What is claimed is:

CLAIMS

1. A composition comprising CD34+ human hematopoietic stem and progenitor cells (hHSPCs) that comprise a genetic modification within a +58 DNase I hypersensitive site (DHS) within the erythroid lineage-specific enhancer of a human *B-cell lymphoma 11A* (*BCL11A)* gene.

2. A composition comprising $CD34^+$ human hematopoietic stem and progenitor cells (hHSPCs) that comprise a genetic modification within a +58 DNase I hypersensitive site (DHS) within the erythroid lineage-specific enhancer of a human *B-cell lymphoma 11A* (*BCL11A)* gene, wherein the genetic modification is produced by delivering to the $CD34^+$ hHSPCs a *S. pyogenes* Cas9 endonuclease or a variant thereof comprising a N-terminal SV40 nuclear localization signal (NLS).

3. The composition of claim 1 or 2, wherein the composition comprises a single dose comprising at least at least 2×10^6 modified CD34+ hHSPCs/kg.

4. The composition of any one of claims 1-3, further comprising a cryopreservation medium substantially free of serum, 5% dimethylsulfoxide (DMSO), and/or dextran-40.

5. The composition of any one of claims 1-4, wherein the genetic modification is produced by delivering to $CD34^+$ hHSPCs a Cas9 endonuclease and a guide RNA (gRNA) that targets the +58 DHS of a human *BCL11A* gene, optionally, wherein the gRNA comprises a nucleotide sequence of SEQ ID NO:1 or SEQ ID NO:2, further optionally, wherein the gRNA comprises three 2'-O-methyl-phosphorothioate residues at or near each of its 5' and 3' ends,

optionally wherein the weight ratio of the gRNA to said endonuclease is 1:1, and

optionally, wherein the Cas9 endonuclease is an *S. pyogenes* Cas9 endonuclease or variant thereof.

6. The composition of any one of claims 1-5, wherein

(i) the modified $CD34^+$ hHSPCs exhibit an increase in γ/(γ+α)-globin mRNA ratios of 0.1 to 0.5 and/or wherein the modified $CD34^+$ hHSPCs exhibit an increase in γ/(γ+β)-globin mRNA ratios of 0.2 to 0.6; and/or

(ii) the modified $CD34^+$ hHSPCs exhibit a HbF mean percentage of HbF/(HbF+HbA) protein levels of 15% to 50%; and/or

(iii) the modified $CD34^+$ hHSPCs exhibit a mean allele editing frequency of 70% to 90%; and/or

(iv) at least 75% of the modified $CD34^+$ hHSPCs maintain multi-lineage potential for at least sixteen weeks after administration of the modified $CD34^+$ hHSPCs to a subject; and/or

(v) the modified CD34+ hHSPCs exhibit an on-target indel rate of at least 40%, preferably wherein the modified $CD34^+$ hHSPCs exhibit an on-target indel rate of at least 80%; and/or

(vi) the modified $CD34^+$ hHSPCs exhibit an off-target indel rate of less than 5%, preferably wherein the modified $CD34^+$ hHSPCs exhibit an off-target indel rate of less than 1%.

7. A dose of $CD34^+$ human hematopoietic stem and progenitor cells (hHSPCs) for use in a method, the method comprising administering to a subject having a hemoglobinopathy a dose of said $CD34^+$ human hematopoietic stem and progenitor cells (hHSPCs) that comprise a genetic modification within a +58 DNase I hypersensitive site (DHS) within the erythroid lineage-specific enhancer of a human *B-cell lymphoma 11A* (*BCL11A)* gene, wherein the hHSPCs are administered in an effective amount to reduce the number of blood transfusions administered to the subject relative to baseline and/or to increase fetal hemoglobin (HbF) levels in the subject to at least 20%, and wherein

the dose is at least 2×10^6 modified $CD34^+$ hHSPCs/kg; and/or

the genetic modification is produced by delivering to the $CD34^+$ hHSPCs a *S. pyogenes* Cas9 endonuclease or a variant thereof comprising a N-terminal SV40 nuclear localization signal (NLS); and/or

the method further comprises administering busulfan to the subject, wherein an intravenous 4 mg/kg to 5 mg/kg dose of busulfan is administered daily for four days or an intravenous 0.5 mg/kg to 1 mg/kg dose is administered every six hours for four days, and wherein the dose of busulfan is adjusted based on pharmacokinetic level to achieve an area under the curve (AUC) of 4500 to 5500 μM/min, preferably 5000 μM/min.

8. A dose of modified $CD34^+$ human hematopoietic stem and progenitor cells (hHSPCs) for use in a method, the method comprising:

(a) mobilizing stem cells in a subject having a hemoglobinopathy;

(b) collecting $CD34^+$ hHSPCs from the subject after step (a);

(c) producing modified $CD34^+$ hHSPCs that comprise a genetic modification in within a +58 DNase I hypersensitive site (DHS) within the erythroid lineage-specific enhancer of a human *BCL11A* gene; and

(d) administering to the subject a dose of the modified $CD34^+$ hHSPCs of step (c) in an effective amount to reduce the number of blood transfusions administered to the subject relative to baseline and/or to increase fetal hemoglobin (HbF) levels in the subject to at least 20%, and wherein

the dose is at least 2×10^6 modified $CD34^+$ hHSPCs/kg; and/or

the genetic modification is produced by delivering to the $CD34^+$ hHSPCs a *S. pyogenes* Cas9 endonuclease or a variant thereof comprising a N-terminal SV40 nuclear localization signal (NLS); and/or

the method further comprises administering busulfan to the subject, wherein the busulfan is administered after step (c) and before step (d), wherein an intravenous 4 mg/kg to 5 mg/kg dose of busulfan is administered daily for four days or an intravenous 0.5 mg/kg to 1 mg/kg dose is administered every six hours for four days, and wherein the dose of busulfan is adjusted based on pharmacokinetic level to achieve an area under the curve (AUC) of 4500 to 5500 μM/min, preferably 5000 μM/min.

9. The dose of modified $CD34^+$ hHSPCs for use in the method of claim 8, wherein step (a) comprises administering an inhibitor of CXCR4 chemokine receptor, optionally wherein the inhibitor of CXCR4 chemokine receptor is

Plerixafor, to the subject, optionally wherein step (a) further comprises administering granulocyte colony stimulating factor to the subject.

10. The dose for use in the method of any one of claims 7 to 9, wherein the genetic modification is produced by delivering to the $CD34^+$ hHSPCs a Cas9 endonuclease and a guide RNA (gRNA) that targets the +58 DHS of a human *BCL11A* gene, optionally,

wherein the gRNA comprises a nucleotide sequence of SEQ ID NO:1 or SEQ ID NO:2, further optionally wherein the gRNA comprises three 2'-O-methyl-phosphorothioate residues at or near each of its 5' and 3' ends.

11. The dose for use in the method of any one of claims 7-10, wherein the hemoglobinopathy is β-thalassemia and wherein

(i) the subject requires fewer blood transfusions within a two-year period of the time of administration of the modified $CD34^+$ hHSPCs, or within a two-year period after the time of administration of the modified $CD34^+$ hHSPCs, relative to a two-year period before the time of administration of the modified $CD34^+$ hHSPCs; and/or

(ii) the subject achieves transfusion reduction or transfusion independence for at least three months following administration of the modified $CD34^+$ hHSPCs starting three months after administration of the modified $CD34^+$ hHSPCs; and/or

(iii) the subject achieves transfusion reduction or transfusion independence for at least six months following administration of the modified $CD34^+$ hHSPCs starting three months after administration of the modified $CD34^+$ hHSPCs; and/or

(iv) the subject achieves transfusion reduction or transfusion independence for at least twelve months following administration of the modified $CD34^+$ hHSPCs starting three months after administration of the modified $CD34^+$ hHSPCs; and/or

(v) in the subject there is a change in patient reported outcomes (PROs) over time using at least one of the following assays selected from: Pain scale (11 point numerical rating scale [NRS]), EuroQol Quality of Life Scale (EQ 5D 5L), functional assessment of cancer therapy-bone marrow transplant

(FACT-BMT), Patient-reported Outcome Measurement Information System (PROMIS)-Fatigue, PROMIS-Cognitive function, and Adult Sickle Cell Quality of Life Measurement System (ASCQ-Me); and/or

(vi) in the subject there is a decrease in parameters of iron overload relative to baseline as assessed by magnetic resonance imaging (MRI); and/or

(vii) in the subject there is a decrease in parameters of iron overload relative to baseline as assessed by change in serum ferritin level over time; and/or

(viii) the subject is no longer in need of iron chelation therapy within a 2 to 5 year period of the time of administration of the modified $CD34^+$ hHSPCs, or within a 2 to 5 year period after the time of administration of the modified $CD34^+$ hHSPCs, relative to a 2 to 5 year period before the time of administration of the modified $CD34^+$ hHSPCs.

12. The dose for use in any one of claims 7-10, wherein the hemoglobinopathy is sickle cell disease and wherein

(i) the HbF level in the subject is at least 20% for at least three months starting at any time at or after the time of administration of the modified $CD34^+$ hHSPCs; and/or

(ii) the HbF level in the subject is at least 20% for at least six months starting at any time from at the time of administration of the modified $CD34^+$ hHSPCs; and/or

(iii) the HbF level in the subject is at least 20% for at least three months starting three months after administration of the modified $CD34^+$ hHSPCs; and/or

(iv) the HbF level in the subject is at least 20% for at least three months starting six months after administration of the modified $CD34^+$ hHSPCs; and/or

(v) the HbF level in the subject is at least 20% in the absence of treatment with a secondary drug, optionally wherein said secondary drug is hydroxyurea (HU); and/or

(vi) there is a relative change in annualized rate of severe vaso-occlusive crises (VOC) from baseline, starting six months after administration of the modified $CD34^+$ hHSPCs; and/or

(vii) there is a reduction in annualized rate of VOC from baseline by at least 50%, starting six months after administration of the modified $CD34^+$ hHSPCs; and/or

(viii) in the subject there is a change in patient reported outcomes (PROs) over time using at least one of the following assays selected from: Pain scale (11 point numerical rating scale [NRS]), EuroQol Quality of Life Scale (EQ 5D 5L), functional assessment of cancer therapy-bone marrow transplant (FACT-BMT), Patient-reported Outcome Measurement Information System (PROMIS)-Fatigue, PROMIS-Cognitive function, and Adult Sickle Cell Quality of Life Measurement System (ASCQ-Me); and/or

(ix) in the subject there is a change in hemolytic index as measured by principal component analysis of the following four markers of hemolysis over time: reticulocyte count, serum concentrations of aspartate transaminase, lactate dehydrogenase [LDH], and total bilirubin and/or

(x) in the subject there is a change in tricuspid regurgitant jet velocity (TRV) over time.

13. The dose for use in the method of any one of claims 7-10 and 12, wherein the subject has a hemoglobin S (HbS) level of less than 30% of total Hb and/or a total Hb concentration of 11 g/dL or less.

14. The dose for use in the method of any one of claims 7-13, wherein in the subject there is an increase, optionally at least a 10% increase, in the proportion of circulating erythrocytes expressing fetal hemoglobin (F-cells) over a period of time, inflammatory and endothelial activation markers over a period of time, proportion of alleles with the genetic modification present in peripheral blood leukocytes over a period of time, and/orproportion of alleles with the genetic modification present in bone marrow cells over a period of time, optionally wherein the period of time is at least three months following administration of the modified $CD34^+$ hHSPCs.

15. The dose for use in the method of any one of claims 7-14, wherein

(i) the modified $CD34^+$ hHSPCs exhibit an increase in $\gamma/(\gamma+\alpha)$-globin mRNA ratios of 0.1 to 0.5 and/or wherein the modified $CD34^+$ hHSPCs exhibit an increase in $\gamma/(\gamma+\beta)$-globin mRNA ratios of 0.2 to 0.6; and/or

(ii) the modified $CD34^+$ hHSPCs exhibit a HbF mean percentage of HbF/(HbF+HbA) protein levels of 15% to 50%; and/or

(iii) the modified $CD34^+$ hHSPCs exhibit a ratio of $(\gamma+\beta)/\alpha$-globin mRNA that is at or above 0.4; and/or

(iv) the modified $CD34^+$ hHSPCs exhibit a mean allele editing frequency of 70% to 90%; and/or

(v) at least 50% of the modified $CD34^+$ hHSPCs maintain multi-lineage potential for at least sixteen weeks after administration of the modified $CD34^+$ hHSPCs; and/or

(vi) the modified $CD34^+$ hHSPCs exhibit an on-target indel rate of at least 40%; and/or

(vii) the modified $CD34^+$ hHSPCs exhibit an on-target indel rate of at least 80%.