developing a hemoglobinopathy such as a thalassemia (*e.g.,* β-thalassemia) or sickle cell disease are also provided, the method comprising administering to the patient the pharmaceutical composition as described herein (*e.g.,* proteins, polynucleotides, cells or a combination selected from proteins, polynucleotides and cells) in an amount sufficient to increase the globin gene expression in the patient.

[0045a] In one aspect, the present disclosure refers to a paired zinc finger nuclease (ZFN) comprising left and right zinc finger nuclease, the left zinc finger nuclease comprising the amino acid sequence as shown in SEQ ID NO:29 and the right zinc finger nuclease comprising the amino acid sequence as shown in SEQ ID NO:31.

[0045b] In another aspect, the present disclosure refers to one or more polynucleotides encoding one or more paired zinc finger nucleases as disclosed herein.

[0045c] In another aspect, the present disclosure refers to an isolated cell comprising the paired zinc finger nuclease as disclosed herein or the one or more polynucleotides as disclosed herein.

[0045d] In another aspect, the present disclosure refers to an isolated population of cells comprising genetically modified cells produced from the cell as disclosed herein, wherein less than .15% genetic modifications are outside the BCL11A locus.

[0045e] In another aspect, the present disclosure refers to an isolated partially or fully differentiated cell descended from the isolated population of genetically modified cells as disclosed herein.

[0045f] In another aspect, the present disclosure refers to pharmaceutical composition comprising the paired zinc finger nuclease, the one or more polynucleotide, the cell or the isolated population of cells as disclosed herein.

[0045g] In yet another aspect, the present disclosure refers to the paired zinc finger nuclease or the polynucleotide as described herein for use in therapy for modifying an endogenous BCL11A enhancer sequence in a cell, wherein the modification is a deletion and/or insertion.

[0045h] In yet another aspect, the present disclosure refers to use of the paired zinc finger nuclease or the one or more polynucleotide as described herein

in the manufacture of a medicament for the treatment of hemoglobinopathy.

[0045i] In still another aspect, the present disclosure refers to the cell or the isolated population of cells as described herein for use in therapy for increasing globin production in a subject.

[0045j] In still another aspect, the present disclosure refers to use of the cell or the isolated population of cells as described herein in the manufacture of a medicament for the treatment of hemoglobinopathy, said use comprises that the cell is to be administered to a subject to increase globin production.

[0045k] In another aspect, the present disclosure refers to a method of producing an isolated genetically modified cell comprising a genomic modification within an endogenous BCL11A enhancer sequence, the method comprising the steps of:

a) contacting an isolated cell with the polynucleotide as disclosed herein;

b) subjecting the cell to conditions conducive to expressing the fusion protein from the polynucleotide; and

c) modifying the endogenous BCL11A enhancer sequence with the expressed fusion protein sufficient to produce the genetically modified cell.

[0045l] In another aspect, the present disclosure refers to a kit comprising the paired zinc finger nuclease, the polynucleotide, the cell, the isolated population of cells and/or the pharmaceutical composition as disclosed herein.

[0046] These and other examples will be readily apparent to the skilled artisan in light of disclosure as a whole.

BRIEF DESCRIPTION OF THE DRAWINGS

[0047] **Figures 1A and 1B** are schematics depicting an overview of the oligonucleotide duplex integration site assay. Figure 1 shows the first step of the assay, in which cells are treated with ZFNs in the presence of supplemental oligonucleotide duplex DNA, which is captured into a fraction of the resulting cleavage events. Figure 1B shows the subsequence step in which cells are cultured for seven days, genomic DNA is isolated, and segments of the genome flanking donor integration sites are amplified via adaptor-mediated PCR using a primer to the integrated oligonucleotide duplex. Amplicons are then sequenced to reveal candidate cleavage sites.

[0048] **Figure 2** depicts the location of 455 potential off-target loci identified by the oligonucleotide duplex integration site assay using the 51857/51949 original or parent ZFN pair. The top 63 loci are highlighted in gray and were analyzed in follow up indel analyses. Each locus is identified by the chromosome and base number indicating the most likely location of cleavage, as well as an indication of the number of integrants detected at that locus.

[0049] **Figures 3A through 3C** depict exemplary phosphate contacting residues within the zinc finger scaffold. Figure 3A depicts a zinc finger interaction with a DNA molecule, and shows the location of the wild type arginine side chain and how it interacts with the phosphate backbone of the DNA molecule. Figure 3B (SEQ ID Nos:7 to 17) depicts exemplary ZFN sequences showing where this arginine is located in the primary sequence of each zinc finger (vertical row of 'R' residues

CLAIMS

1. A paired zinc finger nuclease (ZFN) comprising left and right zinc finger nuclease, the left zinc finger nuclease comprising the amino acid sequence as shown in SEQ ID NO:29 and the right zinc finger nuclease comprising the amino acid sequence as shown in SEQ ID NO:31.

2. One or more polynucleotides encoding one or more paired zinc finger nucleases according to claim 1.

3. The one or more polynucleotides of claim 2, wherein the polynucleotides comprise mRNA.

4. The one or more polynucleotides of claim 3 comprising SEQ ID NO:28 or SEQ ID NO:30.

5. An isolated cell comprising the paired zinc finger nuclease of claim 1 or the one or more polynucleotides of any of claims 2 to 4.

6. The cell of claim 5, wherein the cell is a stem cell or precursor cell.

7. The cell of claim 6, wherein the cell is a human cell.

8. The cell of any of claims 5 to 7, wherein the genome of the cell is modified by the paired zinc finger nuclease.

9. The cell of claim 8, wherein the genomic modification is selected from the group consisting of insertions, deletions and combinations thereof.

10. The cell of claim 8 or 9, wherein the genomic modification comprises an indel in a BCL11A locus.

11. An isolated population of cells comprising genetically modified cells produced from the cell of any of claims 5 to 10, wherein less than .15% genetic modifications are outside the BCL11A locus.

12. An isolated partially or fully differentiated cell descended from the isolated population of genetically modified cells of claim 11.

13. The cell or isolated population of genetically modified cells of any of claims 8 to 12, wherein the cell exhibits increased expression of gamma and/or beta globin as compared to a cell without the paired zinc finger nuclease, the one or more polynucleotides or the genomic modification.

14. A pharmaceutical composition comprising the paired zinc finger nuclease according to claim 1, the one or more polynucleotide of any of claims 2 to 4, the cell of any of claims 5 to 10, 12 or 13 or the isolated population of cells of claim 11.

15. The paired zinc finger nuclease according to claim 1 or the polynucleotide of any of claims 2 to 4 for use in therapy for modifying an endogenous BCL11A enhancer sequence in a cell, wherein the modification is a deletion and/or insertion.

16. Use of the paired zinc finger nuclease according to claim 1 or the one or more polynucleotides of any of claims 2 to 4 in the manufacture of a medicament for the treatment of hemoglobinopathy.

17. The use according to claim 16, wherein the paired zinc finger nuclease or the polynucleotide is to be administered to a cell, such that the endogenous BCL11A enhancer sequence is modified.

18. The paired zinc finger nuclease or the polynucleotide according to claim 15, or the use according to claim 16, wherein an exogenous sequence is inserted into the endogenous BCL11A enhancer sequence.

19. The use according to claim 16 or claim 17, wherein the modification comprises a deletion.

20. The cell of any of claims 5 to 10, 12 or 13 or the isolated population of cells of claim 11 for use in therapy for increasing globin production in a subject.

21. Use of the cell of any of claims 5 to 10, 12 or 13 or the isolated population of cells of claim 11 in the manufacture of a medicament for the treatment of hemoglobinopathy, said use comprises that the cell is to be administered to a subject to increase globin production.

22. The use according to claim 21, wherein the subject is a human and the cell is a human stem cell or human precursor cell.

23. The use according to claim 22, wherein the cell is to be engrafted, differentiated and matured in the subject.

24. The use according to any of claims 21 to 23, wherein the hemoglobinopathy is a beta-thalassemia or sickle cell disease.

25. A method of producing an isolated genetically modified cell comprising a genomic modification within an endogenous BCL11A enhancer sequence, the method comprising the steps of:

a) contacting an isolated cell with the polynucleotide of any of claims 2 to 4;

b) subjecting the cell to conditions conducive to expressing the fusion protein from the polynucleotide; and

c) modifying the endogenous BCL11A enhancer sequence with the expressed fusion protein sufficient to produce the genetically modified cell.

26. The method of claim 25, further comprising stimulating the cell with at least one cytokine.

27. A kit comprising the paired zinc finger nuclease of claim 1, the polynucleotide of any of claims 2 to 4, the cell of any of claims 5 to 10, 12 or 13, the isolated population of cells of claim 11 and/or the pharmaceutical composition of claim 14.