What is claimed is:

CLAIMS

1. A composition comprising a single dose comprising at least 3 x 10^6 modified CD34+ human hematopoietic stem and progenitor cells (hHSPCs)/kg 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 $CD34^+$ hHSPCs a Cas9 endonuclease and a guide RNA (gRNA) that targets the +58 DHS of the human *BCL11A* gene, and wherein the gRNA comprises a nucleotide sequence of SEQ ID NO: 1 or SEQ ID NO: 2.

2. The composition of claim 1, wherein the Cas9 endonuclease is a *S. pyogenes* Cas9 endonuclease, or a variant thereof comprising a N-terminal and/or C-terminal SV40 nuclear localization signal (NLS).

3. The composition of claim 1 or claim 2, further comprising a cryopreservation medium substantially free of serum, 5% dimethylsulfoxide (DMSO), and/or dextran-40.

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

5. The composition of any one of claims 1-4, 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 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%; and/or

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

6. Use of a single dose comprising at least 3 x 10^6 modified $CD34^+$ human hematopoietic stem and progenitor cells (hHSPCs)/kg in the manufacture of a medicament for the treatment of a subject having a β-globin associated hemoglobinopathy, wherein the $CD34^+$(hHSPCs) 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 $CD34^+$ hHSPCs a *S. pyogenes* Cas9 endonuclease, or a variant thereof comprising a N-terminal SV40 nuclear localization signal (NLS), and a gRNA comprising a nucleotide sequence of SEQ ID NO: 1or SEQ ID NO: 2; and

wherein the single dose results in an absence of vaso-occlusive crises (VOCs) for at least 12 months after administration of the modified hHSPCs, and/or reduces the number of blood transfusions administered to the subject relative to baseline, and/or increases fetal hemoglobin (HbF) levels in the subject to at least 20%.

7. The use of claim 6, further comprising administering busulfan to the subject prior to the administration of the single dose.

8. The use of claim 6 or claim 7, wherein the β-globin-associated 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.

9. The use of claim 6 or claim 7, wherein the β-globin-associated 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,; and/or

(vi) 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

(vii) 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

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

10. The use of any one of claims 6-7 and 9 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.

11. The use of any one of claims 6-10, wherein in the subject there is an 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/or proportion of alleles with the genetic modification present in bone marrow cells over a period of time following administration of the modified $CD34^+$ hHSPCs.

12. The use of any one of claims 6-11, 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 $\gamma/\gamma/(\gamma+\beta)$ -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%.

13. A method of producing a dose of modified $CD34^+$ human hematopoietic stem and progenitor cells (hHSPCs):

(a) mobilizing stem cells in a subject having a β-globin-associated hemoglobinopathy;

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

(c) introducing into the $CD34^+$ hHSPCs (i) a Cas9 endonuclease, or a variant thereof comprising a N-terminal SV40 nuclear localization signal (NLS), and (ii) a guide RNA (gRNA) comprising a nucleotide sequence of SEQ ID NO: 1 or SEQ ID NO: 2 that targets the +58 DHS of the human *BCL11A* gene,

wherein the dose comprises a minimum dose of $3x10^6$ modified CD34+ hHSPCs/kg.

14. The method of claim 13, wherein step (a) comprises administering an inhibitor of CXCR4 chemokine receptor.

15. The method of claim 14, wherein the CXCR4 chemokine receptor inhibitor is Plerixafor.

16. The method of claim 13 or claim 14, wherein step (a) further comprises administering granulocyte colony stimulating factor to the subject.