Summary

In one aspect, the present disclosure refers to a process for producing a modified nucleic acid molecule, the process comprising the steps:

(a) contacting a nucleic acid molecule comprising a mutant *HBB* gene encoding a mutant Hb-β polypeptide with a base editor, wherein the mutant *HBB* gene comprises a first non-wild-type codon coding for a first non-wild-type amino acid, and wherein the presence of said first non-wild-type amino acid in the mutant Hb-β polypeptide is the cause of a diseased Hb phenotype; and

(b) incubating the mutant *HBB* gene and base editor under conditions such that the base editor is targeted to the nucleotide sequence of the first non-wild-type codon and wherein the base editor edits one or more nucleotides in the first non-wild-type codon to produce a second non-wild-type codon which codes for a second non-wild-type amino acid, thereby producing a modified nucleic acid molecule comprising an edited *HBB* gene which encodes an edited Hb-β polypeptide,

wherein the edited Hb-β polypeptide has a non-wild-type, yet phenotypically-viable, amino acid sequence.

In another aspect, the present disclosure refers to a population of isolated cells comprising haematopoietic stem cells or progenitor cells comprising edited *HBB* genes, the edited *HBB* genes comprising:

(i) a nucleotide sequence having 90-99.9% nucleotide sequence identity to SEQ ID NO: 1 or a nucleotide sequence encoding an amino acid sequence having 95-99.5% amino acid sequence identity to SEQ ID NO: 2; and wherein

(ii) the nucleotide sequence at the codon which corresponds to codon 7 in SEQ ID NO: 1 codes for glycine or alanine; and/or the nucleotide sequence at the codon which corresponds to codon 27 in SEQ ID NO: 1 codes for glycine, wherein the population of isolated cells comprises at least 20% haematopoietic stem cells or progenitor cells having modified nucleic acid molecules comprising edited *HBB* genes, preferably at least 40%, at least 60%, at least 80% or 100% haematopoietic stem cells or progenitor cells having modified nucleic acid molecules comprising edited *HBB* genes.

CLAIMS

1. A process for producing a modified nucleic acid molecule, the process comprising the steps:

(a) contacting a nucleic acid molecule comprising a mutant *HBB* gene encoding a mutant Hb-β polypeptide with a base editor, wherein the mutant *HBB* gene comprises a first non-wild-type codon coding for a first non-wild-type amino acid, and wherein the presence of said first non-wild-type amino acid in the mutant Hb-β polypeptide is the cause of a diseased Hb phenotype; and

(b) incubating the mutant *HBB* gene and base editor under conditions such that the base editor is targeted to the nucleotide sequence of the first non-wild-type codon and wherein the base editor edits one or more nucleotides in the first non-wild-type codon to produce a second non-wild-type codon which codes for a second non-wild-type amino acid, thereby producing a modified nucleic acid molecule comprising an edited *HBB* gene which encodes an edited Hb-β polypeptide,

wherein the edited Hb-β polypeptide has a non-wild-type, yet phenotypically-viable, amino acid sequence.

2. A process as claimed in claim 1, wherein:

Step (a) comprises contacting a nucleic acid molecule comprising a mutant *HBB* gene encoding a mutant Hb-β polypeptide with a base editor and a gRNA, wherein the mutant *HBB* gene comprises a first non-wild-type codon coding for a first non-wild-type amino acid and wherein the gRNA is capable of targeting the base editor to the nucleotide sequence of the first non-wild-type codon of the mutant *HBB* gene; and

Step (b) comprises incubating the mutant *HBB* gene, base editor and gRNA under conditions such that the gRNA targets the base editor to the nucleotide sequence of the first non-wild-type codon and wherein the base editor edits one or more nucleotides in the first non-wild-type codon to produce a second non-wild-type codon which codes for a second non-wild-type amino acid, thereby producing a modified nucleic acid molecule comprising an edited *HBB* gene.

3. A process as claimed in claim 1 or claim 2, wherein the *HBB* gene is a mammalian gene, preferably a human gene.

4. A process as claimed in any one of the preceding claims, wherein mutant *HBB* gene consists of or comprises a nucleotide sequence which encodes an amino acid sequence having 90-99.5%, more preferably 95-99.5% sequence identity to SEQ ID NO: 2.

5. A process as claimed in claim 4, wherein the nucleotide sequence at the codon which corresponds to codon 7 in SEQ ID NO: 1 codes for lysine or valine; and/or the nucleotide sequence at the codon which corresponds to codon 27 in SEQ ID NO: 1 codes for lysine.

6. A process as claimed in claim 5, wherein the nucleotide sequence at the first non-wild-type codon which corresponds to codon 7 in SEQ ID NO: 1 is AAG or GTG; and/or the nucleotide sequence at the first non-wild-type codon which corresponds to codon 27 in SEQ ID NO: 1 is AAG.

7. A process as claimed in any one of the preceding claims, wherein the base editor is a programmable nucleic acid binding protein (preferably an impaired CRISPR-Cas9 mutant) which is capable of being targeted to a target DNA sequence.

8. A process as claimed in claim 7, wherein the base editor is an adenine deaminating editor.

9. A process as claimed in ~~any one of~~ claim~~s~~ 2 ~~to 8~~, wherein:
(i) the guide RNA sequence for editing codon 7 is an 18-22 nucleotide guide RNA which is complementary to a nucleotide sequence located in SEQ ID NO: 15, wherein the wild-type complement of codon 7 (CTC) is replaced by CAC; or
(ii) the guide RNA sequence for editing codon 27 is an 18-22 nucleotide guide RNA which is located in SEQ ID NO: 16, wherein the wild-type codon 27 (GAG) is replaced by AAG; or
(iii) the guide RNA sequence for editing codon 7 is an 18-22 nucleotide guide RNA which is located in SEQ ID NO: 17, wherein the wild-type of codon 7 (GAG) is replaced by AAG.

10. A process as claimed in any one of the preceding claims, wherein the edited *HBB* gene consists of or comprises:
(i) a nucleotide sequence having 90-99.9% nucleotide sequence identity to SEQ ID NO: 1 or a nucleotide sequence encoding an amino acid sequence having 95-99.5% amino acid sequence identity to SEQ ID NO: 2; and wherein

(ii) the nucleotide sequence at the codon which corresponds to codon 7 in SEQ ID NO: 1 codes for glycine or alanine; and/or the nucleotide sequence at the codon which corresponds to codon 27 in SEQ ID NO: 1 codes for glycine.

11. A process as claimed in any one of the preceding claims, wherein:
(i) the position of the first non-wild-type codon is codon 7, the wild-type codon at this position is GAG (glutamate), the first non-wild-type (mutant) codon sequence is AAG (lysine), the base editor is an adenine base editor and the second non-wild-type codon is GGG (glycine); or
(ii) the position of the first non-wild-type codon is codon 7, the wild-type codon at this position is GAG (glutamate), the first non-wild-type (mutant) codon sequence is GTG (valine), the base editor is an adenine base editor and the second non-wild-type codon is GCG (alanine); or
(iii) the position of the first non-wild-type codon is codon 27, the wild-type codon at this position is GAG (glutamate), the first non-wild-type (mutant) codon sequence is AAG (lysine), the base editor is an adenine base editor and the second non-wild-type codon is GGG (glycine).

~~12. A process as claimed in any one of the preceding claims, which additionally includes, prior to Step (a), the step of obtaining a sample of haematopoietic stem cells from a subject, preferably from a human subject, wherein the stem cells comprise nucleic acid molecules comprising mutant *HBB* genes.~~

~~13~~12. A process as claimed in any one of the preceding claims, which additionally comprises, prior to Step (a), the step of modifying the nucleotide sequences of one or more PAM sites in the vicinity of the first non-wild-type codon in order to increase efficiency of the base-editing process.

~~14~~13. A process as claimed in any one of the preceding claims, wherein the process is performed on haematopoietic stem cells which have previously been obtained from a first subject, preferably from a human subject, wherein the stem cells comprise nucleic acid molecules comprising mutant *HBB* genes.

~~15. A process as claimed in claim 14, the process additionally comprises the subsequent step of introducing a population of haematopoietic stem cells comprising modified nucleic acid molecules comprising edited *HBB* genes, optionally after expansion of the cells, into a second subject, wherein the first and second subjects are the same or related subjects.~~

~~16~~14. A population of isolated cells comprising haematopoietic stem cells or progenitor cells comprising edited *HBB* genes, the edited *HBB* genes comprising:
(i) a nucleotide sequence having 90-99.9% nucleotide sequence identity to SEQ ID NO: 1 or a nucleotide sequence encoding an amino acid sequence having 95-99.5% amino acid sequence identity to SEQ ID NO: 2; and wherein
(ii) the nucleotide sequence at the codon which corresponds to codon 7 in SEQ ID NO: 1 codes for glycine or alanine; and/or the nucleotide sequence at the codon which corresponds to codon 27 in SEQ ID NO: 1 codes for glycine~~.~~

~~17. A population of isolated cells as claimed in claim 16~~, wherein the population of isolated cells comprises at least 20% haematopoietic stem cells or progenitor cells having modified nucleic acid molecules comprising edited *HBB* genes, preferably at least 40%, at least 60%, at least 80% or 100% haematopoietic stem cells or progenitor cells having modified nucleic acid molecules comprising edited *HBB* genes.

15. A process as claimed in any one of claims 1-12, wherein the nucleic acid molecule comprising the mutant *HBB* gene is present in a cell; and the process is carried out *ex vivo* or *in vitro*.

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