

Claims

1. A composition comprising genetically modified red blood precursor cells or genetically modified cells descended therefrom, the genetically modified cells comprising:

(a) a zinc finger nuclease (ZFN) pair comprising left and right zinc finger nucleases, wherein:

(i) the left ZFN binds to a target site within SEQ ID NO: 1 and comprises a zinc finger protein designated 51856, 51862, 51446, 51463 or 51484 in Table 1; and

(ii) the right ZFN binds to a target site within SEQ ID NO: 12 and comprises a zinc finger protein designated 51536, 51990, 51993, 51979, 51982, 52015 or 52032 in Table 1; and

a genomic modification made following cleavage by the ZFN pair, wherein the modification is within an BCL11A enhancer sequence, such that the BCL11A gene is inactivated.

2. The composition of claim 1, wherein the genomic modification is selected from the group consisting of insertions and/or deletions.

3. The composition of claim 1 or claim 2, wherein the genomic modification is within or between the target sites.

4. The composition of claim 1 or claim 2, wherein the cells are hematopoietic stem cells, and wherein the cells are optionally differentiated into a red blood cells (RBC).

5. The composition of claim 1, wherein the nuclease is introduced into the cell as a polynucleotide and the genetically modified cells further comprise a donor polynucleotide encoding a transgene and the genomic modification comprises integration of the donor polynucleotide.

6. A zinc finger nuclease comprising left and right ZFNs, each ZFN comprising a cleavage domain and a zinc finger protein comprising 4, 5, or 6 zinc finger domains designated F1 to F4, F1 to F5 or F1 to F6, wherein

(a) the left ZFN comprises the following recognition helix regions:

(i) F1: DQSNLRA (SEQ ID NO:2);

- F2: RNFSLTMT (SEQ ID NO:9);
 F3: SNGNLRN (SEQ ID NO:10) or SSYNLAN (SEQ ID NO:11);
 F4: TSGSLTR (SEQ ID NO:5);
 F5: DQSNLRA (SEQ ID NO:2); and
 F6: AQCCLFH (SEQ ID NO:6); or
- (ii) F1: STGNLTN (SEQ ID NO:7);
 F2: TSGSLTR (SEQ ID NO:5);
 F3: DQSNLRA (SEQ ID NO:2); and
 F4: AQCCLFH (SEQ ID NO:6); or
- (iii) F1: DQSNLRA (SEQ ID NO:2);
 F2: RPYTLRL (SEQ ID NO:3);
 F3: SRGALKT (SEQ ID NO:8);
 F4: TSGSLTR (SEQ ID NO:5);
 F5: DQSNLRA (SEQ ID NO:2); and
 F6: AQCCLFH (SEQ ID NO:6);
- (b) the right ZFN comprises a ZFP comprising the following recognition helix regions:
- (iv) F1: RNDHRTT (SEQ ID NO:19);
 F2: QKAHLIR (SEQ ID NO:20);
 F3: QKGTGE (SEQ ID NO:15);
 F4: LKRTLKR (SEQ ID NO:25); and
 F5: RRDNLHS (SEQ ID NO:17);
- (v) F1: RSDHLTQ (SEQ ID NO:13);
 F2: QSGHLAR (SEQ ID NO:14);
 F3: QKGTGE (SEQ ID NO:15);
 F4: RHRDLR (SEQ ID NO:18); and
 F5: RRDNLHS (SEQ ID NO:17); or
- (vi) F1: RSDHLTQ (SEQ ID NO:13);
 F2: QRAHLTR (SEQ ID NO:22);
 F3: QKGTGE (SEQ ID NO:15) or QSGTRNH (SEQ ID NO:24);
 F4: HRNTLVR (SEQ ID NO:23); and
 F5: RRDNLHS (SEQ ID NO:17); or
- (vii) F1: RSDHLTQ (SEQ ID NO:13);
 F2: QKAHLIR (SEQ ID NO:20);

- F3: QKGTLLGE (SEQ ID NO:15) or QSGTRNH (SEQ ID NO:24);
 F4: RGRDLSR (SEQ ID NO:21); and
 F5: RRDNLHS (SEQ ID NO:17); or
- (viii) F1: RSDHLTQ (SEQ ID NO:13);
 F2: QSGHLAR (SEQ ID NO:14);
 F3: QSGTRNH (SEQ ID NO:24);
 F4: QSSDLSR (SEQ ID NO:16); and
 F5: RRDNLHS (SEQ ID NO:17).
7. The zinc finger nuclease of claim 6 comprising a wild-type or engineered cleavage domain or wild-type or engineered cleavage half-domain.
8. A polynucleotide encoding one or more zinc finger nucleases of claim 6 or claim 7.
9. An isolated cell comprising one or more zinc finger nucleases of claim 7 or one or more polynucleotides of claim 8.
10. The cell of claim 9, wherein the cell is selected from the group consisting of a red blood cell (RBC) or a precursor cell, such as a CD34⁺ hematopoietic stem cell or a cell descended therefrom.
11. A kit comprising the zinc finger nuclease of claim 6 or claim 7, the polynucleotide or claim 8 or the cell according to claim 9 or claim 10.
12. An *in vitro* method of altering globin gene expression in a cell, the method comprising:
 introducing, into the cell, one or more zinc finger nucleases of claim 6 or claim 7 or one or more polynucleotides of claim 8, under conditions such that the one or more proteins are expressed and expression of the globin gene is altered.
13. The one or more zinc finger nucleases of claim 6 or claim 7 or one or more polynucleotides of claim 8 for use in a method of altering globin gene expression in a cell, the method comprising:

introducing, into the cell, the one or more zinc finger nucleases or one or more polynucleotides, under conditions such that the one or more proteins are expressed and expression of the globin gene is altered.

14. The method of claim 12 or the one or more zinc finger nucleases of claim 6 or claim 7 or one or more polynucleotides of claim 8 for use of claim 13, wherein:

- (a) expression of a globin gene is increased, such as a gamma globin or beta globin gene;
- (b) the method further comprises integrating a donor sequence into the genome of the cell, for example, by introducing the donor sequence to the cell using a viral vector, an oligonucleotide or a plasmid;
- (c) the cell is a red blood cell (RBC) precursor cell, such as a CD34+ hematopoietic stem cell; and/or
- (d) the donor sequence comprises a transgene under the control of an endogenous promoter or an exogenous promoter.

15. The method of claim 12 or 14, or the one or more zinc finger nucleases or one or more polynucleotides for use according to claim 13, wherein globin gene expression is the level of globin mRNA or globin protein produced.

16. A composition of any of claims 1 to 5 for use in the treatment of hemoglobinopathies.

17. Use of a composition of any of claims 1 to 5 in the manufacture of medicament for the treatment of a beta-thalassemia or sickle cell disease wherein upon administration, the medicament increases globin production in a subject.

18. The use of claim 17, wherein the subject is a human and the composition comprises human stem cells or human precursor cells.

19. The use of claim 17 or claim 18, wherein the composition is to be administered in a bone marrow transplant whereupon the cells engraft, differentiate and mature in the subject.