Claims

1. A donor polynucleotide comprising a non-replicative double-stranded DNA molecule (dsDNA) comprising a nucleotide sequence which corrects a mutation that causes Glycogen Storage Disease 1a in a genomic DNA (gDNA) molecule molecule in a cell, wherein the mutation is a protein-coding mutation proximal to a 3' splice site in the human G6PC gene, the donor polynucleotide comprising,

(i) a first strand comprising from 5' to 3': (a) a nucleotide sequence comprising splicing signals to control processing of a precursor mRNA (pre-mRNA) transcribed from the gDNA, wherein the splicing signals are a branch point, a polypyrimidine tract, and a natural or enhanced 3' splice site; and (b) a nucleotide sequence comprising an exonic sequence which corrects the mutation; and

(ii) a second strand comprising a nucleotide sequence complementary to the first strand, wherein the donor polynucleotide is at least 25 and up to 500 nucleotides in length, and wherein when the donor polynucleotide is introduced into the cell in combination with a site-directed nuclease, a non-homologous end-joining (NHEJ) DNA repair pathway inserts the donor polynucleotide into a double-stranded DNA break (DSB) introduced into the gDNA by the site-directed nuclease at a location proximal to the mutation, thereby correcting the mutation;

wherein the branch point sequence comprises the nucleotide sequence TTCAT, wherein the polypyrimidine tract comprises the nucleotide sequence CTTGTTCTGTTTTTTT (SEQ ID NO: 109), wherein the 3' splice site comprises the nucleotide sequence TAG.

2. The donor polynucleotide of claim 1, wherein the donor polynucleotide is up to 400, 300, 200, 100, 80, 70, or 60 nucleotides in length.

3. The donor polynucleotide of claim 1, wherein the donor polynucleotide is 30-70 or 40-60 nucleotides in length.

4. The donor polynucleotide of claim 1, wherein the mutation is an amino acid substitution of arginine (R) at position 83 in the G6PC gene, and wherein the exonic sequence comprises a codon encoding arginine (R) corresponding to the codon at position 83 in the G6PC gene.

5. The donor polynucleotide of claim 1, wherein insertion of the donor polynucleotide into the DSB results in the formation of an exon in the gDNA comprising the exonic sequence.

6. The donor polynucleotide of claim 1, wherein the splicing signals direct the inclusion of the exon comprising the exonic sequence which corrects the mutation into an mRNA.

7. The donor polynucleotide of claim 1, wherein the exonic sequence comprises the nucleotide sequence GATTCTCTTTGGACAGCGCCCTTACT (SEQ ID NO: 110).

8. The donor polynucleotide of claim 1, wherein the donor polynucleotide comprises two blunt ends.

9. A pharmaceutical composition comprising the donor polynucleotide according to claim 1 and a pharmaceutically acceptable carrier.