fragile than normal RBCs, and tend towards hemolysis, eventually leading to anemia in the patient.

[0013] Treatment and management of sickle cell patients is a life-long proposition involving antibiotic treatment, pain management and transfusions during acute episodes. One approach is the use of hydroxyurea, which exerts its effects in part by increasing the production of γ globin. Long term side effects of chronic hydroxyurea therapy are still unknown, however, and treatment gives unwanted side effects and can have variable efficacy from patient to patient. Despite an increase in the efficacy of sickle cell treatments, the life expectancy of patients is still only in the mid to late 50's and the associated morbidities of the disease have a profound impact on a patient's quality of life.

[0014] Thus, there remains a need for additional methods and compositions that can be used for genome editing, to correct an aberrant gene or alter the expression of others for example to treat hemoglobinopathies such as sickle cell disease.

SUMMARY

[0015] In one aspect, the present disclosure refers to a genetically modified cell comprising: a pair of zinc finger nucleases that bind to SEQ ID NO:23 and SEQ ID NO:24 and make a double-stranded break in an endogenous human beta-hemoglobin (Hbb) gene, wherein the pair comprises the zinc finger proteins designated 33488 and 33501; 33488 and 47817; 47773 and 33501; or 47773 and 47817 having the recognition helix regions ordered as shown in a single row of Table 1; and a genomic modification within or between SEQ ID NO:23 or SEQ ID NO:24 of an endogenous human beta-hemoglobin (Hbb) gene following double-stranded break by the pair of zinc finger nucleases, wherein the genomic modification comprises an insertion and/or deletion. In another aspect, the present disclosure refers to a genetically modified differentiated cell descended from the stem cell of the present disclosure. In yet another aspect, the present disclosure refers to pharmaceutical composition comprising the genetically modified cell of the present disclosure. In another aspect, the present disclosure refers to a composition comprising a pair of zinc finger nucleases, each zinc finger nuclease comprising a zinc finger protein and a cleavage domain or cleavage half-domain, wherein the zinc finger protein comprises 5 or 6 zinc finger domains comprising a recognition helix region, wherein the pair comprises the zinc finger proteins designated 33488 and 33501 or 47773 and 47817 having the recognition helix regions ordered as shown in a single row of Table 1. In another aspect, the present disclosure refers to an isolated cell comprising the composition of any of the present disclosure. In another aspect, the present disclosure refers to a kit comprising a composition according to the present disclosure or a cell

according to the present disclosure. In yet another aspect, the present disclosure refers to an *in vitro* method of altering Hbb expression in a cell, the method comprising: introducing, into the cell, one or more compositions according to the present disclosure, under conditions such that the pair of zinc finger nucleases is expressed and expression of the Hbb gene is altered. In still another aspect, the present disclosure refers to an *in vitro* method of producing a genetically modified cell comprising a genomic modification within an endogenous Hbb gene, the method comprising the steps of: a) contacting a cell with a composition according to the present invention, b) subjecting the cell to conditions conducive to expressing the pair of zinc finger nuclease; and c) modifying the endogenous Hbb gene with the expressed fusion protein sufficient to produce the genetically modified cell. In one other aspect, the present disclosure refers to the use of the isolated cell of the present disclosure in the manufacture of a medicament for treating a patient with sickle cell anemia. Also disclosed herein are methods and compositions for altering the expression or for correcting one or more genes encoding proteins involved in a genetic disease (*e.g.,* producing proteins lacking, deficient or aberrant in the disease and/or proteins that regulate these proteins) such as sickle cell disease. The present disclosure describes compositions and methods for use in gene therapy and genome engineering.

[0016] Alteration of such proteins can result in the treatment of these genetic diseases. In particular, genome editing is used to correct an aberrant gene, insert a wild type gene, or change the expression of an endogenous gene. One approach involves the use of gene correction where a faulty endogenous β globin gene is targeted and the mutant sequence replaced. One approach further involves the use of modification of a stem cell (*e.g.,* hematopoietic stem cell or RBC precursor), which stem cell can then be used to engraft into a patient, for treatment of a hemoglobinopathy.

[0017] In one example, described herein is a genetically modified cell or cell line, for example as compared to the wild-type genomic sequence of the same type of cell or cell line. In certain examples, the cells comprise genetically modified

CLAIMS

What is claimed is:

1. A genetically modified cell comprising:

a pair of zinc finger nucleases that bind to SEQ ID NO:23 and SEQ ID NO:24 and make a double-stranded break in an endogenous human beta-hemoglobin (Hbb) gene, wherein the pair comprises the zinc finger proteins designated 33488 and 33501; 33488 and 47817; 47773 and 33501; or 47773 and 47817 having the recognition helix regions ordered as shown in a single row of Table 1; and

a genomic modification within or between SEQ ID NO:23 or SEQ ID NO:24 of an endogenous human beta-hemoglobin (Hbb) gene following double-stranded break by the pair of zinc finger nucleases, wherein the genomic modification comprises an insertion and/or deletion.

2. The genetically modified cell of claim 1, wherein the cell is a stem cell.

3. The genetically modified cell of claim 2, wherein the stem cell is a hematopoietic stem cell.

4. The genetically modified cell of claim 3, wherein the hematopoietic stem cell is a CD34+ cell.

5. A genetically modified differentiated cell descended from the stem cell of any of claims 1 to 4.

6. The genetically modified cell of claim 5, wherein the cell is a red blood cell (RBC).

7. The genetically modified cell of any of claims 1 to 6, wherein the insertion comprises integration of a donor polynucleotide encoding a transgene.

8. The genetically modified cell of claim 7, wherein the donor polynucleotide corrects a sickle cell mutation.

9. A pharmaceutical composition comprising the genetically modified cell of any of claims 1 to 8.

10. A composition comprising a pair of zinc finger nucleases, each zinc finger nuclease comprising a zinc finger protein and a cleavage domain or cleavage half-domain, wherein the zinc finger protein comprises 5 or 6 zinc finger domains comprising a recognition helix region, wherein the pair comprises the zinc finger proteins designated 33488 and 33501 or 47773 and 47817 having the recognition helix regions ordered as shown in a single row of Table 1.

11. The composition of claim 10, wherein the cleavage half-domain is a wild-type or engineered cleavage half-domain.

12. The composition of claim 10 or 11, wherein the composition comprises one or more polynucleotides encoding the pair of zinc finger nucleases.

13. An isolated cell comprising the composition of any of claims 10 to 12.

14. The cell of claim 13, wherein the cell is selected from the group consisting of a red blood cell (RBC) or a precursor cell (e.g., CD4+ hematopoietic stem cell).

15. A kit comprising a composition according to any of claims 10 to 12 or a cell according to claim 13 or 14.

16. An *in vitro* method of altering Hbb expression in a cell, the method comprising:

introducing, into the cell, one or more compositions according to claim 12, under conditions such that the pair of zinc finger nucleases is expressed and expression of the Hbb gene is altered.

17. The method of claim 16, wherein expression of the Hbb gene is increased.

18. The method of claim 16 or claim 17, further comprising integrating a donor sequence into the genome of the cell.

19. The method of any of claims 16 to 18, wherein the cell is selected from the group consisting of a red blood cell (RBC) precursor cell and a hematopoietic stem cell.

20. The method of any of claims 16 to 19, wherein the donor sequence comprises an oligonucleotide that corrects a mutation that alters the splice donor site for mRNA processing of the Hbb gene.

21. An *in vitro* method of producing a genetically modified cell comprising a genomic modification within an endogenous Hbb gene, the method comprising the steps of:

a) contacting a cell with a composition according to claim 12,

b) subjecting the cell to conditions conducive to expressing the pair of zinc finger nuclease; and

c) modifying the endogenous Hbb gene with the expressed fusion protein sufficient to produce the genetically modified cell.

22. The method of claim 21, wherein the method further comprises stimulating the cells with at least one cytokine.

23. Use of the isolated cell of claim 14 in the manufacture of a medicament for treating a patient with sickle cell anemia.