Claims

What is claimed is:

1. A *ex vivo* method for editing a B-cell lymphoma 11A (BCL11A) gene in a human cell by genome editing, the method comprising the step of:
introducing into the human cell *ex vivo* one or more deoxyribonucleic acid (DNA) endonucleases and a single-molecule guide ribonucleic acid (sgRNA) comprising the nucleic acid sequence of SEQ ID NO: 71,959 to effect one or more single-strand breaks (SSBs) or double-strand breaks (DSBs), within or near the BCL11A gene or other DNA sequence that encodes a regulatory element of the BCL11A gene, that results in a permanent deletion, modulation, or inactivation of a transcriptional control sequence of the BCL11A gene.

2. An *ex vivo* method, the method comprising the steps of:
creating a subject specific induced pluripotent stem cell (iPSC) or obtaining a mesenchymal stem cell isolated from the subject;
editing within or near a BCL11A gene or other DNA sequence that encodes a regulatory element of the BCL11A gene of the iPSC or the mesenchymal stem cell by introducing one or more deoxyribonucleic acid (DNA) endonucleases and a single-molecule guide ribonucleic acid (sgRNA) comprising the nucleic acid sequence of SEQ ID NO: 71,959;
differentiating the genome-edited iPSC or the mesenchymal stem cell into a hematopoietic progenitor cell; and
recovering the hematopoietic progenitor cell for implanting into the subject.

3. The method of claim 2, wherein:
the creating step comprises recovering a somatic cell isolated from the subject, or the isolated cell is isolated by aspiration of bone marrow and isolation of mesenchymal cells using density gradient centrifugation media; and
introducing a set of pluripotency-associated genes into the somatic cell to induce the somatic cell to become a pluripotent stem cell.

4. The method of claim 3, wherein the set of pluripotency-associated genes is one or more of the genes selected from the group consisting of OCT4, SOX2, KLF4, Lin28, NANOG and cMYC.

5. The method of claim 2, wherein the method meets any one or more of the following:
(i) the differentiating step comprises one or more of the following to differentiate the genome-edited iPSC or the mesenchymal stem cell into a hematopoietic progenitor cell: treatment with a combination of small molecules, delivery of master transcription factors, delivery of mRNA encoding master transcription factors, or delivery of mRNA encoding transcription factors; or
(ii) the recovered cell is for being implanted into the patient by transplantation, local injection, systemic infusion, or combinations thereof.

6. An *ex vivo* method, the method comprising the steps of:
obtaining a hematopoietic progenitor cell isolated from a subject;
editing within or near a BCL11A gene or other DNA sequence that encodes a regulatory element of the BCL11A gene of the hematopoietic progenitor cell by introducing one or more deoxyribonucleic acid (DNA) endonucleases and a single-molecule guide ribonucleic acid (sgRNA) comprising the nucleic acid sequence of SEQ ID NO: 71,959; and
recovering the genome-edited hematopoietic progenitor cell for implanting into the patient.

7. The method of any one of claims 1, 2, or 5, wherein the one or more DNA endonucleases is selected from a Cas1, Cas1B, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8, Cas9 (also known as Csn1 and Csx12), Cas100, Csy1, Csy2, Csy3, Cse1, Cse2, Csc1, Csc2, Csa5, Csn2, Csm2, Csm3, Csm4, Csm5, Csm6, Cmr1, Cmr3, Cmr4, Cmr5, Cmr6, Csb1, Csb2, Csb3, Csx17, Csx14, Csx10, Csx16, CsaX, Csx3, Csx1, Csx15, Csf1, Csf2, Csf3, Csf4, or Cpf1 endonuclease; a homolog thereof, a recombination of the naturally occurring molecule thereof, codon-optimized thereof, or modified versions thereof, and combinations thereof.

8. The method of claim 7, wherein the method comprises (i) introducing into the cell one or more polynucleotides encoding the one or more DNA endonucleases; or (ii) introducing into the cell one or more ribonucleic acids (RNAs) encoding the one or more DNA endonucleases.

9. The method of claim 7, wherein the one or more DNA endonuclease is one or more proteins or polypeptides.

10. The method of claim 9, wherein the one or more proteins or polypeptides is flanked at the N-terminus, the C-terminus, or both the N-terminus and C-terminus by one or more nuclear localization signals (NLSs).

11. The method of any one of claims 1, 2, or 5, wherein the sgRNA comprises three 2'-O-methyl-phosphorothioate residues at or near each of its 5' and 3' ends.

12. The method of any one of claims 1, 2, or 5, wherein the one or more DNA endonucleases is pre-complexed with the sgRNA to form one or more ribonucleoproteins (RNPs).

13. The method of claim 12, wherein the one or more DNA endonucleases is combined with the sgRNA in a weight ratio of 1:1 DNA endonuclease to sgRNA to form the one or more ribonucleoproteins (RNPs).

14. The method of claim 12, wherein the sgRNA comprises the nucleic acid sequence of SEQ ID NO: 71,959, the DNA endonuclease is a *S. pyogenes* Cas9 comprising a N-terminus SV40 NLS and a C-terminus SV40 NLS, wherein the one or more DNA endonucleases is combined with the sgRNA in a weight ratio of 1:1 DNA endonuclease to sgRNA to form the one or more ribonucleoproteins (RNPs).

15. The method of any one of claims 1-6, wherein the method further comprises introducing into the cell a polynucleotide donor template comprising a wild-type BCL11A gene or cDNA comprising a modified transcriptional control sequence.

16. The method of any one of claims 1, 2, or 5, wherein:

(i) the method further comprises introducing into the cell a polynucleotide donor template comprising a wild-type BCL11A gene or cDNA comprising a modified transcriptional control sequence, and wherein the one or more DNA endonucleases is one or more Cas9 or Cpf1 endonucleases that effect one single-strand break (SSB) or double-strand break (DSB), at a locus within or near the BCL11A gene or other DNA sequence that encodes a regulatory element of the BCL11A gene, that facilitates insertion of a new sequence from the polynucleotide donor template into the chromosomal DNA at the locus that results in a permanent insertion, modulation, or inactivation of the transcriptional control sequence of the chromosomal DNA proximal to the locus, and wherein the gRNA comprises a spacer sequence that is complementary to a segment of the locus; or

(ii) the method further comprises introducing into the cell a polynucleotide donor template comprising a wild-type BCL11A gene or cDNA comprising a modified transcriptional control sequence, and wherein the one or more DNA endonucleases is one or more Cas9 or Cpf1 endonucleases that effect or create a pair of single-strand breaks (SSBs) or double-strand breaks (DSBs), the first break at a 5’ locus and the second break at a 3’ locus, within or near the BCL11A gene or other DNA sequence that encodes a regulatory element of the BCL11A gene, that facilitates insertion of a new sequence from the polynucleotide donor template into the chromosomal DNA between the 5’ locus and the 3’ locus that results in a permanent insertion, modulation, or inactivation of the transcriptional control sequence of the chromosomal DNA between the 5’ locus and the 3’ locus.

17. The method of claim 16, wherein the one or more Cas9 endonucleases is pre-complexed with the sgRNA to form one or more ribonucleoproteins (RNPs).

18. The method of claim 17, wherein the one or more Cas9 endonucleases is combined with the sgRNA in a weight ratio of 1:1 Cas9 endonuclease to sgRNA to form the one or more ribonucleoproteins (RNPs).

19. The method of claim 16, wherein

(i) the donor template is either single or double stranded; or

(ii) the modified transcriptional control sequence is located within a second intron of the BCL11A gene or the modified transcriptional control sequence is located within a +58 DNAse hypersensitive site (DHS) of the BCL11A gene.

20. The method of claim 16, wherein
(i) the Cas9 or Cpf1 are introduced by mRNA, and wherein the mRNA, sgRNA, and donor template are either each formulated into separate lipid nanoparticles or all co-formulated into a lipid nanoparticle; or
(ii) the Cas9 or Cpf1 are introduced by mRNA, and wherein the mRNA is formulated into a lipid nanoparticle, and both the sgRNA and donor template are delivered to the cell by an adeno-associated virus (AAV) vector; or
(iii) the Cas9 or Cpf1 are introduced by mRNA, and wherein the mRNA is formulated into a lipid nanoparticle, and the sgRNA is delivered to the cell by electroporation and donor template is delivered to the cell by an adeno-associated virus (AAV) vector.

21. The method of claim 12, wherein the one or more RNP is delivered to the cell by electroporation.

22. The method of claim 17, wherein the one or more RNP is delivered to the cell by electroporation.

23. A single-molecule guide RNA (sgRNA) comprising the nucleic acid sequence of SEQ ID NO: 71,959.

24. A genetically engineered cell, characterized in that is obtainable by a method comprising:
introducing into a human cell one or more S. pyogenes Cas9 endonucleases and one or more guide RNAs (gRNAs) to effect one or more double-strand breaks (DSBs) within or near a B-cell lymphoma 11A (BCL11A) gene or other DNA sequence that encodes a regulatory element of the BCL11A gene, that results in a permanent deletion, modulation, or inactivation of a transcripstional control sequence of the BCL11A gene, wherein the one or more gRNAs comprise a single-molecule guide RNA (sgRNA) of SEQ ID NO: 71,959.

25. A genetically engineered cell, characterized in that comprises a genetic mutation, which is one of a permanent deletion, modulation, or inactivation of a transcriptional control sequence of a B-cell lymphoma 11A (BCL11A) gene, wherein the genetic mutation occurs at the site target by a gRNA comprising a single-molecule guide RNA (sgRNA) of SEQ ID NO: 71,959.

26. The sgRNA of claim 23, wherein the sgRNA comprises three 2'-O-methyl-phosphorothioate residues at or near each of its 5' and 3' ends.

27. The genetically engineered cell of claim 24 or 25, wherein the sgRNA comprises three 2'-O-methyl-phosphorothioate residues at or near each of its 5' and 3' ends.