

CLAIMS

1. A capsid-free closed-ended DNA (ceDNA) vector comprising:
at least one nucleic acid sequence positioned between flanking inverted terminal repeat sequences (ITRs), wherein the at least one nucleic acid sequence encodes at least one beta glucocerebrosidase (GBA) protein.
2. The ceDNA vector of claim 1, wherein the least one nucleic acid sequence that encodes at least one GBA protein is selected from the group consisting of: any of the sequences set forth in Table 1 ~~or~~ and any of the open reading frame sequences (ORFs) set forth in Table 13.
3. The ceDNA vector of claims 1 or 2, wherein:
the ceDNA vector comprises a promoter selected from any of ~~those~~ the sequences set forth in in Table 7 operatively linked to the least one nucleic acid sequence that encodes the at least one GBA protein;
- ~~4. The ceDNA vector of any of claims 1 to 3, wherein~~ the ceDNA vector comprises an enhancer selected from the group consisting of any of the sequences set forth ~~any of those~~ in Table 8;
- ~~5. The ceDNA vector of any of claims 1 to 4, wherein~~ the ceDNA vector comprises a 5' UTR and/or intron sequence selected the group consisting of any of the sequences set forth from ~~any of those~~ those in Table 9A;
- ~~6. The ceDNA vector of any of claims 1 to 5, wherein~~ the ceDNA vector comprises a 3' UTR sequence selected from the group consisting of any of the sequences set forth ~~any of those~~ in Table 9B;
- ~~7. The ceDNA vector of any of claims 1 to 6, wherein~~ the ceDNA vector comprises at least one poly A sequence selected from the group consisting of any of the sequences set forth ~~any of those~~ in Table 10; and/or;
- ~~8. The ceDNA vector of any one of claims 1-7, wherein~~ the ceDNA vector comprises at least one promoter sequence operably linked to at least one nucleic acid sequence.
- ~~9.4. The ceDNA vector of any one of claims 1-83, wherein~~ the at least one nucleic acid sequence is cDNA.
- ~~10.5. The ceDNA vector of any one of claims 1-49, wherein~~ the at least one ITR comprises a functional terminal resolution site and a Rep binding site.
- ~~11.6. The ceDNA vector of any one of claims 1-105, wherein one or both of the~~ flanking ITRs are derived from a virus selected from the group consisting of: a *Parvovirus*, a *Dependovirus*, and an adeno-associated virus (AAV).
- ~~12.7. The ceDNA vector of any one of claims 1-611, wherein~~
the flanking ITRs are symmetric or asymmetric with respect to ~~one another each other;~~
- ~~13. The ceDNA vector of claim 12, wherein~~ the flanking ITRs are symmetrical ~~or~~ substantially symmetric; or at.

- ~~14.~~ The ~~ceDNA vector of claim 12, wherein~~ the flanking ITRs are asymmetric.
- ~~15-8.~~ The ceDNA vector of any one of claims 1-~~7~~~~4~~~~4~~, wherein:
- ~~one or both of the flanking ITRs is~~~~are~~ wild-type, or wherein both of the flanking ITRs are wild-type ITRs:-
- one of the flanking ITRs is not a wild-type ITR, or wherein both of the flanking ITRs are not wild-type ITRs;
- ~~16.~~ The ~~ceDNA vector of any one of claims 1-15, wherein~~ the flanking ITRs are derived from different viral serotypes:-
- one of both of the flanking ITRs are derived from an AAV serotype selected from the group consisting of: AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, and AAV12;
- ~~17.~~ The ~~ceDNA vector of any one of claims 1-16, wherein~~ the flanking ITRs are selected from the group consisting of: any pair of viral serotypes shown in Table 2; ~~and/or:~~
- ~~18.~~ The ~~ceDNA vector of any one of claims 1-17, wherein~~ one or both of the flanking ITRs comprises a sequence selected from the group consisting of: one or more of the sequences set forth in Table 3.
- ~~19-9.~~ The ceDNA vector of any one of claims 1-~~8~~~~18~~~~8~~, wherein at least one of the flanking ITRs is altered from a wild-type AAV ITR sequence by a deletion, an addition, or a substitution that affects the overall three-dimensional conformation of the ITR.
- ~~20.~~ The ~~ceDNA vector of any one of claims 1-19, wherein one or both of the ITRs are derived from an AAV serotype selected from AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, and AAV12.~~
- ~~21-10.~~ The ceDNA vector of any one of claims 1-~~9~~~~20~~~~20~~, wherein one or both of the ITRs are synthetic.
- ~~22.~~ The ~~ceDNA vector of any one of claims 1-21, wherein one or both of the ITRs are not a wild type ITR, or wherein both of the ITRs are not wild type ITRs.~~
- ~~23-11.~~ The ceDNA vector of any one of claims 1-~~10~~~~22~~~~22~~, wherein:
- one or both of the flanking ITRs are modified by a deletion, an insertion, and/or a substitution in at least one of the ITR regions selected from the group consisting of: the A, A', B, B', C, C', D, and D' regions;
- ~~24.~~ The ~~ceDNA vector of claim 23, wherein the deletion, the insertion, and/or the substitution results in the deletion of all or part of a stem-loop structure normally formed by the A, A', B, B', C, or C' regions.~~
- ~~25.~~ The ~~ceDNA vector of any one of claims 1-24, wherein~~ one or both of the flanking ITRs are modified by a deletion, an insertion, and/or a substitution that results in the deletion of all or part of a stem-loop structure ~~normally~~ formed by the B and B' regions:-
- ~~26.~~ The ~~ceDNA vector of any one of claims 1-24, wherein~~ one or both of the flanking ITRs are

modified by a deletion, an insertion, and/or a substitution that results in the deletion of all or part of a stem-loop structure ~~normally~~ formed by the C and C' regions;

~~27. The ceDNA vector of any one of claims 1-24, wherein~~ one or both of the flanking ITRs are modified by a deletion, an insertion, and/or a substitution that results in the deletion of part of a stem-loop structure ~~normally~~ formed by the B and B' regions and/or part of a stem-loop structure normally formed by the C and C' regions;

~~28. The ceDNA vector of any one of claims 1-27, wherein~~ one or both of the flanking ITRs comprise a single stem-loop structure in the region that ~~normally, in a wild-type ITR, would~~ comprises a first stem-loop structure formed by the B and B' regions and a second stem-loop structure formed by the C and C' regions;

~~29. The ceDNA vector of any one of claims 1-28, wherein~~ one or both of the flanking ITRs comprise a single stem and two loops in the region that, in a wild-type ITR, normally would comprises a first stem-loop structure formed by the B and B' regions and a second stem-loop structure formed by the C and C' regions; or

~~30. The ceDNA vector of any one of claims 1-29, wherein~~ one or both of the flanking ITRs comprise a single stem and a single loop in the region that, in a wild-type ITR, normally would comprises a first stem-loop structure formed by the B and B' regions and a second stem-loop structure formed by the C and C' regions.

12. The ceDNA vector of claim 11, wherein the deletion, the insertion, and/or the substitution results in the deletion of all or part of a stem-loop structure formed by the A, A', B, B', C, or C' regions.

~~31.13. The ceDNA vector of any one of claims 1-3012, wherein both~~ ~~of the~~ flanking ITRs are altered in a manner that results in an overall three-dimensional symmetry when the flanking ITRs are inverted relative to each other.

~~32.14. The ceDNA vector of any one of claims 1-1334, wherein one or both of the~~ flanking ITRs comprise a sequence selected from the group consisting of one or more of the sequences set forth in Tables 3, 5A, 5B, and 6.

~~33.15. The ceDNA vector of any one of claims 1-1432, wherein~~ the ceDNA vector comprises at least one nucleic acid sequence is under the control of at least one regulatory switch.

~~34. The ceDNA vector of claim 33, wherein at least one regulatory switch is selected from a binary regulatory switch, a small molecule regulatory switch, a passcode regulatory switch, a nucleic acid-based regulatory switch, a post-transcriptional regulatory switch, a radiation-controlled or ultrasound-controlled regulatory switch, a hypoxia-mediated regulatory switch, an inflammatory response regulatory switch, a shear-activated regulatory switch, and a kill switch.~~

~~35.16. A capsid-free close-ended DNA (ceDNA) vector comprising a nucleic acid sequence selected from~~ the group consisting of: any of the sequences set forth in Table 13.

~~36-17.~~ A capsid-free close-ended DNA (ceDNA) vector comprising a nucleic acid sequence at least 85% identical to a nucleic acid selected from SEQ ID NO: 385, SEQ ID NO: 386, SEQ ID NO: 387, SEQ ID NO: 388, SEQ ID NO: 389 or SEQ ID NO: 390.

~~37-18.~~ A capsid-free close-ended DNA (ceDNA) vector consisting of a nucleic acid sequence selected from the group consisting of SEQ ID NO: 385, SEQ ID NO: 386, SEQ ID NO: 387, SEQ ID NO: 388, SEQ ID NO: 389 or SEQ ID NO: 390.

~~38-19.~~ A method of Use of the ceDNA vector of any one of claims 1 to 18 in the manufacture of a medicament for expressing a beta glucocerebrosidase (GBA) protein in a cell ~~comprising contacting the cell with the ceDNA vector of any one of claims 1-34.~~

~~39-20.~~ A GBA protein produced by translation of the ORF nucleic acid sequence ~~set forth in~~ selected from the group consisting of: any one of SEQ ID NO: 385, SEQ ID NO: 386, SEQ ID NO: 387, SEQ ID NO: 388, SEQ ID NO: 389 ~~or and~~ SEQ ID NO: 390.

~~40.~~ The method of claim 38, wherein the cell is a liver cell or muscle cell.

~~41.~~ The method of claim 38 or 40, wherein the cell is *in vitro* or *in vivo*.

~~42-21.~~ The ~~use method~~ of ~~any one of claims 19 or 20~~ ~~38-41~~, wherein the at least one nucleic acid sequence is codon optimized for expression in ~~the a~~ eukaryotic cell.

~~43-22.~~ The ~~use method~~ of ~~any one of claims 19 or 20~~ ~~38-41~~, wherein the at least one nucleic acid sequence ~~is codon optimized~~ is selected from the group consisting of any of the sequences set forth in Table 5.

~~44-23.~~ Use of the ceDNA vector of any one of claims 1-18 or the GBA protein of claim 20 in the manufacture of a medicament for ~~A method of treating a subject with Gaucher's disease, comprising administering to the subject a ceDNA vector of any one of claims 1-34, or a GBA protein of claim 39, wherein at least one nucleic acid sequence encodes at least one beta glucocerebrosidase (GBA) protein.~~

~~45-24.~~ The ~~method use~~ of claim ~~20~~ ~~41~~, wherein the least one nucleic acid sequence that encodes at least one GBA protein is selected from the group consisting of: any of the sequences set forth in Table 1 ~~or any of the ORFs included set forth~~ in Table 13.

~~46.~~ The method of claim 44 or 45, wherein the ceDNA vector is administered via intravenous injection.

~~47-25.~~ The ~~use method of any of claims 23 or 24~~ ~~44 to 46~~, wherein the ceDNA vector expresses the GBA protein in a liver cell, or in a muscle cell, or both.

~~48.~~ The method of any of claims 44-47, wherein the ceDNA vector is administered by ~~any one or more of: intramuscular injection, intrathecal injection or intravenous injection.~~

~~49-26.~~ A pharmaceutical composition comprising the ceDNA vector of any one of claims 1-~~18~~ ~~37~~.

~~50-27.~~ A cell containing a the ceDNA vector of any of claims 1-~~18~~ ~~37~~.

~~51.~~ The cell of claim 50, wherein the cell is a liver cell, or a muscle cell or both.

~~52-28.~~ A composition comprising a the ceDNA vector of any of claims 1-~~18~~ ~~37~~ and a lipid, wherein

the lipid is a lipid nanoparticle (LNP).

~~53. — The composition of claim 52, wherein the lipid is a lipid nanoparticle (LNP).~~

~~54-29.~~ A kit comprising the ceDNA vector of any one of claims 1-~~1837~~ or the composition of claim ~~52 or 53~~28 or the cell of claim ~~2750~~and instructions for use.