours, 100µl/well of complete media was added. One day post-transduction, the media for each cell line was changed to complete media (+25mM HEPES for secretion analysis).

[0155] GCase activity was determined fluorometrically with 4-Methylumbelliferyl-β-D-glucopyranoside (4MU-Glc) as substrate.

[0156] GCase activity was measured from the culture supernatant for each cell line to determine the levels of GCase secreted following transduction with AAV-FLF-PL64 (Figure 10).

When the LSP-L promoter drives the GBA transgene, GCase secretion was detected in the HUH-7 cell line alone. The level of active GCase observed in HUH-7 cells was approximately 5.0 nmol/h/ml [5.1 ± 0.1 nmol/h/ml]. No detectable levels of active GCase were observed for any of the other cell lines analysed.

Example 8 - Comparison with ERT therapy

[0157] The goal of this example was to compare FLF-PL64 with VPRIV ® (60 U/kg BW) when administered in mice as a single injection. VPRIV® contains the same amino acid sequence and a similar glycosylation pattern as the native enzyme, GCase (i.e. SEQ ID NO: 25), and therefore provides a suitable comparison. Patients undergoing enzyme replacement therapy (ERT) would be typically treated with an IV infusion of ERT (duration of infusion of 1-2 hours, clinical dose of VPRIV® is 60 U/kg) on alternate weeks.

[0158] VPRIV ® powder (400 Units, Shire), for preparation of a solution for infusion, was obtained and maintained under refrigeration and protection from light until reconstitution. One vial (400U) was reconstituted with 4.3 ml of sterile water to achieve a solution at 100 U/ml, as recommended by the manufacturer. Following reconstitution, VPRIV solution was promptly snap frozen as single-use aliquots and stored at (-80 °C) for later use.

[0159] A single IV injection of either VPRIV ® (60 U/kg BW) or FLF-PL64 (formulated as AAV2/8 particles, 2×10^{12} vg/kg), was administered to wild type mice. Levels of active GCase in serum and tissue were determined at various time points for up to one week and also at three weeks and five weeks post-injection. The levels of active GCase were determined fluorometrically with 4-Methylumbelliferyl-β-D-glucopyranoside (4MU-Glc).

[0160] As shown in Figure 11(A), VPRIV is rapidly cleared from murine blood. VPRIV reached a Cmax of 12.7 µmol/h/ml at two minutes post-injection; with an estimated half-life of approximately 5.6 minutes. At approximately 20 minutes post-injection, only residual levels of active GCase could be detected in serum. These levels remained close to untreated controls for the remainder of the study period. A comparison of VPRIV with FLF-PL64 was made by analysing mice with stable expression of GCase (Figure 11B). Treatment with FLF-PL64 also led to increased levels of active GCase in murine blood (Cmax 9.4 µmol/h/ml) (Figure 11B). However, although levels of active GCase were not as high as observed post-VPRIV ® injection, these levels remained constant for the duration of the study. Table 2 below shows the predicted bioavailability during a 2-week interval in mice following injection of either ERT or FLF-PL64.

Table 2. Predicted bioavailability (AUC) during a 2-week interval in C57BL/6 mice upon single injection of ERT (60 U/kg BW) or AAV-FLF-PL64 (2×10^{12} vg/kg).

	ERT (VPRIV®)	FLF-PL64
AUC_{2week} (min.U/ml)	18.6 ± 5.6	3,161.6 ± 348.2

[0161] Figure 12 shows GCase immunostaining in murine liver, spleen and bone marrow following administration of VPRIV ® or AAV2/8-FLF-PL64. A representative image for each animal group is shown. DAB (3,3'-Diaminobenzidine) was used to visualise GCase and haematoxylin was used as counterstain. FLF-PL64 treated samples were obtained at five weeks post-injection, while VPRIV ® treated samples were collected as labelled. A semi-quantitative analysis of the images are represented in Table 3 below:

Table 3: Relative levels of GCase immunoreactivity observed in murine liver, spleen and bone marrow post-administration of either ERT (VPRIV ®) or FLF-PL64. "-" refers to negative staining for GCase; "+" represents positive staining for GCase.

Group	Liver	Spleen	Bone Marrow
Naive	-	-	-
ERT - 20 min	+	++	++
ERT - 60 min	+	+++	++
ERT - 240 min	+	-/+	-
ERT - 1440 min	-/+	-	-
FLF-PL64	+++	+++	++/+++

Example 9 - *in vivo* study of therapeutic potential

1. Methods

Mouse methods

[0162] 9V/null mice carrying the *Gba1* mutation D409V/D409V (9V/9V) were used as the Gaucher disease model in this study. 9V/null mice have a nearly normal lifespan with visceral abnormalities (inflammation and storage cells) and substrate accumulation (Xu et al. Am J Pathol. 2003 Nov;163(5):2093-101; Xu et al. PLoS One. 2010 May 20;5(5):e10750). 9V/null mice were generated by crossing mice carrying *Gba1* mutation D409V/D409V (9V/9V) and Gba1 null/WT. There are approximately two 9V/null produced in each litter. The strain background of 9V/null and WT mice are C57BL/6, 129SvEvBrd and FVB. 9V/null mice from multiple litters were randomly assigned into each treatment group on a rolling basis. Both male and female mice were enrolled in each group with an attempt to balance gender in the groups.

All mice were housed under pathogen-free conditions and were monitored daily and weighed weekly. All AAV treated mice showed normal growth and weight gain.

[0163] At the end of the study, mice were euthanized by pentobarbital (100 mg/kg). Mice were transcardially perfused with saline. Liver, spleen and lung were then dissected.

AAV/VPRIV preparation and administration

[0164] Aliquots of AAV8-FLF-PL64 were stored at -80°C. Before injection, the aliquot was thawed on ice and diluted with X-VIVO 10 (Lonza, pH7.4, 4°C), and gently mixed by vortexing briefly at low speed. The diluted AAV was kept on ice before injection and used within 2 hours.

[0165] VPRIV ® was resuspended and aliquoted (25, 50, 100 µl) and stored at -80°C. Before injection, the aliquot was thawed on ice and diluted with acidified X-VIVO 10 (Lonza, pH5.5, 4°C) to indicated dose, and gently mixed by vortexing briefly at low speed. The diluted enzyme was kept on ice before injection and used within 2 hours.

[0166] AAV (2×10^{12} vg/kg) and vehicle (X-vivo) were given one time to 9V/null mice at 8 weeks of age with indicated doses at 5 µL/g body weight (BW). WT mice were administrated with vehicle. AAV and vehicle administration were via tail vein to the mice while briefly under isoflurane. VPRIV ® was administered by tail vein bolus injection to 9V/null mice anesthetized with mixture of isoflurane and oxygen in bio-bubble room at 60U/kg and 2.5 µL/g BW, starting at 8 weeks of age, biweekly, for 7 injections.

Tissue collection

[0167] Blood (~100 µL) was collected from tail vein in a tube containing 0.5 M EDTA (5 µL) at 12 weeks, 16 weeks and 20 weeks of age. Freshly collected blood samples were kept on ice and separated to plasma to assay for GCase activity within 2 hours. Each plasma collection and activity assay from the VPRIV ® treatment group was performed within 2 hours after the scheduled enzyme injection. A separate portion of blood (~400 µL) was processed to isolate white blood cells (WBC) for GCase activity assay. Collected WBC was stored at -80°C.

[0168] Tissues (liver, lung, spleen, bone marrow) were collected at experimental endpoint (20 weeks of age). Tissue collection from the VPRIV ® group was performed within 2 hours after the final scheduled enzyme injection. Liver, lung and spleen samples were divided into 4 parts, with 3 parts frozen in individual tubes and stored at -80°C prior to GCase activity assay, protein and substrate analysis. The remaining part was fixed in 10% Formalin for histology analysis. Bone marrow cells were collected from femurs and tibias of both legs of the mice and frozen in two tubes stored at -80°C freezer for GCase activity and substrate assays.

GCase activity assay

[0169] Tissues were homogenized in 1% Na taurocholate and 1% Triton X-100 (Tc/Tx) using a Precellys Evolution tissue homogenizer for two cycles (20 seconds each, 30 seconds interval) at 4°C. Cells (bone marrow (BM) and white blood cells (WBC)) were homogenized in 1% Tc/Tx with sonication at 4°C. Tissue and cell lysates (2 µL) were diluted (5 x) with reaction buffer in assay mixture (0.025 M Citrate-phosphate buffer, pH5.6). Diluted lysates (10 µL) (in triplicate per sample) were loaded to reaction plate. GCase activity was determined fluorometrically with 4-methylumberlliferyl-β-D-glucopyranoside (4MU-Glucose, 4 mM) (Biosynth AG, Switzerland) in the presence and absence of 2 mM Conduritol B epoxide (Millipore. CA) incubated for 1 hour at 37°C. Protein concentrations were determined using BCA Protein Assay Reagent (Pierce, Rockford, IL).

[0170] Plasma was diluted in 0.025 M Citrate-phosphate buffer, pH5.6. GCase activity was determined fluorometrically with 4-methylumberlliferyl-β-D-glucopyranoside (4MU-Glucose, 4 mM) (Biosynth AG, Switzerland) as above.

Substrate analysis

[0171] Frozen tissues were weighed and homogenized in 3.6 mL of Methanol/Chloroform/H_2O (2:1:0.6 v/v/v). Aliquots (500 µL) of lysate were subjected to LC/MS analysis. The quantitated hexosylceramide and hexosylsphingosine were normalized by tissue weight.

[0172] Plasma was diluted in water (40 µL plasma + 60 µL water) and subjected to LC/MS analysis. Substrate level was normalized by plasma volume.

[0173] Bone marrow cells were suspended in 200 µL water and sonicated and vortexed to make cell lysate. 160 µL lysate was subjected to LC/MS analysis. Remaining lysate was determined for protein concentration. Substrate level was normalized by mg protein.

[0174] LC/MS analysis was performed to analyse hexosylceramide and hexosylsphingosine concentrations. Since galatosylceramide and galatosylsphingosine levels are very low in this mouse model model, measured hexosylceramide and hexosylsphingosine concentrations represent levels of glucosylceramide and glucosylsphingosine, respectively.

Histology analysis

[0175] Liver, lung, spleen and bone marrow were dissected from saline perfused mice and fixed in Formalin (10%) and paraffin embedded. Fixed tissues were cut into 4 µm sections and

mounted on slides.

Storage cell count

[0176] Tissue sections were stained with hematoxylin and eosin (H&E) by Autostainner (Leica Autostainner XL). The stained tissues were scanned with Aperio AT2 (Leica, 40X). The tissue images were processed with Aperio ImageScope (V12.4.0.0543). 10 photos of 20X magnitude (500 µm X 800 µm image) from liver and lung per mouse were chosen for analysis. Storage cells were counted from each image. Average of cell counts from 10 images was calculated for data graph. Definition of "storage cells" is based on the size of cells (macrophage), *e.g.* size of storage cells in liver is > 10 µm, in lung is > 15 µm.

CD68 staining and quantification

[0177] Tissue sections were stained with rabbit anti-mouse CD68 antibody (1:25. Abcam Ab53444) in Discover Ultra automated IHC/ISH slide staining machine. The tissues were counter-stained with hematoxylin on cell nuclei. Stained tissues were scanned with Aperio AT2 (Leica, 40X) and the images were acquired by Aperio ImageScope (V12.4.0.0543). The images of liver and lung at 20X magnitude (500 µm X 800 µm) were used for quantitative analysis. IHC signals from 5 images of liver or lung per mouse were analysed using Image J (Fiji, v5.1). Average CD68 signals per mouse was calculated for data graph.

Statistical analyses

[0178] The data was analysed by Student's t-test or OneWay ANOVA. Figure graphs and statistical analysis were generated by PRISM 8 software (PRISM version 8.0.1).

2. Results

GCase activity

[0179] AAV-FLF-PL64 treatment to restore active GCase levels in 9V/null mice was studied by measuring GCase activity in cells and tissues. White blood cells (WBC), bone marrow and tissue samples were collected at the experimental end point as above (i.e. 12 weeks post AAV-FLF-PL64 injection or on the final VPRIV ® administration), when the mice are at 20 weeks of age.

[0180] VPRIV ® was shown to increase the activity across all cells and tissues tested (Figure 13). As stated above, tissues of the VPRIV ® treated group were collected within 2 hours post last injection, and this is in line with previous data showing that this is within the period where VPRIV ® is at its C-max in the tissues.

[0181] AAV-FLF-PL64 was shown to also significantly increase GCase activity in all tissues following only a single administration (Figure 13). Compared to Vehicle-9V/null, liver GCase activity increased by 4.7-fold, and spleen GCase activity increased by 2.5-fold. In white blood cells GCase activity was seen to significantly increase in the AAV-FLF-PL64 treated groups by 7-9 fold. In particular, the GCase activity level in white blood cells reached to about 82% of WT activity levels.

Tissue Histology

[0182] Visceral pathology in 9V/null mice was determined by counting foamy macrophages as storage cells and quantitating CD68 staining signals on activated macrophages. The storage cells were counted in H&E stained liver sections. CD68 signal (brown colour) intensity was quantified on anti-CD68 antibody stained liver and lung sections.

[0183] Storages cells at size ≥ 10 μm in liver were counted from 10 images per tissues of each mouse. In liver, the number of storage cells was undetectable in the AAV-FLF-PL64 treated groups, as well as in the VPRIV ® group. (Figure 14)
CD68 signals in liver were also significantly decreased in the AAV-FLF-PL64 treated groups. AAV-FLF-PL64 treatment reduced CD68 signals to about 25% of Vehicle-9V/null level. In comparison, CD68 signals in VPRIV group was about 37% of Vehicle-9V/null level. (Figure 14)

Substrate accumulation

[0184] 9V/null mice are known to develop glycolipid substrates accumulation in liver, lung and spleen (Xu et al. PLoS One. 2010 May 20;5(5):e10750). For example, the study showed that hexosylceramide in the control Vehicle-9V/null group is above WT level by 7.97-fold in liver and 3.57-fold in spleen (data not shown).

[0185] AAV-FLF-PL64 treated groups showed significant reduction of hexosylceramide and hexosylsphingosine in the liver and spleen compared to Vehicle-9V/null (Figure 15). In particular, AAV-FLF-PL64 treated groups had hexosylceramide levels reduced to 1.20-fold times the wild-type level in liver and 1.03-fold times the wild-type level in spleen (data not shown). Similar reduction to close to the WT level was seen upon analysis of bone marrow (data not shown).

[0186] On the other hand, VPRIV ® treatment only showed a significant reduction of hexosylceramide in liver, with the other tested tissues showing no significant changes in the hexosylceramide levels. VPRIV ® did not appear to have any significant effect on hexosylphingosine levels in any tested tissue.

REFERENCES CITED IN THE DESCRIPTION

Cited references

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PATENTKRAV

1. Polynukleotid, der omfatter en GBA-nukleotidsekvens, hvor GBA-nukleotidsekvensen koder for et β-Glucocerebrosidase- (GCase) protein, og hvor GBA-nukleotidsekvensen omfatter en sekvens, der er:

(i) 100 % identisk med SEQ ID NO: 1 eller SEQ ID NO: 5;

(ii) mindst 95 %, mindst 96 %, mindst 97 %, mindst 98 %, mindst 99 %, mindst 99,5 % eller mindst 99,8 % identisk med SEQ ID NO: 1 eller SEQ ID NO: 5, hvor GCase kodet for af GBA-nukleotidsekvensen har GCase-aktivitet og udtrykkes i humane leverceller ved højere niveauer sammenlignet med en GCase kodet for af en vildtype-GBA-nukleotidsekvens i et andet ellers identisk referencepolypeptid; og/eller

(iii) en variant af SEQ ID NO: 1 eller SEQ ID NO: 5, der koder for et GCase-protein med GCase-aktivitet, hvor varianten er identisk med henholdsvis SEQ ID NO: 1 eller SEQ ID NO: 5 bortset fra, at den omfatter nukleotidsubstitutioner, således at GCase-proteinet har 1, op til 2, op til 3, op til 4, op til 5, op til 6, op til 7, op til 8, op til 9 eller op til 10 aminosyresubstitutioner i forhold til vildtype-GCase-aminosyresekvensen ifølge SEQ ID NO: 25, endvidere GCase kodet for af GBA-nukleotidet udtrykkes i humane leverceller ved højere niveauer sammenlignet med a GCase kodet for af en vildtype-GBA-nukleotidsekvens i et andet ellers identisk referencepolypeptid.

2. Polynukleotid ifølge krav 1, hvor:

(a) varianten er en variant af SEQ ID NO: 1 og varianten af SEQ ID NO: 1:

(i) er identisk med SEQ ID NO: 1 bortset fra, at den omfatter nukleotidsubstitutioner, således at GCase-proteinet har 1, op til 2, op til 3, op til 4, op til 5, op til 6, op til 7, op til 8, op til 9 eller op til 10 aminosyresubstitutioner i forhold til vildtype-GCase-aminosyresekvensen ifølge SEQ ID NO: 25;

(ii) har 1, op til 2, op til 3, op til 4, op til 5, op til 6, op til 7, op til 8, op til 9, op til 10, op til 20 eller op til 30 nukleotidsubstitutioner i forhold til sekvensen ifølge SEQ ID NO: 1;

(iii) har 1, op til 2, op til 3, op til 4, op til 5 eller op til 6 nukleotidsubstitutioner i forhold til sekvensen ifølge SEQ ID NO: 1;

(iv) har op til 4 nukleotidsubstitutioner i forhold til sekvensen ifølge SEQ ID NO: 1 og/eller koder for et GCase-protein med op til 3 aminosyresubstitutioner i forhold til vildtype-aminosyre-GCase-sekvensen ifølge SEQ ID NO: 25;

(v) har op til 3 nukleotidsubstitutioner i forhold til sekvensen ifølge SEQ ID NO: 1 og/eller koder for et GCase-protein med op til 2 aminosyresubstitutioner i forhold til vildtype-GCase-aminosyresekvensen ifølge SEQ ID NO: 25; og/eller

(vi) har 1 nukleotidsubstitution i forhold til sekvensen ifølge SEQ ID NO: 1 og/eller koder for et GCase-protein med op til 1 aminosyresubstitution i forhold til vildtype-GCase-aminosyresekvensen ifølge SEQ ID NO: 25; eller

(b) varianten er en variant af SEQ ID NO: 5 og varianten af SEQ ID NO: 5:

(i) er identisk med SEQ ID NO: 5 bortset fra, at den omfatter nukleotidsubstitutioner, således at GCase-proteinet har 1, op til 2, op til 3, op til 4, op til 5, op til 6, op til 7, op til 8, op til 9 eller op til 10 aminosyresubstitutioner i forhold til vildtype-GCase-aminosyresekvensen ifølge SEQ ID NO: 25;

(ii) har 1, op til 2, op til 3, op til 4, op til 5, op til 6, op til 7, op til 8, op til 9, op til 10, op til 20 eller op til 30 nukleotidsubstitutioner i forhold til sekvensen ifølge SEQ ID NO: 5;

(iii) har 1, op til 2, op til 3, op til 4, op til 5 eller op til 6 nukleotidsubstitutioner i forhold til sekvensen ifølge SEQ ID NO: 5;

(iv) har op til 4 nukleotidsubstitutioner i forhold til sekvensen ifølge SEQ ID NO: 5 og/eller koder for et GCase-protein med op til 3 aminosyresubstitutioner i forhold til vildtype-aminosyre-GCase-sekvensen ifølge SEQ ID NO: 25;

(v) har op til 3 nukleotidsubstitutioner i forhold til sekvensen ifølge SEQ ID NO: 5 og/eller koder for et GCase-protein med op til 2 aminosyresubstitutioner i forhold til vildtype-GCase-aminosyresekvensen ifølge SEQ ID NO: 25; og/eller

(vi) har 1 nukleotidsubstitution i forhold til sekvensen ifølge SEQ ID NO: 5 og/eller koder for et GCase-protein med op til 1 aminosyresubstitution i forhold til vildtype-GCase-aminosyresekvensen ifølge SEQ ID NO: 25.

3. Polynukleotid ifølge krav 1 eller 2, hvor varianten har:

(i) op til 3 aminosyresubstitutioner i forhold til vildtype-GCase-aminosyresekvensen ifølge SEQ ID NO: 25;

(ii) op til 2 aminosyresubstitutioner i forhold til vildtype-GCase-aminosyresekvensen ifølge SEQ ID NO: 25; og/eller

(iii) op til 1 aminosyresubstitution i forhold til vildtype-GCase-aminosyresekvensen ifølge SEQ ID NO: 25.

4. Polynukleotid ifølge et hvilket som helst af de foregående krav, hvor GCase kodet for af GBA-nukleotidsekvensen udtrykkes i humane leverceller ved højere niveauer sammenlignet med GCase kodet for af en vildtype-GBA-nukleotidsekvens i et andet ellers identisk referencepolypeptid og hvor:

(a) GBA-nukleotidsekvensen er kodonoptimeret for ekspression i humane leverceller; og/eller

(b) GBA-nukleotidsekvensen omfatter et reduceret antal CpG'er sammenlignet med en vildtype-GBA-nukleotidsekvens; eventuelt hvor GBA-nukleotidsekvensen omfatter færre end 40, færre end 20, færre end 18, færre end 10, eller færre end 5 CpG'er, mere eventuelt hvor GBA-nukleotidsekvensen omfatter færre end 5, færre end 4, færre end 3, eller færre end 2 CpG'er pr. 100 nukleotider, mere eventuelt hvor GBA-nukleotidsekvensen er CpG-fri, fortrinsvis hvor vildtype-GBA-nukleotidsekvensen er SEQ ID NO: 9.

5. Polynukleotid ifølge et hvilket som helst af de foregående krav, hvor polynukleotidet endvidere omfatter et regulatorisk transskriptionselement, eventuelt hvor det regulatoriske transskriptionselement omfatter en leverspecifik promotor og/eller en forstærker; mere eventuelt hvor:

(I)

(a) det regulatoriske transskriptionselement omfatter en A1AT-promotor eller et fragment af en A1AT-promoter, eventuelt hvor:

(i) A1AT-promotoren eller fragmentet af en A1AT-promotor er mindst 100, mindst 120, mindst 150, mindst 180, færre end 255, mellem 100 og 255, mellem 150 og 225, mellem 150 og 300 eller mellem 180 og 255 nukleotider i længden, mere eventuelt hvor fragmentet af en A1AT-promotor er mellem 180 og 255 nukleotider i længden;

(ii) A1AT-promotoren eller fragmentet af en A1AT-promotor er mindst 200, mindst 250, mindst 300, færre end 500, mellem 200 og 500, mellem 250 og 500, mellem 350 og 450 eller 418 nukleotider i længden, mere eventuelt hvor fragmentet af en A1AT-promotor er mellem 350 og 450 nukleotider i længden; og/eller

(iii) polynukleotidet omfatter en promotor, der er mindst 80 %, mindst 85 %, mindst 90 %, mindst 95 %, mindst 98 %, mindst 99 %, mindst 99,5 %, mindst 99,8 %, eller 100 % identisk med SEQ ID NO: 15 eller SEQ ID NO: 12; og/eller

(b) hvor forstærkeren er en HCR-forstærker eller et fragment af en HCR-forstærker, eventuelt hvor:

(i) HCR-forstærkeren eller fragmentet af en HCR-forstærker er et fragment af mindst 80, mindst 90, mindst 100, færre end 192, mellem 80 og 192, mellem 90 og 192, mellem 100 og 250 eller mellem 117 og 192 nukleotider i længden, mere eventuelt hvor fragmentet af en HCR-forstærker er mellem 117 og 192 nukleotider i længden;

(ii) HCR-forstærkeren eller fragmentet af en HCR-forstærker er et fragment af mindst 150, mindst 190, mindst 230, færre end 400, mellem 150 og 400, mellem 190 og 370, mellem 230 og 340, mellem 250 og 340 eller 321 nukleotider i længden, mere eventuelt hvor fragmentet af en HCR-forstærker er mellem 250 og 340 nukleotider i længden; eller

(iii) polynukleotidet omfatter en forstærker, der er mindst 80 %, mindst 85 %, mindst 90 %, mindst 95 % mindst 98 %, mindst 99 %, mindst 99,5 %, mindst 99,8 % eller 100 % % identisk med SEQ ID NO: 16 eller SEQ ID NO: 11; eller

(II) hvor det regulatoriske transskriptionselement er mindst 80 %, mindst 85 %, mindst 90 %, mindst 95 %, mindst 98 %, mindst 99 %, mindst 99,5 %, mindst 99,8 %, eller 100 % identisk med SEQ ID NO: 14 eller 10.

6. Polynukleotid ifølge et hvilket som helst af de foregående krav, hvor GCase kodet for af GBA-nukleotidsekvensen udtrykkes i humane leverceller mindst 1,1x, mindst 1,2x, mindst 1,3x, mindst 1,4x eller mindst 1,5x højere sammenlignet med en GCase kodet for af en vildtype-GBA-nukleotidsekvens i et andet ellers identisk referencepolypeptid, eventuelt hvor referencepolypeptidet omfatter en vildtype-GBA-nukleotidsekvens ifølge SEQ ID NO: 9, mere eventuelt hvor referencepolypeptidet omfatter en promotor af SEQ ID NO: 13.

7. Viruspartikel, der omfatter et rekombinant genom omfattende polynukleotidet ifølge et hvilket som helst af de foregående krav; eventuelt hvor:

(a) viruspartiklen er en AAV, adenoviral, eller lentiviral viruspartikel, mere eventuelt en AAV viruspartikel;

(b) viruspartiklen omfatter et lever-tropisk eller CNS-tropisk capsid, mere eventuelt hvor: (i) det lever-tropiske capsid omfatter en sekvens mindst 98 %, mindst 99 %, eller mindst 99,5 % identisk med et fragment af mindst 600, mindst 650, mindst 700, mellem 600 og 736, mellem 650 og 736 eller mellem 700 og 736 aminosyrer ifølge SEQ ID NO: 19, 20 eller 24, eventuelt hvor det lever-tropiske capsid omfatter en sekvensmindst 99 % identisk med SEQ ID NO: 19 eller 20; eller (ii) hvor CNS-tropiske capsid omfatter en sekvens mindst 98 %, mindst 99 % eller mindst 99,5 % identisk med et fragment af mindst 600, mindst 650, mindst 700, mellem 600 og 736, mellem 650 og 736 eller mellem 700 og 736 aminosyrer ifølge SEQ ID NO: 21, eventuelt hvor det CNS-tropiske capsid omfatter en sekvens mindst 99 % identisk med SEQ ID NO: 21;

(c) det rekombinante genom endvidere omfatter:

i) AAV2 ITR'er;

ii) en poly-A-sekvens og/eller

iii) en intron;

eventuelt hvor det rekombinante genom er enkeltstrenget; og/eller

(d) ved transduktion ind i Huh-7-celler, viruspartiklen udtrykker GCase, således at GCase-aktiviteten i den transducerede celle er større end aktiviteten af GCase i en celle transduceret med en ellers identisk viruspartikel omfattende en GBA-nukleotidsekvens ifølge SEQ ID NO: 9, eventuelt hvor ved transduktion ind i Huh-7-celler,

viruspartiklen udtrykker GCase, således at GCase-aktiviteten i den transducerede celle er mindst 2x, mindst 3x, mindst 4x, mindst 5x, mindst 10x eller mindst 20x større end aktiviteten af GCase i en celle transduceret med en ellers identisk viruspartikel omfattende en GBA-nukleotidsekvens ifølge SEQ ID NO: 9, mere eventuelt hvor aktiviteten måles ved hjælp af et fluorometrisk substrat, der er specifikt for GCase.

8. Sammensætning, der omfatter polynukleotidet eller viruspartiklen ifølge et hvilket som helst af de foregående krav og et farmaceutisk acceptabelt hjælpestof.

9. Polynukleotid, viruspartikel eller sammensætning ifølge et hvilket som helst af de foregående krav til anvendelse i en behandlingsfremgangsmåde, eventuelt hvor behandlingsfremgangsmåden omfatter administration af en virksom mængde af polynukleotidet, sammensætningen eller viruspartiklen ifølge et hvilket som helst af krav 1 til 8 til en patient; mere eventuelt hvor behandlingsfremgangsmåden er:

(a) en fremgangsmåde til behandling af Parkinsons sygdom; eller

(b) en fremgangsmåde til behandling af Gauchers sygdom, eventuelt hvor:

(i) Gauchers sygdom er Gauchers sygdom type I, II eller III; og/eller

(ii) patienten har antistoffer eller hæmmere mod en rekombinant GCase, hvormed patienten tidligere er behandlet som led i en enzymerstatningsterapi.

10. Polynukleotid, viruspartikel eller sammensætning ifølge et hvilket som helst af krav 1 til 8 til anvendelse i en behandlingsfremgangsmåde, hvilken fremgangsmåde omfatter: (a) ekspression af GBA-nukleotidsekvensen og opnåelse af en stabil GCase-aktivitet hos et individ; eller (b) ekspression af GBA-nukleotidsekvensen og tilvejebringelse af større GCase-biotilgængelighed hos et

individ sammenlignet med tilgængeligheden fra GCase-enzymerstatningsterapi, hvor tilgængeligheden måles over en periode på 2 uger fra administration.

11. Polynukleotid, viruspartikel eller sammensætning til anvendelse i behandlingsfremgangsmåden ifølge krav 10 hvor:

(a) opnåelse af en stabil GCase-aktivitet og/eller tilvejebringelse af større GCase-biotilgængelighed fører til behandling af en sygdom hos individet, eventuelt hvor sygdommen er Gauchers sygdom, mere eventuelt hvor Gauchers sygdom er Gauchers sygdom type I, II eller III; og/eller

(b)

(i) GCase-aktiviteten er stabil ved et niveau på mindst 1, mindst 2, mindst 3, mindst 4, mindst 5, mindst 6, mindst 7, mindst 8, eller mindst 9 µmol/h/ml hos individet;

(ii) GCase-aktiviteten er stabil ved et niveau på mindst 3 µmol/h/ml hos individet;

(iii) GCase-aktiviteten er stabil ved et niveau på mindst 5 µmol/h/ml hos individet;

(iv) GCase-aktiviteten er stabil ved et niveau på mindst 9 µmol/h/ml hos individet;

(v) fremgangsmåden omfatter administration af en virksom dosis af polynukleotidet, viruspartiklen eller sammensætningen til individet;

(vi) den stabile GCase-aktivitet er en GCase-aktivitet på mindst 10 %, mindst 20 %, mindst 30 %, mindst 40 %, eller mindst 50 % i forhold til GCase-aktiviteten hos et sundt individ;

(vii) den stabile GCase-aktivitet er en GCase-aktivitet på mellem 10 % og 100 %, mellem 20 % og 90 %, mellem 30 % og 70 %, mellem 40 % og 70 %, eller mellem 50 % og 70 % i forhold til GCase-aktiviteten hos et sundt individ;

(viii) den stabile GCase-aktivitet er stabil i mindst 5 uger fra administration;

(ix) den stabile GCase-aktivitet er stabil i mindst 10 uger fra administration;

(x) den stabile GCase-aktivitet er stabil i mindst 15 uger fra administration;

(xi) den stabile GCase-aktivitet er stabil i mindst 20 uger fra administration;

(xii) den stabile GCase-aktivitet er stabil i mindst 25 uger fra administration;

(xiii) den stabile GCase-aktivitet er stabil i mindst 30 uger fra administration;

(xiv) den stabile GCase-aktivitet er stabil i mindst 35 uger fra administration;

(xv) den stabile GCase-aktivitet er stabil i mindst 40 uger efter administration;

(xvi) GCase-aktiviteten i individets lever, milt og/eller knoglemarv mindst 5, mindst 10, mindst 15, mindst 20, mindst 25, mindst 30 eller mindst 35 uger efter administration er større ved sammenligning med aktiviteten målt hos et individ, der har fået administreret dosis af en GCase-enzymerstatningsterapi, ved måling i det samme assay på det samme tidspunkt efter administration; og/eller

(xvii) GCase-biotilgængeligheden i individets lever, milt og/eller knoglemarv over en periode på mindst 5, mindst 10, mindst 15, mindst 20, mindst 25, mindst 30 eller mindst 35 uger efter administration er større ved sammenligning med tilgængeligheden målt hos et individ, der har fået administreret dosis af en GCase-enzymerstatningsterapi, ved måling i det samme assay på det samme tidspunkt efter administration;

og eventuelt hvor sygdommen er Gauchers sygdom, mere eventuelt hvor Gauchers sygdom er Gauchers sygdom type I, II eller III.

12. Polynukleotid, viruspartikel eller sammensætning ifølge et hvilket som helst af krav 1 til 8, til anvendelse i en fremgangsmåde til reduktion af hexosylceramid- og/eller hexosylsphingosinniveauer hos et individ, der har Parkinsons sygdom eller Gauchers sygdom, eventuelt hvor reduktion af hexosylceramid- og/eller hexosylsphingosinniveauer fører til behandling af Parkinsons sygdom eller Gauchers sygdom, mere eventuelt hvor:

(i) hexosylceramid- og/eller hexosylsphingosinniveauerne reduceres med 2 gange eller mere, 3 gange eller mere, 4 gange eller mere, 5 gange eller mere, 6 gange eller mere, 2 til 3 gange, 2 til 4 gange, 2 til 5 gange, 2 til 6 gange eller 3 til 5 gange ved sammenligning med hexosylceramid- og/eller hexosylsphingosinniveauerne på tidspunktet for administration af polynukleotidet, viruspartiklen eller sammensætningen ifølge et hvilket som helst af krav 1 til 8;

(ii) reduktionen i hexosylceramid- og/eller hexosylsphingosinniveauer er større end reduktionen opnået hos et individ, der har fået administreret dosis af en GCase-enzymerstatningsterapi, eventuelt når hexosylceramid- og/eller hexosylsphingosinniveauerne måles mindst 6 uger, mindst 8 uger, mindst 10 uger eller mindst 12 uger efter administration;

(iii) hexosylceramid- og/eller hexosylsphingosinniveauerne måles i individets makrofager;

(iv) hexosylceramid- og/eller hexosylsphingosinniveauerne måles i individets milt;

(v) hexosylceramid- og/eller hexosylsphingosinniveauerne måles i individets lever;

(vi) hexosylceramid- og/eller hexosylsphingosinniveauerne måles i individets serum;

(vii) hexosylceramid- og/eller hexosylsphingosinniveauerne måles ved massespektrometri; og/eller

(viii) Gauchers sygdom er Gauchers sygdom type I, II eller III.

13. Polynukleotid, viruspartikel eller sammensætning til anvendelse ifølge et hvilket som helst af krav 9 til 12, hvor patienten har antistoffer eller hæmmere mod en rekombinant GCase, hvormed patienten tidligere er behandlet som led i en enzymerstatningsterapi.

DRAWINGS

FIGURE 1

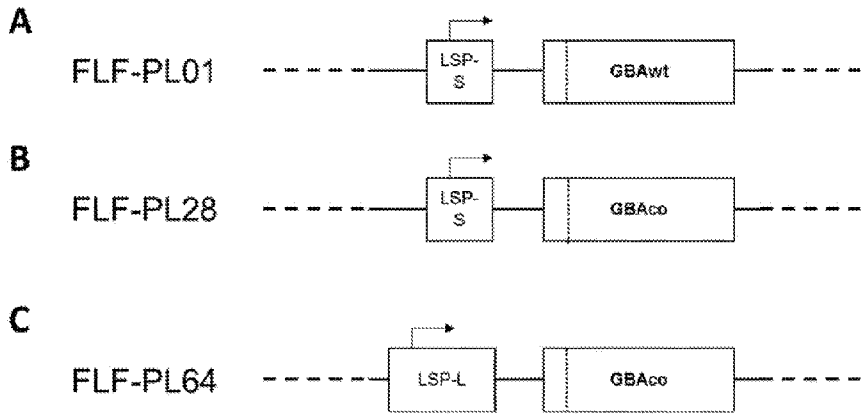


FIGURE 2

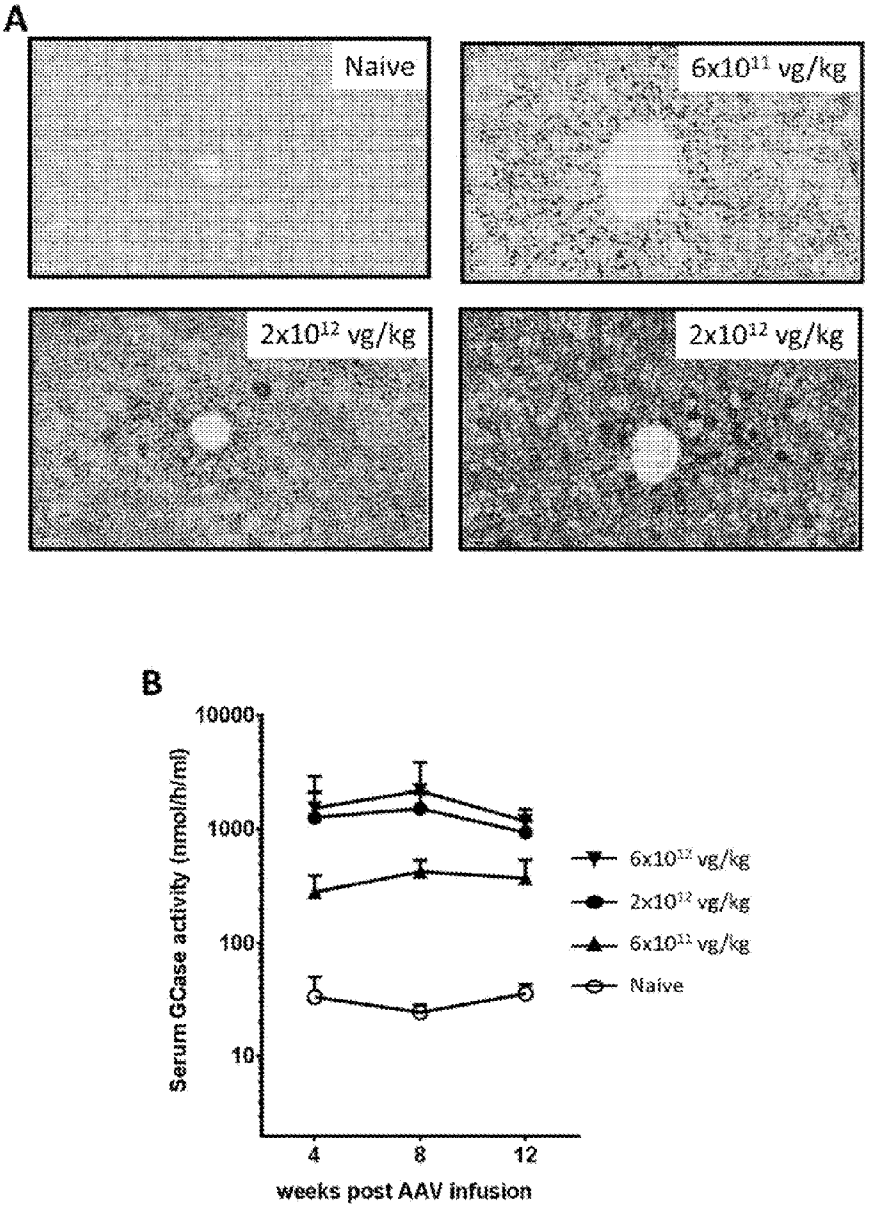

A
Naive
6x10^11 vg/kg
2x10^12 vg/kg
2x10^12 vg/kg
B
Serum GCase activity (nmol/h/ml)
10000
1000
100
10
4
8
12
weeks post AAV infusion
6x10^12 vg/kg
2x10^12 vg/kg
6x10^11 vg/kg
Naive

FIGURE 3

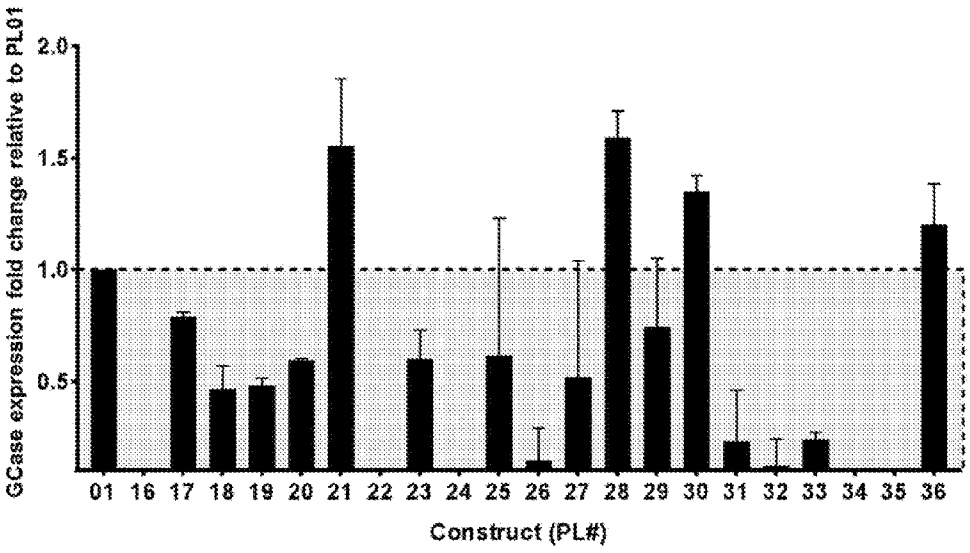

GCase expression fold change relative to PL01
2.0
1.5
1.0
0.5
01 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36
Construct (PL#)

FIGURE 4

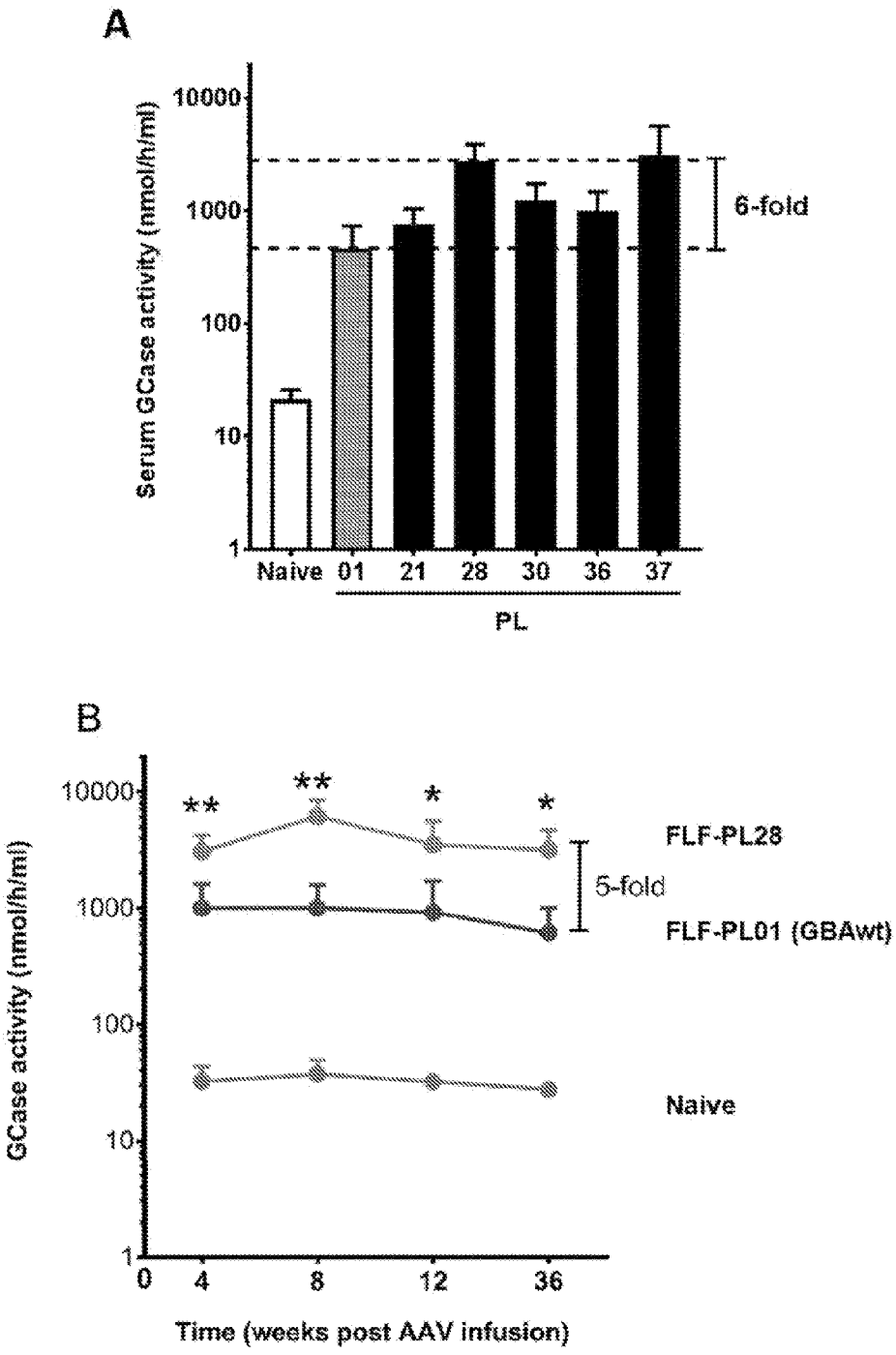

A
Serum GCase activity (nmol/h/ml)
10000
1000
100
10
1
6-fold
Naive
01
21
28
30
36
37
PL
B
GCase activity (nmol/h/ml)
10000
1000
100
10
1
**
**
*
*
FLF-PL28
5-fold
FLF-PL01 (GBAwt)
Naive
0
4
8
12
36
Time (weeks post AAV infusion)

FIGURE 5

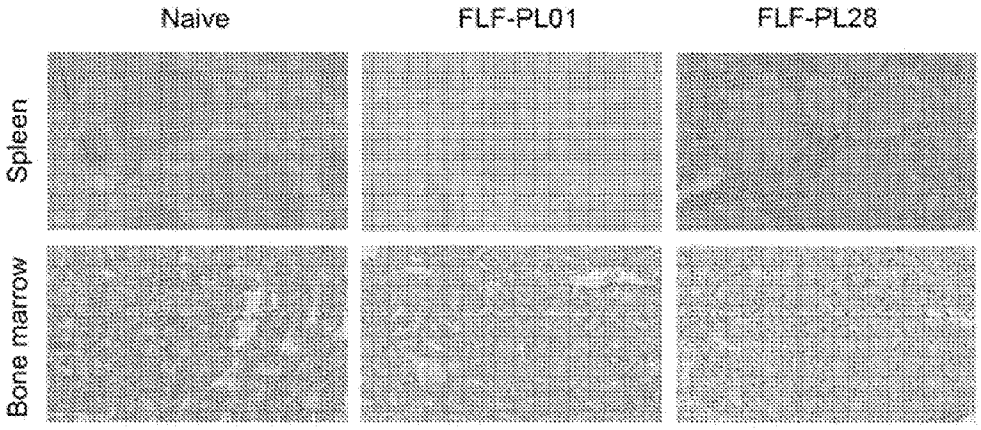


FIGURE 6

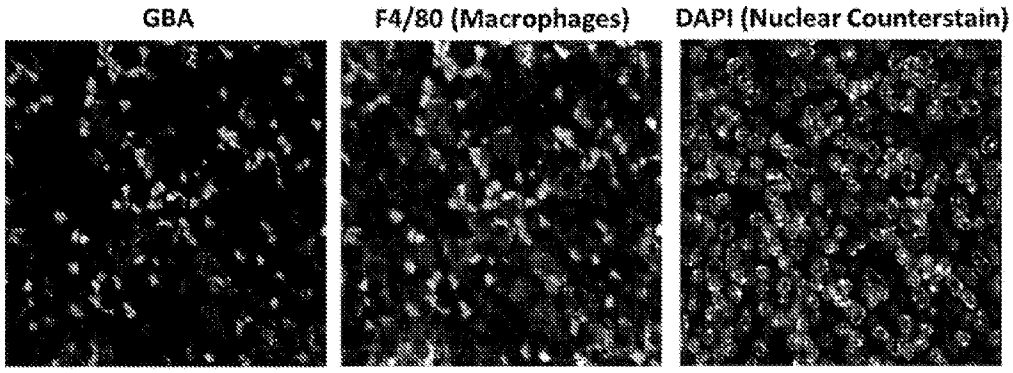


FIGURE 7

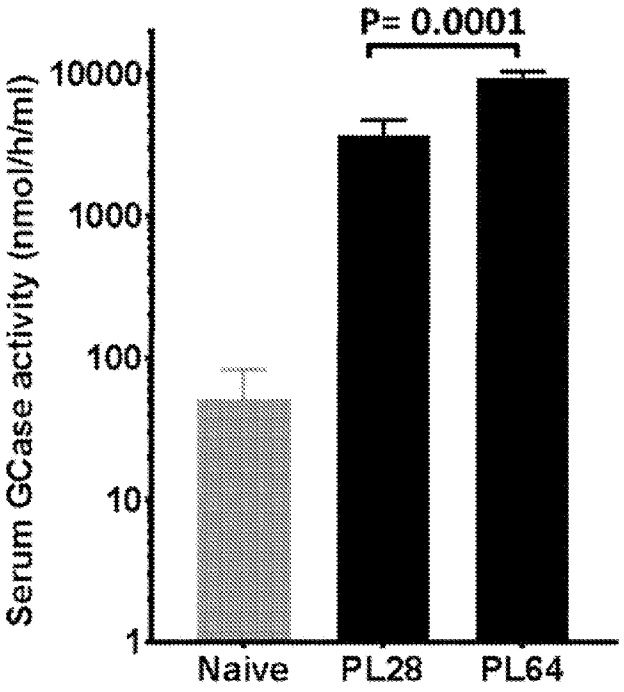

P= 0.0001
Serum GCase activity (nmol/h/ml)
10000
1000
100
10
1
Naive
PL28
PL64

FIGURE 8

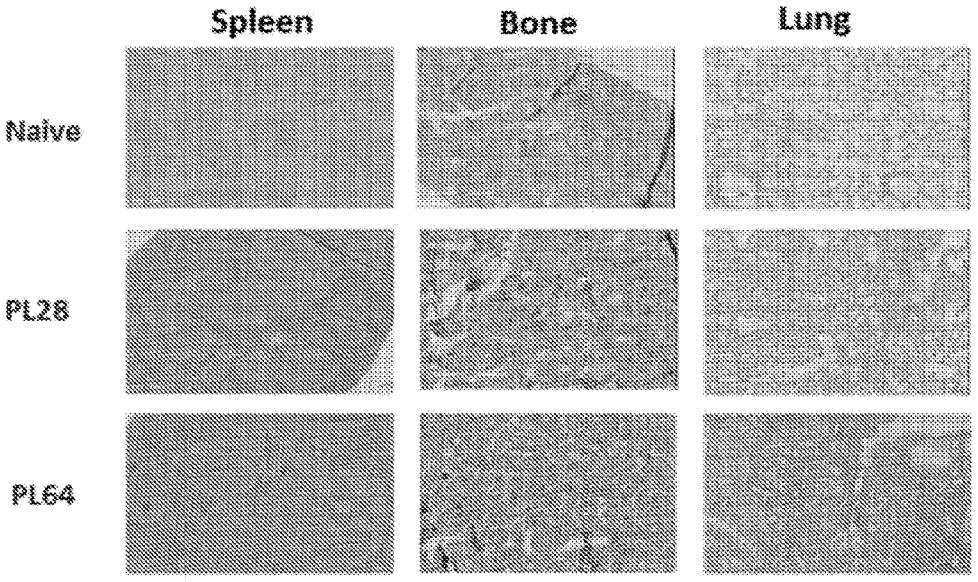


FIGURE 9

SEQ ID NO: 1 - Codon-optimised GBA nucleotide sequence from FLF-PL28, without signal peptide portion

GCCAGGCCCTGCATCCCTAAGAGCTTTGGCTACAGCTCTGTGGTGTGTGTGTGCAATGCCACCTAC
TGTGACAGCTTTGACCCCCCCACCTTTCCTGCCCTGGGCACCTTCAGCAGATATGAGAGCACCAGGT
CTGGGAGGAGGATGGAGCTGAGCATGGGGCCCATCCAGGCTAATCACACTGGCACTGGCCTGCTG
CTGACCCTGCAGCCTGAGCAGAAGTTCCAGAAAGTAAAGGGCTTTGGAGGGGCCATGACTGATGCT
GCTGCTCTGAACATCCTGGCCCTGAGCCCCCCTGCCCAGAATCTGCTGCTGAAGAGCTACTTCTCTG
AGGAGGGCATTGGCTATAACATCATCAGGGTGCCCATGGCCAGCTGTGACTTCAGCATCAGGACCT
ACACCTATGCTGACACCCCTGATGATTTCCAGCTGCACAACTTCAGCCTGCCTGAGGAGGATACCAA
GCTGAAGATCCCACTGATCCACAGGGCTCTGCAGCTGGCCCAGAGGCCTGTGAGCCTGCTGGCCAG
CCCCTGGACCAGCCCCACTTGGCTGAAGACCAATGGGGCTGTGAATGGGAAGGGGAGCCTGAAGG
GACAGCCTGGAGACATCTACCACCAGACCTGGGCCAGATACTTTGTGAAGTTCCTGGATGCCTATGC
TGAGCACAAGCTGCAGTTCTGGGCTGTGACTGCTGAGAATGAGCCTTCTGCTGGGCTGCTGTCTGG
CTACCCCTTCCAATGCCTGGGCTTCACCCCTGAGCATCAGAGGGACTTCATTGCCAGGGACCTGGG
CCCTACCCTGGCCAACAGCACTCACCATAATGTTAGGCTGCTGATGCTGGATGACCAGAGGCTGCT
GCTGCCCCACTGGGCTAAGGTGGTGCTGACTGACCCTGAGGCTGCTAAATATGTGCATGGCATTGC
TGTGCATTGGTACCTGGACTTTCTGGCTCCTGCCAAGGCCACCCTGGGGGAGACCCACAGGCTGTT
CCCCAACACCATGCTGTTTGCCTCTGAGGCCTGTGTGGGCAGCAAGTTCTGGGAGCAGTCTGTGAG
GCTGGGCAGCTGGGATAGGGGGATGCAGTACAGCCACAGCATCATCACCAACCTGCTGTACCATGT
GGTGGGCTGGACTGACTGGAACCTGGCCCTGAACCCTGAGGGAGGACCTAACTGGGTCAGAAACTT
TGTGGACAGCCCCATCATTGTGGACATCACCAAGGACACCTTTTACAAGCAGCCCATGTTCTACCAC
CTGGGCCACTTCAGCAAGTTCATCCCTGAGGGCAGCCAGAGAGTGGGGCTGGTGGCCAGCCAGAA
GAATGACCTGGATGCTGTGGCTCTGATGCATCCTGATGGCTCTGCTGTGGTGGTGGTGCTGAACAG
GAGCTCTAAGGATGTGCCTCTGACCATCAAGGATCCTGCTGTGGGCTTCCTGGAGACCATCAGCCC
TGGCTACAGCATCCACACCTACCTGTGGAGGAGGCAGTGA

SEQ ID NO: 2 - Codon-optimised GBA nucleotide sequence from FLF-PL21, without signal peptide portion

GCCAGGCCCTGTATCCCTAAGAGCTTtGGCTACAGCTCAGTaGTtTGTGTCTGTAATGCCACATACTG
TGACTCCTTtGACCCCCCTACCTTCCCTGCCCTGGGAACCTTCAGCAGaTATGAGTCAACAAGaTCAG
GAAGGAGGATGGAGCTGTCAATGGGACCCATCCAGGCTAATCACACAGGCACAGGCCTGCTGCTGA
CCCTGCAGCCAGAACAGAAGTTCCAGAAaGTGAAGGGATTtGGAGGAGCCATGACAGATGCTGCTG
CTCTCAACATCCTGGCCCTGTCACCCCCTGCCCAGAATCTGCTGCTGAAGTCATACTTCTCTGAAGA
AGGAATtGGATATAACATCATCAGGGTGCCCATGGCCAGCTGTGACTTCTCCATCAGGACCTACACC
TATGCtGACACCCCTGATGATTTCCAGCTGCACAACTTCAGCCTCCCAGAGGAAGATACCAAGCTCAA
GATCCCtCTGATaCAtAGgGCaCTGCAGCTGGCCCAGAGGCCtGTGTCACTCCTGGCCAGCCCCTGGA
CATCACCCACTTGGCTCAAGACCAATGGAGCtGTGAATGGAAAGGGATCACTCAAGGGACAGCCtGG
AGACATCTACCACCAGACCTGGGCCAGaTACTTtGTGAAGTTCCTGGATGCCTATGCTGAGCACAAG
CTGCAGTTCTGGGCaGTGACAGCTGAAAATGAGCCTTCTGCTGGACTGCTGTCAGGATACCCCTTCC
AGTGTCTGGGCTTCACCCCTGAACATCAGAGGGACTTCATtGCCAGGGACCTGGGACCTACCCTtGC
CAACTCAACTCACCACAATGTCAGGCTGCTCATGCTGGATGACCAGAGGCTGCTGCTGCCCCACTGG
GCCAAGGTGGTGCTGACAGACCCAGAAGCtGCTAAaTATGTGCATGGCATtGCTGTGCATTGGTACC
TGGACTTCCTGGCTCCAGCCAAGGCCACCCTGGGAGAGACACACAGGCTGTTCCCCAACACCATGCT
CTTtGCCTCtGAGGCCTGTGTGGGCTCCAAGTTCTGGGAGCAGTCAGTGAGGCTGGGCTCCTGGGA
TAGGGGAATGCAGTACAGCCACAGCATCATCACAAACCTCCTGTACCATGTGGTgGGCTGGACtGAC
TGGAACCTGGCCCTGAACCCtGAAGGAGGACCCAAcTGGGTcagaAAtTTtGTgGACTCACCCATCATt
GTGGACATCACCAAGGACACATTCTACAAGCAGCCCATGTTCTACCACCTGGGCCACTTCAGCAAGT
TCATCCCTGAGGGCTCCCAGAGGGTGGGACTGGTGGCCTCACAGAAGAAtGACCTGGAtGCaGTGG

CCCTGATGCATCCtGATGGCTCTGCTGTGGTGGTtGTGCTGAAtAGaTCCTCTAAGGATGTGCCTCT
GACCATCAAGGATCCTGCTGTGGGCTTCCTGGAGACAATCTCACCTGGCTACTCCATCCACACCTAC
CTGTGGAGGAGGCAGTGA

SEQ ID NO: 3 - Codon-optimised GBA nucleotide sequence from FLF-PL30, without signal peptide portion

GCCAGGCCCTGCATCCCTAAGAGCTTTGGCTACAGCTCTGTGGTGTGTGTGTGCAATGCCACATAC
TGTGACTCCTTTGACCCCCCCACCTTTCCTGCCCTGGGCACaTTctccAGaTATGAGAGCACAAGATC
TGGGAGAAGGATGGAGCTGAGCATGGGGCCCATCCAGGCTAATCACACTGGCACAGGCCTGCTGCT
GACCCTGCAGCCTGAACAGAAGTTTCAGAAaGTGAAGGGATTTGGAGGGGCCATGACAGATGCTGC
TGCTCTGAATATCCTGGCCCTGTCACCCCCTGCCCAGAATCTGCTGCTGAAGAGCTACTTTTCAGAA
GAAGGAATTGGATATAATATCATCAGAGTGCCCATGGCCAGCTGTGACTTTTCCATCAGAACCTACA
CCTATGCAGACACCCCTGATGATTTTCAGCTGCACAATTTTAGCCTGCCTGAGGAAGATACCAAGCT
GAAGATACCCCTGATTCACAGGGCCCTGCAGCTGGCCCAGAGGCCTGTTTCACTGCTGGCCAGCCC
CTGGACATCACCCACCTGGCTGAAGACCAATGGAGCTGTGAATGGGAAGGGGTCACTGAAGGGACA
GCCTGGAGACATCTACCACCAGACCTGGGCCAGATACTTTGTGAAGTTTCTGGATGCCTATGCTGA
GCACAAGCTGCAGTTTTGGGCAGTGACAGCTGAAAATGAGCCTTCAGCTGGGCTGCTGTCAGGATA
CCCCTTTCAGTGCCTGGGCTTTACCCCTGAACATCAGAGGGACTTTATTGCCAGGGACCTGGGCCCT
ACCCTGGCCAATAGCACCCAcCAtAATGTgAGgttgCTGATGCTGGATGACCAGAGGCTGCTGCTGCC
CCACTGGGCAAAGGTGGTGCTGACAGACCCTGAAGCAGCTAAaTATGTTCATGGCATTGCTGTGCA
TTGGTACCTGGACTTTCTGGCTCCTGCCAAGGCCACCCTGGGGGAGACACACAGGCTGTTTCCCAA
TACCATGCTGTTTGCCTCtGAGGCCTGTGTGGGCTCCAAGTTTTGGGAGCAGTCTGTGAGGCTGGG
CTCCTGGGATAGAGGGATGCAGTACAGCCACAGCATCATCACCAATCTGCTGTACCATGTGGTGGG
CTGGACTGACTGGAATCTGGCCCTGAATCCTGAAGGAGGACCtAAcTGGGTcAGgAATTTTGTGGAC
AGCCCCATCATTGTGGACATCACCAAGGACACCTTTTACAAGCAGCCCATGTTTTACCACCTGGGCC
ACTTTAGCAAGTTTATTCCTGAGGGCTCCCAGAGAGTGGGGCTGGTTGCCAGCCAGAAGAATGACC
TGGATGCAGTGGCACTGATGCATCCTGATGGCTCAGCTGTTGTGGTGGTGCTGAATAGATCCAGCA
AGGATGTGCCTCTGACCATCAAGGATCCTGCTGTGGGCTTTCTGGAGACAATCTCACCTGGCTACTC
CATTCACACCTACCTGTGGAGAAGGCAGTGA

SEQ ID NO: 4 - Codon-optimised GBA nucleotide sequence from FLF-PL36, without signal peptide portion

GCCAGGCCTTGCATCCCAAAGTCTTTCGGCTACAGCTCCGTGGTGTGCGTGTGCAACGCCACCTATT
GTGACTCCTTCGATCCCCCTACCTTTCCCGCCCTGGGCACATTTTCTAGATACGAGTCTACACGCAG
CGGCCGGAGAATGGAGCTGAGCATGGGCCCTATCCAGGCCAATCACACAGGAACAGGCCTGCTGCT
GACCCTGCAGCCAGAGCAGAAGTTCCAGAAGGTGAAGGGCTTTGGCGGAGCCATGACAGATGCAGC
CGCCCTGAACATCCTGGCCCTGTCCCCACCCGCCCAGAATCTGCTGCTGAAGTCCTACTTCTCTGAG
GAGGGCATCGGCTATAACATCATCCGGGTGCCCATGGCCAGCTGCGACTTTTCCATCAGAACCTACA
CATATGCCGATACCCCTGACGATTTCCAGCTGCACAATTTTTCCCTGCCAGAGGAGGATACAAAGCT
GAAGATCCCCCTGATTCACCGGGCCCTGCAGCTGGCACAGCGGCCCGTGAGCCTGCTGGCCAGCCC
CTGGACCTCCCCTACATGGCTGAAGACCAACGGCGCCGTGAATGGCAAGGGCTCTCTGAAGGGACA
GCCTGGCGACATCTACCACCAGACATGGGCCAGATATTTCGTGAAGTTTCTGGATGCCTACGCCGA
GCACAAGCTGCAGTTCTGGGCCGTGACAGCAGAGAATGAGCCTTCTGCCGGCCTGCTGAGCGGCTA
TCCCTTCCAGTGCCTGGGCTTTACACCTGAGCACCAGCGGGACTTTATCGCCAGAGATCTGGGCCC
AACCCTGGCCAACTCCACACACCACAATGTGAGGCTGCTGATGCTGGACGATCAGCGCCTGCTGCT
GCCTCACTGGGCCAAGGTGGTGCTGACCGACCCAGAGGCCGCCAAGTACGTGCACGGCATCGCCGT
GCACTGGTATCTGGATTTCCTGGCACCTGCAAAGGCCACCCTGGGAGAGACACACCGGCTGTTCCC
TAACACCATGCTGTTTGCCAGCGAGGCCTGCGTGGGCTCCAAGTTTTGGGAGCAGTCCGTGAGGCT
GGGATCTTGGGACAGAGGCATGCAGTACTCCCACTCTATCATCACCAATCTGCTGTATCACGTGGTG
GGCTGGACAGACTGGAACCTGGCCCTGAATCCAGAGGGCGGCCCCAACTGGGTGAGAAATTTCGTG
GATAGCCCCATCATCGTGGACATCACCAAGGATACATTCTACAAGCAGCCAATGTTTTATCACCTGG
GCCACTTCTCTAAGTTTATCCCTGAGGGCAGCCAGAGGGTGGGCCTGGTGGCCAGCCAGAAGAACG

ACCTGGATGCCGTGGCCCTGATGCACCCTGATGGCTCCGCCGTGGTGGTGGTGCTGAATCGCTCTA
GCAAGGACGTGCCTCTGACCATCAAGGATCCAGCCGTGGGATTTCTGGAGACTATTTCACCTGGCT
ATTCAATTCATACCTACCTGTGGAGGAGGCAGTGA

SEQ ID NO: 5 - Codon-optimised GBA nucleotide sequence from FLF-PL28, with signal peptide portion

ATGGAGTTTTCAAGTCCTTCCAGAGAGGAATGTCCCAAGCCTTTGAGTAGGGTAAGCATCATGGCT
GGCAGCCTCACAGGATTGCTTCTACTTCAGGCAGTGTCGTGGGCATCAGGTGCCAGGCCCTGCATC
CCTAAGAGCTTTGGCTACAGCTCTGTGGTGTGTGTGTGCAATGCCACCTACTGTGACAGCTTTGAC
CCCCCCACCTTTCCTGCCCTGGGCACCTTCAGCAGaTATGAGAGCACCAGGTCTGGGAGGAGGATG
GAGCTGAGCATGGGGCCCATCCAGGCTAATCACACTGGCACTGGCCTGCTGCTGACCCTGCAGCCT
GAGCAGAAGTTCCAGAAaGTaAAGGGCTTTGGAGGGGCCATGACTGATGCTGCTGCTCTGAACATC
CTGGCCCTGAGCCCCCCTGCCCAGAATCTGCTGCTGAAGAGCTACTTCTCTGAGGAGGGCATTGGC
TATAACATCATCAGGGTGCCCATGGCCAGCTGTGACTTCAGCATCAGGACCTACACCTATGCTGACA
CCCCTGATGATTTCCAGCTGCACAACTTCAGCCTGCCTGAGGAGGATACCAAGCTGAAGATCCCaCT
GATCCACAGGGCtCTGCAGCTGGCCCAGAGGCCTGTGAGCCTGCTGGCCAGCCCCTGGACCAGCCC
CACTTGGCTGAAGACCAATGGGGCTGTGAATGGGAAGGGGAGCCTGAAGGGACAGCCTGGAGACA
TCTACCACCAGACCTGGGCCAGATACTTTGTGAAGTTCCTGGATGCCTATGCTGAGCACAAGCTGCA
GTTCTGGGCTGTGACTGCTGAGAATGAGCCTTCTGCTGGGCTGCTGTCTGGCTACCCCTTCCAaTG
CCTGGGCTTCACCCCTGAGCATCAGAGGGACTTCATTGCCAGGGACCTGGGCCCTACCCTGGCCAA
CAGCACTCACCAtAATGTtAGGCTGCTGATGCTGGATGACCAGAGGCTGCTGCTGCCCCACTGGGCT
AAGGTGGTGCTGACTGACCCTGAGGCTGCTAAaTATGTGCATGGCATTGCTGTGCATTGGTACCTG
GACTTTCTGGCTCCTGCCAAGGCCACCCTGGGGGAGACCCACAGGCTGTTCCCCAACACCATGCTG
TTTGCCTCTGAGGCCTGTGTGGGCAGCAAGTTCTGGGAGCAGTCTGTGAGGCTGGGCAGCTGGGA
TAGGGGGATGCAGTACAGCCACAGCATCATCACCAACCTGCTGTACCATGTGGTGGGCTGGACTGA
CTGGAACCTGGCCCTGAACCCTGAGGGAGGACCtAAcTGGGTcAGaAACTTTGTGGACAGCCCCATC
ATTGTGGACATCACCAAGGACACCTTTTACAAGCAGCCCATGTTCTACCACCTGGGCCACTTCAGCA
AGTTCATCCCTGAGGGCAGCCAGAGAGTGGGGCTGGTGGCCAGCCAGAAGAATGACCTGGATGCT
GTGGCTCTGATGCATCCTGATGGCTCTGCTGTGGTGGTGGTGCTGAACAGGAGCTCTAAGGATGTG
CCTCTGACCATCAAGGATCCTGCTGTGGGCTTCCTGGAGACCATCAGCCCTGGCTACAGCATCCACA
CCTACCTGTGGAGGAGGCAGTGA

SEQ ID NO: 6 - Codon-optimised GBA nucleotide sequence from FLF-PL21, with signal peptide portion

ATGGAGTTTTCAAGTCCTTCCAGAGAGGAATGTCCCAAGCCTTTGAGTAGGGTAAGCATCATGGCT
GGCAGCCTCACAGGATTGCTTCTACTTCAGGCAGTGTCGTGGGCATCAGGTGCCAGGCCCTGTATC
CCTAAGAGCTTtGGCTACAGCTCAGTaGTtTGTGTCTGTAATGCCACATACTGTGACTCCTTtGACCC
CCCTACCTTCCCTGCCCTGGGAACCTTCAGCAGaTATGAGTCAACAAGaTCAGGAAGGAGGATGGAG
CTGTCAATGGGACCCATCCAGGCTAATCACACAGGCACAGGCCTGCTGCTGACCCTGCAGCCAGAAC
AGAAGTTCCAGAAaGTGAAGGGATTtGGAGGAGCCATGACAGATGCTGCTGCTCTCAACATCCTGGC
CCTGTCACCCCCTGCCCAGAATCTGCTGCTGAAGTCATACTTCTCTGAAGAAGGAATtGGATATAAC
ATCATCAGGGTGCCCATGGCCAGCTGTGACTTCTCCATCAGGACCTACACCTATGCtGACACCCCTG
ATGATTTCCAGCTGCACAACTTCAGCCTCCCAGAGGAAGATACCAAGCTCAAGATCCCtCTGATaCAt
AGgGCaCTGCAGCTGGCCCAGAGGCCtGTGTCACTCCTGGCCAGCCCCTGGACATCACCCACTTGGC
TCAAGACCAATGGAGCtGTGAATGGAAAGGGATCACTCAAGGGACAGCCtGGAGACATCTACCACCA
GACCTGGGCCAGaTACTTtGTGAAGTTCCTGGATGCCTATGCTGAGCACAAGCTGCAGTTCTGGGCa
GTGACAGCTGAAAATGAGCCTTCTGCTGGACTGCTGTCAGGATACCCCTTCCAGTGTCTGGGCTTC
ACCCCTGAACATCAGAGGGACTTCATTgCCAGGGACCTGGGACCTACCCTtGCCAACTCAACTCACCA
CAATGTCAGGCTGCTCATGCTGGATGACCAGAGGCTGCTGCTGCCCCACTGGGCCAAGGTGGTGCT
GACAGACCCAGAAGCtGCTAAaTATGTGCATGGCATtGCTGTGCATTGGTACCTGGACTTCCTGGCT
CCAGCCAAGGCCACCCTGGGAGAGACACACAGGCTGTTCCCCAACACCATGCTCTTtGCCTCtGAGG
CCTGTGTGGGCTCCAAGTTCTGGGAGCAGTCAGTGAGGCTGGGCTCCTGGGATAGGGGAATGCAG

TACAGCCACAGCATCATCACAAACCTCCTGTACCATGTGGTgGGCTGGACtGACTGGAACCTGGCCC
TGAACCCtGAAGGAGGACCCAAcTGGGTcagaAAtTTtGTgGACTCACCCATCATtGTGGACATCACCA
AGGACACATTCTACAAGCAGCCCATGTTCTACCACCTGGGCCACTTCAGCAAGTTCATCCCTGAGGG
CTCCCAGAGGGTGGGACTGGTGGCCTCACAGAAGAAtGACCTGGAtGCaGTGGCCCTGATGCATCCt
GATGGCTCTGCTGTGGTGGTtGTGCTGAAtAGaTCCTCTAAGGATGTGCCTCTGACCATCAAGGATC
CTGCTGTGGGCTTCCTGGAGACAATCTCACCTGGCTACTCCATCCACACCTACCTGTGGAGGAGGC
AGTGA

SEQ ID NO: 7 - Codon-optimised GBA nucleotide sequence from FLF-PL30, with signal peptide portion

ATGGAGTTTTCAAGTCCTTCCAGAGAGGAATGTCCCAAGCCTTTGAGTAGGGTAAGCATCATGGCT
GGCAGCCTCACAGGATTGCTTCTACTTCAGGCAGTGTCGTGGGCATCAGGTGCCAGGCCCTGCATC
CCTAAGAGCTTTGGCTACAGCTCTGTGGTGTGTGTGTGCAATGCCACATACTGTGACTCCTTTGACC
CCCCCACCTTTCCTGCCCTGGGCACaTTctccAGaTATGAGAGCACAAGATCTGGGAGAAGGATGGA
GCTGAGCATGGGGCCCATCCAGGCTAATCACACTGGCACAGGCCTGCTGCTGACCCTGCAGCCTGA
ACAGAAGTTTCAGAAaGTGAAGGGATTTGGAGGGGCCATGACAGATGCTGCTGCTCTGAATATCCT
GGCCCTGTCACCCCCTGCCCAGAATCTGCTGCTGAAGAGCTACTTTTCAGAAGAAGGAATTGGATAT
AATATCATCAGAGTGCCCATGGCCAGCTGTGACTTTTCCATCAGAACCTACACCTATGCAGACACCC
CTGATGATTTTCAGCTGCACAATTTTAGCCTGCCTGAGGAAGATACCAAGCTGAAGATACCCCTGAT
TCACAGGGCCCTGCAGCTGGCCCAGAGGCCTGTTTCACTGCTGGCCAGCCCCTGGACATCACCCAC
CTGGCTGAAGACCAATGGAGCTGTGAATGGGAAGGGGTCACTGAAGGGACAGCCTGGAGACATCTA
CCACCAGACCTGGGCCAGATACTTTGTGAAGTTTCTGGATGCCTATGCTGAGCACAAGCTGCAGTTT
TGGGCAGTGACAGCTGAAAATGAGCCTTCAGCTGGGCTGCTGTCAGGATACCCCTTTCAGTGCCTG
GGCTTTACCCCTGAACATCAGAGGGACTTTATTGCCAGGGACCTGGGCCCTACCCTGGCCAATAGC
ACCCAcCAtAATGTgAGgttgCTGATGCTGGATGACCAGAGGCTGCTGCTGCCCCACTGGGCAAAGGT
GGTGCTGACAGACCCTGAAGCAGCTAAaTATGTTCATGGCATTGCTGTGCATTGGTACCTGGACTTT
CTGGCTCCTGCCAAGGCCACCCTGGGGGAGACACACAGGCTGTTTCCCAATACCATGCTGTTTGCC
TCtGAGGCCTGTGTGGGCTCCAAGTTTTGGGAGCAGTCTGTGAGGCTGGGCTCCTGGGATAGAGG
GATGCAGTACAGCCACAGCATCATCACCAATCTGCTGTACCATGTGGTGGGCTGGACTGACTGGAA
TCTGGCCCTGAATCCTGAAGGAGGACCtAAcTGGGTcAGgAATTTTGTGGACAGCCCCATCATTGTG
GACATCACCAAGGACACCTTTTACAAGCAGCCCATGTTTTACCACCTGGGCCACTTTAGCAAGTTTA
TTCCTGAGGGCTCCCAGAGAGTGGGGCTGGTTGCCAGCCAGAAGAATGACCTGGATGCAGTGGCAC
TGATGCATCCTGATGGCTCAGCTGTTGTGGTGGTGCTGAATAGATCCAGCAAGGATGTGCCTCTGA
CCATCAAGGATCCTGCTGTGGGCTTTCTGGAGACAATCTCACCTGGCTACTCCATTCACACCTACCT
GTGGAGAAGGCAGTGA

SEQ ID NO: 8 - Codon-optimised GBA nucleotide sequence from FLF-PL36, with signal peptide portion

ATGGAGTTTTCAAGTCCTTCCAGAGAGGAATGTCCCAAGCCTTTGAGTAGGGTAAGCATCATGGCT
GGCAGCCTCACAGGATTGCTTCTACTTCAGGCAGTGTCGTGGGCATCAGGTGCCAGGCCTTGCATC
CCAAAGTCTTTCGGCTACAGCTCCGTGGTGTGCGTGTGCAACGCCACCTATTGTGACTCCTTCGATC
CCCCTACCTTTCCCGCCCTGGGCACATTTTCTAGATACGAGTCTACACGCAGCGGCCGGAGAATGGA
GCTGAGCATGGGCCCTATCCAGGCCAATCACACAGGAACAGGCCTGCTGCTGACCCTGCAGCCAGA
GCAGAAGTTCCAGAAGGTGAAGGGCTTTGGCGGAGCCATGACAGATGCAGCCGCCCTGAACATCCT
GGCCCTGTCCCCACCCGCCCAGAATCTGCTGCTGAAGTCCTACTTCTCTGAGGAGGGCATCGGCTA
TAACATCATCCGGGTGCCCATGGCCAGCTGCGACTTTTCCATCAGAACCTACACATATGCCGATACC
CCTGACGATTTCCAGCTGCACAATTTTTCCCTGCCAGAGGAGGATACAAAGCTGAAGATCCCCCTGA
TTCACCGGGCCCTGCAGCTGGCACAGCGGCCCGTGAGCCTGCTGGCCAGCCCCTGGACCTCCCCTA
CATGGCTGAAGACCAACGGCGCCGTGAATGGCAAGGGCTCTCTGAAGGGACAGCCTGGCGACATCT
ACCACCAGACATGGGCCAGATATTTCGTGAAGTTTCTGGATGCCTACGCCGAGCACAAGCTGCAGTT
CTGGGCCGTGACAGCAGAGAATGAGCCTTCTGCCGGCCTGCTGAGCGGCTATCCCTTCCAGTGCCT
GGGCTTTACACCTGAGCACCAGCGGGACTTTATCGCCAGAGATCTGGGCCCAACCCTGGCCAACTC

CACACACCACAATGTGAGGCTGCTGATGCTGGACGATCAGCGCCTGCTGCTGCCTCACTGGGCCAA
GGTGGTGCTGACCGACCCAGAGGCCGCCAAGTACGTGCACGGCATCGCCGTGCACTGGTATCTGGA
TTTCCTGGCACCTGCAAAGGCCACCCTGGGAGAGACACACCGGCTGTTCCCTAACACCATGCTGTTT
GCCAGCGAGGCCTGCGTGGGCTCCAAGTTTTGGGAGCAGTCCGTGAGGCTGGGATCTTGGGACAG
AGGCATGCAGTACTCCCACTCTATCATCACCAATCTGCTGTATCACGTGGTGGGCTGGACAGACTGG
AACCTGGCCCTGAATCCAGAGGGCGGCCCCAACTGGGTGAGAAATTTCGTGGATAGCCCCATCATC
GTGGACATCACCAAGGATACATTCTACAAGCAGCCAATGTTTTATCACCTGGGCCACTTCTCTAAGT
TTATCCCTGAGGGCAGCCAGAGGGTGGGCCTGGTGGCCAGCCAGAAGAACGACCTGGATGCCGTG
GCCCTGATGCACCCTGATGGCTCCGCCGTGGTGGTGGTGCTGAATCGCTCTAGCAAGGACGTGCCT
CTGACCATCAAGGATCCAGCCGTGGGATTTCTGGAGACTATTTCACCTGGCTATTCAATTCATACCT
ACCTGTGGAGGAGGCAGTGA

SEQ ID NO: 9 - Wild type human GBA nucleotide sequence with signal peptide (from GenBank NM_000157.3)

ATGGAGTTTTCAAGTCCTTCCAGAGAGGAATGTCCCAAGCCTTTGAGTAGGGTAAGCATCATGGCT
GGCAGCCTCACAGGATTGCTTCTACTTCAGGCAGTGTCGTGGGCATCAGGTGCCCGCCCCTGCATC
CCTAAAAGCTTCGGCTACAGCTCGGTGGTGTGTGTCTGCAATGCCACATACTGTGACTCCTTTGACC
CCCCGACCTTTCCTGCCCTTGGTACCTTCAGCCGCTATGAGAGTACACGCAGTGGGCGACGGATGG
AGCTGAGTATGGGGCCCATCCAGGCTAATCACACGGGCACAGGCCTGCTACTGACCCTGCAGCCAG
AACAGAAGTTCCAGAAAGTGAAGGGATTTGGAGGGGCCATGACAGATGCTGCTGCTCTCAACATCC
TTGCCCTGTCACCCCCTGCCCAAAATTTGCTACTTAAATCGTACTTCTCTGAAGAAGGAATCGGATA
TAACATCATCCGGGTACCCATGGCCAGCTGTGACTTCTCCATCCGCACCTACACCTATGCAGACACC
CCTGATGATTTCCAGTTGCACAACTTCAGCCTCCCAGAGGAAGATACCAAGCTCAAGATACCCCTGA
TTCACCGAGCCCTGCAGTTGGCCCAGCGTCCCGTTTCACTCCTTGCCAGCCCCTGGACATCACCCAC
TTGGCTCAAGACCAATGGAGCGGTGAATGGGAAGGGGTCACTCAAGGGACAGCCCGGAGACATCTA
CCACCAGACCTGGGCCAGATACTTTGTGAAGTTCCTGGATGCCTATGCTGAGCACAAGTTACAGTTC
TGGGCAGTGACAGCTGAAAATGAGCCTTCTGCTGGGCTGTTGAGTGGATACCCCTTCCAGTGCCTG
GGCTTCACCCCTGAACATCAGCGAGACTTCATTGCCCGTGACCTAGGTCCTACCCTCGCCAACAGTA
CTCACCACAATGTCCGCCTACTCATGCTGGATGACCAACGCTTGCTGCTGCCCCACTGGGCAAAGGT
GGTACTGACAGACCCAGAAGCAGCTAAATATGTTCATGGCATTGCTGTACATTGGTACCTGGACTTT
CTGGCTCCAGCCAAAGCCACCCTAGGGGAGACACACCGCCTGTTCCCCAACACCATGCTCTTTGCCT
CAGAGGCCTGTGTGGGCTCCAAGTTCTGGGAGCAGAGTGTGCGGCTAGGCTCCTGGGATCGAGGG
ATGCAGTACAGCCACAGCATCATCACGAACCTCCTGTACCATGTGGTCGGCTGGACCGACTGGAACC
TTGCCCTGAACCCCGAAGGAGGACCCAATTGGGTGCGTAACTTTGTCGACAGTCCCATCATTGTAGA
CATCACCAAGGACACGTTTTACAAACAGCCCATGTTCTACCACCTTGGCCACTTCAGCAAGTTCATTC
CTGAGGGCTCCCAGAGAGTGGGGCTGGTTGCCAGTCAGAAGAACGACCTGGACGCAGTGGCACTG
ATGCATCCCGATGGCTCTGCTGTTGTGGTCGTGCTAAACCGCTCCTCTAAGGATGTGCCTCTTACCA
TCAAGGATCCTGCTGTGGGCTTCCTGGAGACAATCTCACCTGGCTACTCCATTCACACCTACCTGTG
GCGTCGCCAGTGA

SEQ ID NO: 10 - LSP-S transcription regulatory element

CCCTAAAATGGGCAAACATTGCAAGCAGCAAACAGCAAACACACAGCCCTCCCTGCCTGCTGACCTT
GGAGCTGGGGCAGAGGTCAGACACCTCTCTGGGCCCATGCCACCTCCAACTGGACACAGGACGCTG
TGGTTTCTGAGCCAGGGGGCGACTCAGATCCCAGCCAGTGGACTTAGCCCCTGTTTGCTCCTCCGA
TAACTGGGGTGACCTTGGTTAATATTCACCAGCAGCCTCCCCCGTTGCCCCTCTGGATCCACTGCTT
AAATACGGACGAGGACAGGGCCCTGTCTCCTCAGCTTCAGGCACCACCACTGACCTGGGACAGTGA
AT

SEQ ID NO: 11 - HCR enhancer portion of LSP-S

CCCTAAAATGGGCAAACATTGCAAGCAGCAAACAGCAAACACACAGCCCTCCCTGCCTGCTGACCTT
GGAGCTGGGGCAGAGGTCAGACACCTCTCTGGGCCCATGCCACCTCCAAC

SEQ ID NO: 12 - A1AT promoter portion of LSP-S

GGGCGACTCAGATCCCAGCCAGTGGACTTAGCCCCTGTTTGCTCCTCCGATAACTGGGGTGACCTT
GGTTAATATTCACCAGCAGCCTCCCCCGTTGCCCCTCTGGATCCACTGCTTAAATACGGACGAGGAC
AGGGCCCTGTCTCCTCAGCTTCAGGCACCACCACTGACCTGGGACAGTGAAT

SEQ ID NO: 13 - CAG promoter

GACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATG
GAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCAT
TGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGT
GGAGTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCT
ATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTC
CTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTCGAGGTGAGCCCCACGTTCTG
CTTCACTCTCCCCATCTCCCCCCCCTCCCCACCCCCAATTTTGTATTTATTTATTTTTTAATTATTTTG
TGCAGCGATGGGGGCGGGGGGGGGGGGGGGCGCGCGCCAGGCGGGGCGGGGCGGGGCGAGG
GGCGGGGCGGGGCGAGGCGGAGAGGTGCGGCGGCAGCCAATCAGAGCGGCGCGCTCCGAAAGTT
TCCTTTTATGGCGAGGCGGCGGCGGCGGCGGCCCTATAAAAAGCGAAGCGCGCGGCGGGCG

SEQ ID NO: 14 - LSP-L transcription regulatory element

AGGCTCAGAGGCACACAGGAGTTTCTGGGCTCACCCTGCCCCCTTCCAACCCCTCAGTTCCCATCCT
CCAGCAGCTGTTTGTGTGCTGCCTCTGAAGTCCACACTGAACAAACTTCAGCCTACTCATGTCCCTA
AAATGGGCAAACATTGCAAGCAGCAAACAGCAAACACACAGCCCTCCCTGCCTGCTGACCTTGGAGC
TGGGGCAGAGGTCAGAGACCTCTCTGGGCCCATGCCACCTCCAACATCCACTCGACCCCTTGGAATT
TCGGTGGAGAGGAGCAGAGGTTGTCCTGGCGTGGTTTAGGTAGTGTGAGAGGGGTACCCGGGGAT
CTTGCTACCAGTGGAACAGCCACTAAGGATTCTGCAGTGAGAGCAGAGGGCCAGCTAAGTGGTACT
CTCCCAGAGACTGTCTGACTCACGCCACCCCCTCCACCTTGGACACAGGACGCTGTGGTTTCTGAGC
CAGGTACAATGACTCCTTTCGGTAAGTGCAGTGGAAGCTGTACACTGCCCAGGCAAAGCGTCCGGG
CAGCGTAGGCGGGCGACTCAGATCCCAGCCAGTGGACTTAGCCCCTGTTTGCTCCTCCGATAACTG
GGGTGACCTTGGTTAATATTCACCAGCAGCCTCCCCCGTTGCCCCTCTGGATCCACTGCTTAAATAC
GGACGAGGACAGGGCCCTGTCTCCTCAGCTTCAGGCACCACCACTGACCTGGGACAGTGAATGATC
CCCCTGATCTGCGGCC

SEQ ID NO: 15 - A1AT promoter portion of LSP-L

GGATCTTGCTACCAGTGGAACAGCCACTAAGGATTCTGCAGTGAGAGCAGAGGGCCAGCTAAGTGG
TACTCTCCCAGAGACTGTCTGACTCACGCCACCCCCTCCACCTTGGACACAGGACGCTGTGGTTTCT
GAGCCAGGTACAATGACTCCTTTCGGTAAGTGCAGTGGAAGCTGTACACTGCCCAGGCAAAGCGTC
CGGGCAGCGTAGGCGGGCGACTCAGATCCCAGCCAGTGGACTTAGCCCCTGTTTGCTCCTCCGATA
ACTGGGGTGACCTTGGTTAATATTCACCAGCAGCCTCCCCCGTTGCCCCTCTGGATCCACTGCTTAA
ATACGGACGAGGACAGGGCCCTGTCTCCTCAGCTTCAGGCACCACCACTGACCTGGGACAGTGAAT
GATCCCCCTGATCTGCGGCC

SEQ ID NO: 16 - HCR enhancer portion of LSP-L

AGGCTCAGAGGCACACAGGAGTTTCTGGGCTCACCCTGCCCCCTTCCAACCCCTCAGTTCCCATCCT
CCAGCAGCTGTTTGTGTGCTGCCTCTGAAGTCCACACTGAACAAACTTCAGCCTACTCATGTCCCTA
AAATGGGCAAACATTGCAAGCAGCAAACAGCAAACACACAGCCCTCCCTGCCTGCTGACCTTGGAGC
TGGGGCAGAGGTCAGAGACCTCTCTGGGCCCATGCCACCTCCAACATCCACTCGACCCCTTGGAATT
TCGGTGGAGAGGAGCAGAGGTTGTCCTGGCGTGGTTTAGGTAGTGTGAGAGGG

SEQ ID NO: 17 - Wild type GBA nucleotide sequence corresponding to signal peptide

ATGGAGTTTTCAAGTCCTTCCAGAGAGGAATGTCCCAAGCCTTTGAGTAGGGTAAGCATCATGGCT
GGCAGCCTCACAGGATTGCTTCTACTTCAGGCAGTGTCGTGGGCATCAGGT

SEQ ID NO: 18 - Wild type GCase polypeptide sequence of signal peptide

MEFSSPSREECPKPLSRVSIMAGSLTGLLLLQAVSWASG

SEQ ID NO: 19 - Polypeptide sequence of liver-tropic capsid

MAADGYLPDWLEDNLSEGIREWWALKPGVPQPKANQQHQDNRRGLVLPGYKYLGPGNGLDKGEPVNEA
DAAALEHDKAYDQQLKAGDNPYLKYNHADAEFQERLQEDTSFGGNLGRAVFQAKKRILEPLGLVEEAAKT
APGKKRPVDQSPQEPDSSSGVGKSGKQPARKRLNFGQTGDSESVPDPQPLGEPPAAPTSLGSNTMASGGG
APMADNNEGADGVGNSSGNWHCDSQWLGDRVITTSTRTWALPTYNNHLYKQISSQSGASNDNHYFGYST
PWGYFDFNRFHCHFSPRDWQRLINNNWGFRPKKLSFKLFNIQVKEVTQNDGTTTIANNLTSTVQVFTDSE
YQLPYVLGSAHQGCLPPFPADVFMVPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNFQFSYTFED
VPFHSSYAHSQSLDRLMNPLIDQYLYYLNRTQGTTSGTTNQSRLLFSQAGPQSMSLQARNWLPGPCYRQQ
RLSKTANDNNNSNFPWTAASKYHLNGRDSLVNPGPAMASHKDDEEKFFPMHGNLIFGKEGTTASNAELDN
VMITDEEEIRTTNPVATEQYGTVANNLQSSNTAPTTRTVNDQGALPGMVWQDRDVYLQGPIWAKIPHTD
GHFHPSPLMGGFGLKHPPPQIMIKNTPVPANPPTTFSPAKFASFITQYSTGQVSVEIEWELQKENSKRWNP
EIQYTSNYNKSVNVDFTVDTNGVYSEPRPIGTRYLTRNL

SEQ ID NO: 20 - Polypeptide sequence of liver-tropic capsid

MAADGYLPDWLEDNLSEGIREWWALKPGAPKPKANQQKQDDGRGLVLPGYKYLGPFNGLDKGEPVNAA
DAAALEHDKAYDQQLQAGDNPYLRYNHADAEFQERLQEDTSFGGNLGRAVFQAKKRVLEPLGLVEEGAK
TAPGKKRPVDQSPQEPDSSSGVGKSGKQPARKRLNFGQTGDSESVPDPQPLGEPPAAPTSLGSNTMASGG
GAPMADNNEGADGVGNSSGNWHCDSQWLGDRVITTSTRTWALPTYNNHLYKQISSQSGASNDNHYFGY
STPWGYFDFNRFHCHFSPRDWQRLINNNWGFRPKKLSFKLFNIQVKEVTQNDGTTTIANNLTSTVQVFTD
SEYQLPYVLGSAHQGCLPPFPADVFMVPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNFQFSYTF
EDVPFHSSYAHSQSLDRLMNPLIDQYLYYLNRTQGTTSGTTNQSRLLFSQAGPQSMSLQARNWLPGPCYR
QQRLSKTANDNNNSNFPWTAASKYHLNGRDSLVNPGPAMASHKDDEEKFFPMHGNLIFGKEGTTASNAEL
DNVMITDEEEIRTTNPVATEQYGTVANNLQSSNTAPTTRTVNDQGALPGMVWQDRDVYLQGPIWAKIPHT
DGHFHPSPLMGGFGLKHPPPQIMIKNTPVPANPPTTFSPAKFASFITQYSTGQVSVEIEWELQKENSKRWN
PEIQYTSNYNKSVNVDFTVDTNGVYSEPRPIGTRYLTRNL

SEQ ID NO: 21 - Polypeptide sequence of CNS-tropic capsid

MAADGYLPDWLEDNLSEGIREWWALKPGAPQPKANQQHQDNARGLVLPGYKYLGPGNGLDKGEPVNAA
DAAALEHDKAYDQQLKAGDNPYLKYNHADAEFQERLKEDTSFGGNLGRAVFQAKKRLLEPLGLVEEAAKT
APGKKRPVEQSPQEPDSSAGIGKSGAQPAKKRLNFGQTGDTESVPDPQPIGEPPAAPSGVGSLTMASGGG
APVADNNEGADGVGSSSGNWHCDSQWLGDRVITTSTRTWALPTYNNHLYKQISNSTSGGSSNDNAYFGY
STPWGYFDFNRFHCHFSPRDWQRLINNNWGFRPKRLNFKLFNIQVKEVTDNNGVKTIANNLTSTVQVFTD
SDYQLPYVLGSAHEGCLPPFPADVFMIPQYGYLTLNDGSQAVGRSSFYCLEYFPSQMLRTGNNFQFSYEFE
NVPFHSSYAHSQSLDRLMNPLIDQYLYYLSKTINGSGQNQQTLKFSVAGPSNMAVQGRNYIPGPSYRQQRV
STTVTQNNNSEFAWPGASSWALNGRNSLMNPGPAMASHKEGEDRFFPLSGSLIFGKQGTGRDNVDADKV
MITNEEEIKTTNPVATESYGQVATNHQSAQAQAQTGWVQNQGILPGMVWQDRDVYLQGPIWAKIPHTDG
NFHPSPLMGGFGMKHPPPQILIKNTPVPADPPTAFNKDKLNSFITQYSTGQVSVEIEWELQKENSKRWNPEI
QYTSNYYKSNNVEFAVNTEGVYSEPRPIGTRYLTRNL

SEQ ID NO: 22 - Nucleotide sequence of SV40 intron

GTAAATATAAAATTTTTAAGTGTATAATGTGTTAAACTACTGATTCTAATTGTTTCTCTCTTTTAG

SEQ ID NO: 23 - Nucleotide sequence of bovine growth hormone poly A sequence

CTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGG
TGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCAT
TCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCA
TGCTGGGGA

SEQ ID NO: 24 - Polypeptide sequence of liver-tropic capsid

MAADGYLPDWLEDNLSEGIREWWALQPGAPKPKANQQHQDNARGLVLPGYKYLGPGNGLDKGEPVNAA
DAAALEHDKAYDQQLKAGDNPYLKYNHADAEFQERLKEDTSFGGNLGRAVFQAKKRLLEPLGLVEEAAKT
APGKKRPVDQSPQEPDSSSGVGKSGKQPARKRLNFGQTGDSESVPDPQPLGEPPAAPTSLGSNTMASGGG
APMADNNEGADGVGNSSGNWHCDSQWLGDRVITTSTRTWALPTYNNHLYKQISSQSGASNDNHYFGYST
PWGYFDFNRFHCHFSPRDWQRLINNNWGFRPKKLSFKLFNIQVKEVTQNDGTTTIANNLTSTVQVFTDSE
YQLPYVLGSAHQGCLPPFPADVFMVPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNFQFSYTFED
VPFHSSYAHSQSLDRLMNPLIDQYLYYLNRTQGTTSGTTNQSRLLFSQAGPQSMSLQARNWLPGPCYRQQ
RLSKTANDNNNSNFPWTAASKYHLNGRDSLVNPGPAMASHKDDEEKFFPMHGNLIFGKEGTTASNAELDN
VMITDEEEIRTTNPVATEQYGTVANNLQSSNTAPTTRTVNDQGALPGMVWQDRDVYLQGPIWAKIPHTD
GHFHPSPLMGGFGLKHPPPQIMIKNTPVPANPPTTFSPAKFASFITQYSTGQVSVEIEWELQKENSKRWNP
EIQYTSNYNKSVNVDFTVDTNGVYSEPRPIGTRYLTRPL

SEQ ID NO: 25 - Polypeptide sequence of wild type human GCase

MEFSSPSREECPKPLSRVSIMAGSLTGLLLLQAVSWASGARPCIPKSFGYSSVVCVCNATYCDSFDPPTFPAL
GTFSRYESTRSGRRMELSMGPIQANHTGTGLLLTLQPEQKFQKVKGFGGAMTDAAALNILALSPPAQNLLL
KSYFSEEGIGYNIIRVPMASCDFSIRTYTYADTPDDFQLHNFSLPEEDTKLKIPLIHRALQLAQRPVSLLASPW
TSPTWLKTNGAVNGKGSLKGQPGDIYHQTWARYFVKFLDAYAEHKLQFWAVTAENEPSAGLLSGYPFQC
LGFTPEHQRDFIARDLGPTLANSTHHNVRLLMLDDQRLLLPHWAKVVLTDPEAAKYVHGIAVHWYLDFLA
PAKATLGETHRLFPNTMLFASEACVGSKFWEQSVRLGSWDRGMQYSHSIITNLLYHVVGWTDWNLALNPE
GGPNWVRNFVDSPIIVDITKDTFYKQPMFYHLGHFSKFIPEGSQRVGLVASQKNDLDAVALMHPDGSAVVV
VLNRSSKDVPLTIKDPAVGFLETISPGYSIHTYLWRRQ

FIGURE 10

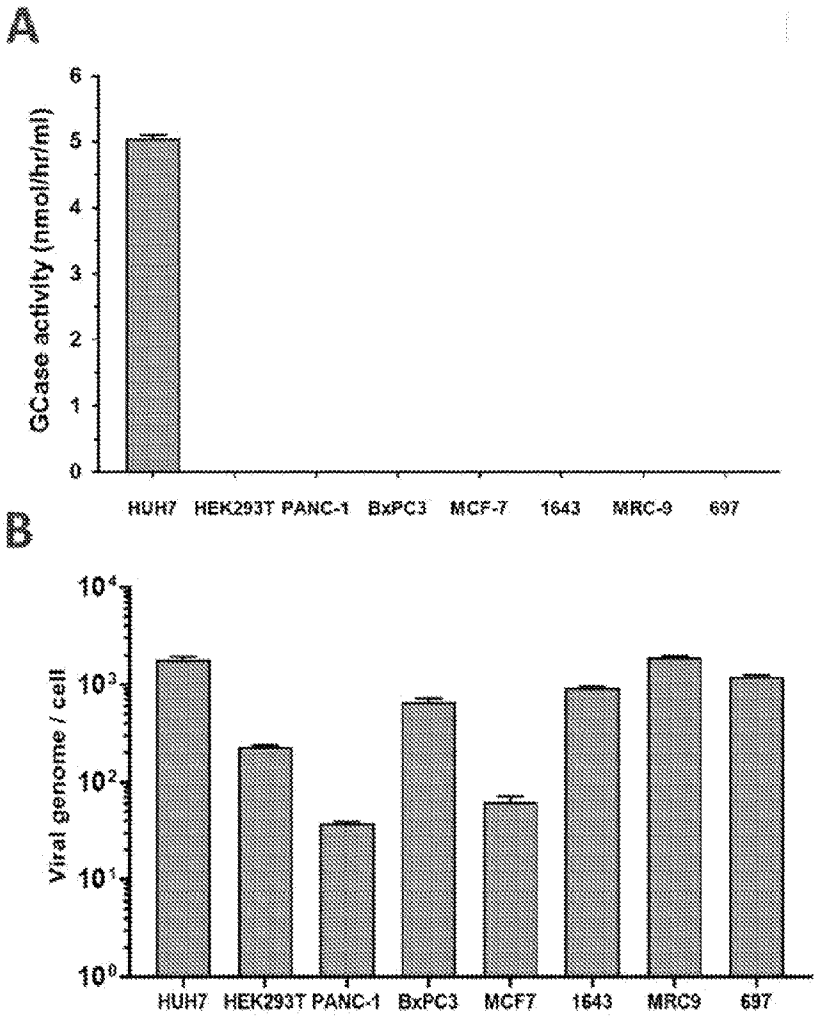
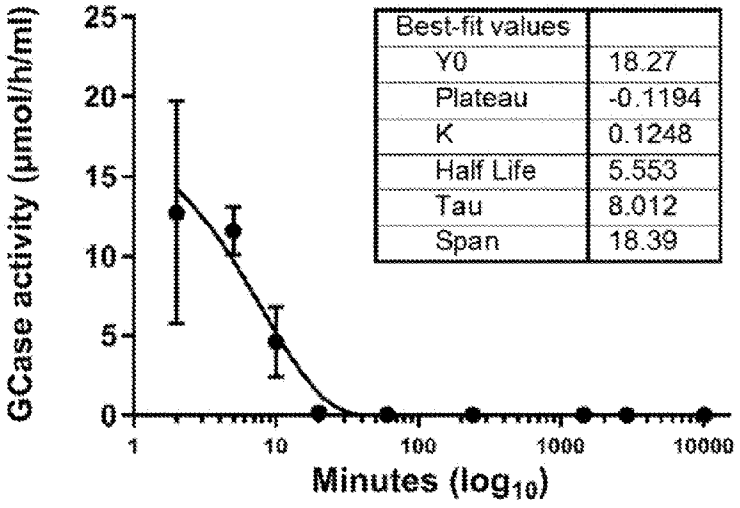

A
GCase activity (nmol/hr/ml)
0
1
2
3
4
5
6
HUH7
HEK293T
PANC-1
BxPC3
MCF-7
1643
MRC-9
697
B
Viral genome / cell
10^0
10^1
10^2
10^3
10^4
HUH7
HEK293T
PANC-1
BxPC3
MCF7
1643
MRC9
697

FIGURE 11

A


GCase activity (µmol/h/ml)
25
20
15
10
5
0
1
10
100
1000
10000
Minutes (log10)
Best-fit values
Y0 18.27
Plateau -0.1194
K 0.1248
Half Life 5.553
Tau 8.012
Span 18.39

B

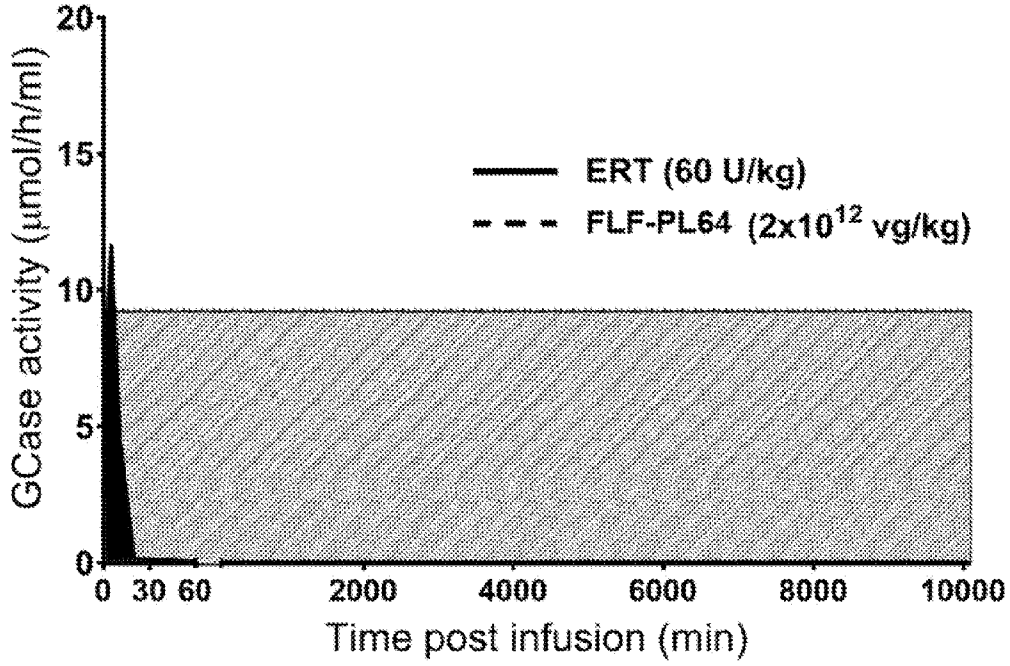

GCase activity (µmol/h/ml)
20
15
10
5
0
ERT (60 U/kg)
FLF-PL64 (2x10^12 vg/kg)
0 30 60
2000
4000
6000
8000
10000
Time post infusion (min)

FIGURE 12

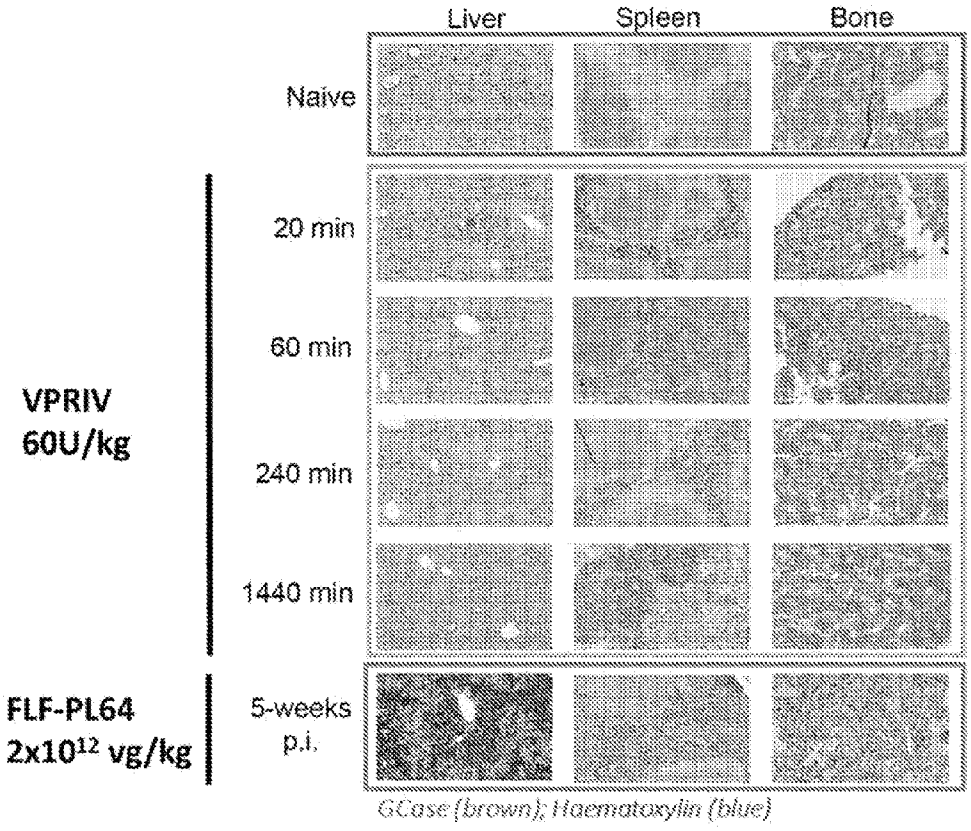

Liver
Spleen
Bone
Naive
20 min
60 min
240 min
1440 min
VPRIV
60U/kg
FLF-PL64
2x10^12 vg/kg
5-weeks
p.i.
GCase (brown); Haematoxylin (blue)

FIGURE 13

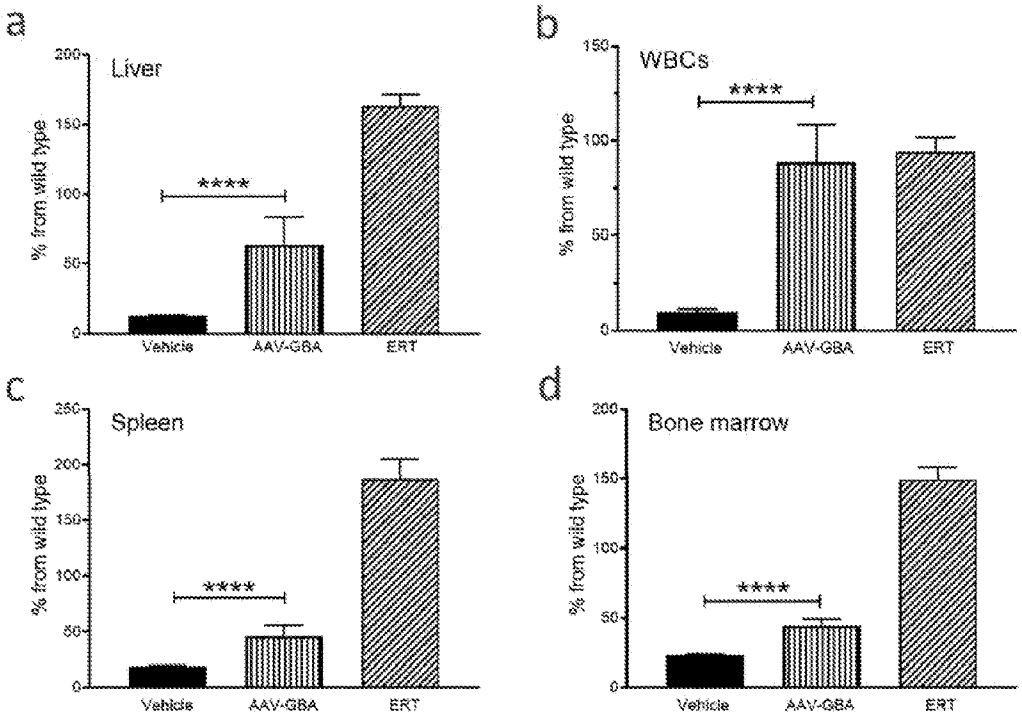

a
Liver
% from wild type
200
150
100
50
0
****
Vehicle
AAV-GBA
ERT
b
WBCs
% from wild type
150
100
50
0
****
Vehicle
AAV-GBA
ERT
c
Spleen
% from wild type
250
200
150
100
50
0
****
Vehicle
AAV-GBA
ERT
d
Bone marrow
% from wild type
200
150
100
50
0
****
Vehicle
AAV-GBA
ERT

FIGURE 14

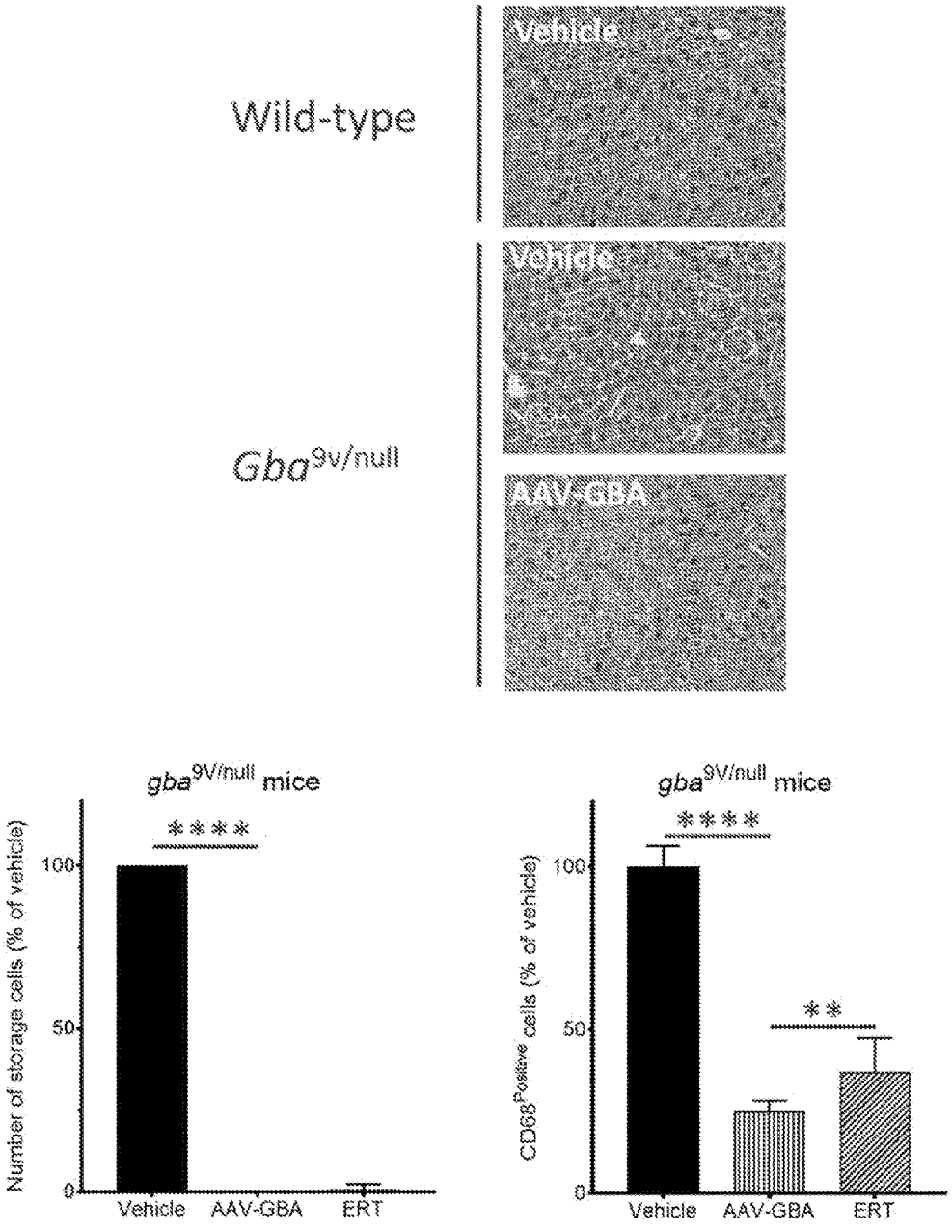
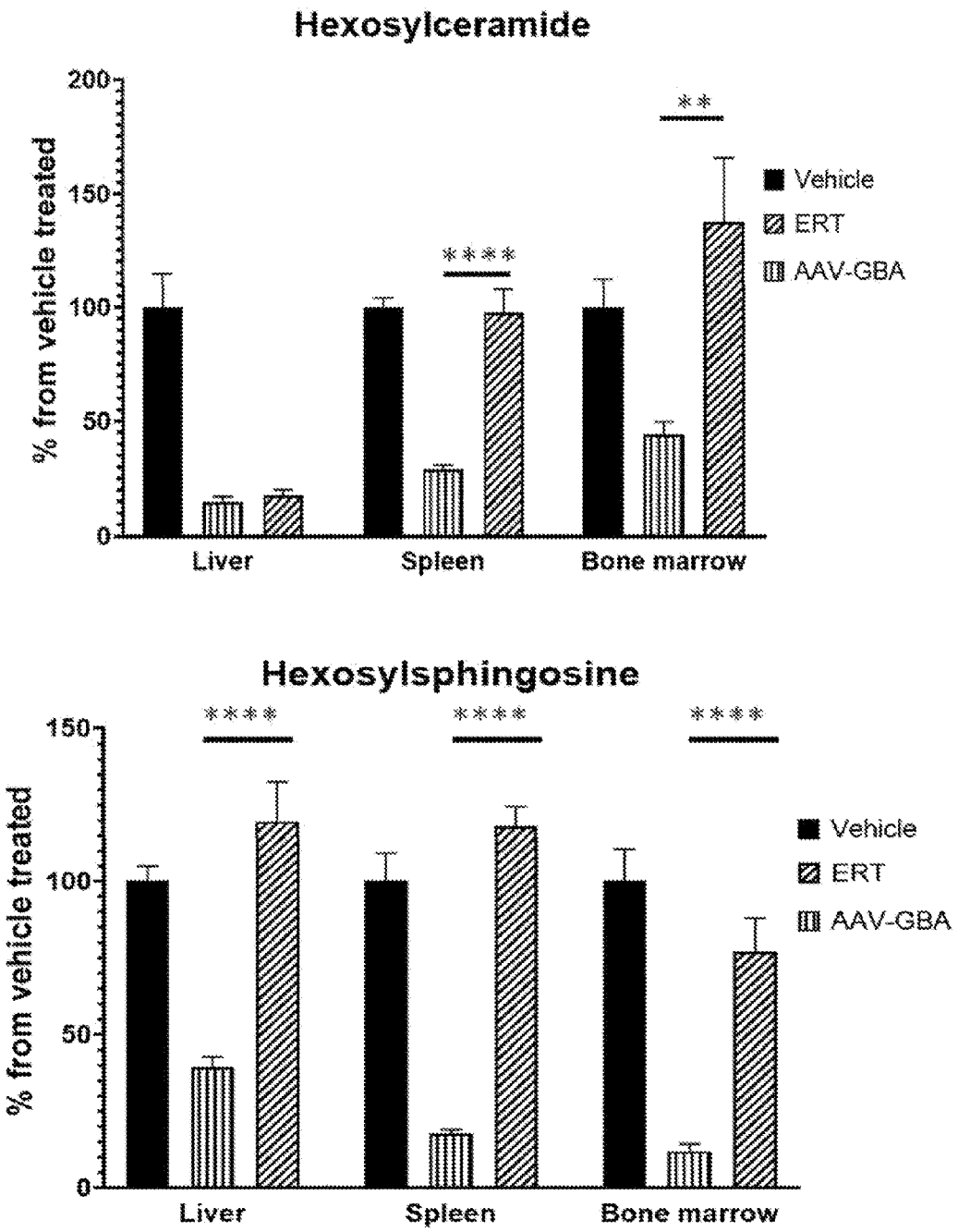

Wild-type
Gba9v/null
Vehicle
Vehicle
AAV-GBA
gba9V/null mice
Number of storage cells (% of vehicle)
100
50
0
****
Vehicle
AAV-GBA
ERT
gba9V/null mice
CD68Positive cells (% of vehicle)
100
50
0
****
**
Vehicle
AAV-GBA
ERT

FIGURE 15


Hexosylceramide
% from vehicle treated
200
150
100
50
0
**
****
Vehicle
ERT
AAV-GBA
Liver
Spleen
Bone marrow
Hexosylsphingosine
% from vehicle treated
150
100
50
0
****
****
****
Vehicle
ERT
AAV-GBA
Liver
Spleen
Bone marrow

SEKVENSLISTE

Sekvenslisten er udeladt af skriftet og kan hentes fra det Europæiske Patent Register.

The Sequence Listing was omitted from the document and can be downloaded from the European Patent Register.