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

3. The composition of claim 1 or 2, wherein the genetic modification comprises an indel.

4. The composition of any one of claims 1-3, wherein the genetic modification is producing by delivering to CD34+ hHSPCs a Cas9 endonuclease and a guide RNA (gRNA) that targets the +58 DHS of a human *BCL11A* gene.

5. The composition of claim 4, wherein the gRNA comprises a nucleotide sequence of SEQ ID NO:1 or SEQ ID NO:2.

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

7. The composition of any one of claims 4-6, wherein the Cas9 endonuclease is an *S. pyogenes* Cas9 endonuclease or variant thereof.

8. The composition of any one of claims 1-7, wherein 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.

9. The composition of any one of claims 1-8, wherein the modified CD34+ hHSPCs exhibit a HbF mean percentage of HbF/(HbF+HbA) protein levels of 15% to 50%.

10. The composition of any one of claims 1-9, wherein the modified CD34+ hHSPCs exhibit a mean allele editing frequency of 70% to 90%.

11. The composition of any one of claims 1-10, wherein 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.

12. The composition of any one of claims 1-11, wherein the modified CD34+ hHSPCs exhibit an on-target indel rate of at least 40%.

13. The composition of claim 12, wherein the modified CD34+ hHSPCs exhibit an on-target indel rate of at least 80%.

14. The composition of any one of claims 1-13, wherein the modified CD34+ hHSPCs exhibit an off-target indel rate of less than 5%.

15. The composition of any one of claims 1-14, wherein the modified CD34+ hHSPCs exhibit an off-target indel rate of less than 1%.

16. The composition of any one of claims 1-15 comprising a single dose of the modified CD34+ hHSPCs.

17. The composition of claim 16, wherein the single dose comprises at least at least 2 x 10^6 modified CD34+ hHSPCs/kg.

18. The composition of any one of claims 1-17, 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).

19. The composition of claim 18, wherein the *S. pyogenes* Cas9 endonuclease or a variant thereof further comprises a C-terminal NLS, optionally a C-terminal SV40 NLS.

20. The composition of claim 18 or 19 further comprising a gRNA (gRNA) comprising a nucleotide sequence of SEQ ID NO:1 or SEQ ID NO:2.

21. The composition of claim 20, wherein the weight ratio of the gRNA to said endonuclease is 1:1.

22. A method comprising administering to a subject having a hemoglobinopathy a dose of 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%.

23. A 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%.

24. The method of claim 23, wherein step (a) comprises administering an inhibitor of CXCR4 chemokine receptor, optionally wherein the inhibitor of CXCR4 chemokine receptor is Plerixafor, to the subject.

25. The method of claim 23 or 24, wherein step (a) further comprises administering granulocyte colony stimulating factor to the subject.

26. The method of any one of claims 22-24 further comprising administering red blood cells to the subject.

27. The method of claim 26, wherein the red blood cells are administered before step (a) and/or after step (b).

28. The method of any one of claims 23-27, wherein at least 15 x 10^6 CD34+ hHSPCs/kg are collected in step (b).

29. The method of any one of claims 22-28 further comprising administering busulfan to the subject.

30. The method of claim 29, wherein the busulfan is administered after step (c) and before step (d).

31. The method of claim 30, 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.

32. The method of claim 30, wherein the dose is adjusted based on pharmacokinetic level to achieve an area under the curve (AUC) of 4500 to 5500 μM/min, preferably 5000 μM/min.

33. The method of any one of claims 22-32, wherein the genetic modification is an indel.

34. The method of any one of claims 22-31, 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.

35. The method of claim 34, wherein the gRNA comprises a nucleotide sequence of SEQ ID NO:1 or SEQ ID NO:2.

36. The method of claim 35, wherein the gRNA comprises three 2'-O-methyl-phosphorothioate residues at or near each of its 5' and 3' ends.

37. The method of any one of claims 34-36, wherein the Cas9 endonuclease is an *S. pyogenes* Cas9 endonuclease.

38. The method of any one of claims 22-37, wherein neutrophil engraftment occurs in the subject within 35-45 days after administration of the modified CD34+ hHSPCs.

39. The method of claim 38, wherein neutrophil engraftment occurs in the subject within 42 days after administration of the modified CD34+ hHSPCs.

40. The method of any one of claims 22-39, wherein the hemoglobinopathy is β-thalassemia.

41. The method of any one of claims 22-40, wherein 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.

42. The method of any one of claims 22-41, wherein 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.

43. The method of any one of claims 22-42, wherein 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.

44. The method of any one of claims 22-43, wherein 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.

45. The method of any one of claims 22-44, wherein 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).

46. The method of any one of claims 22-45, wherein in the subject there is a decrease in parameters of iron overload relative to baseline as assessed by magnetic resonance imaging (MRI).

47. The method of any one of claims 22-46, wherein 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.

48. The method of claim 46 or 47, wherein the decrease in parameters of iron overload includes a decrease in liver iron concentration (LIC) and/or cardiac iron content (CIC).

49. The method of any one of claims 22-48, wherein 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.

50. The method of any one of claims 22-39, wherein the hemoglobinopathy is sickle cell disease.

51. The method of any one of claims 22-39 or 50, wherein 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.

52. The method of any one of claims 22-39 or 50-51, wherein 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.

53. The method any one of claims 22-39 or 50-52, wherein 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.

54. The method any one of claims 22-39 or 50-53, wherein 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.

55. The method of any one of claims 22-39 or 50-54, wherein the HbF level in the subject is at least 20% in the absence of treatment with a secondary drug.

56. The method of claim 55, wherein said secondary drug is hydroxyurea (HU).

57. The method of any one of claims any one of claims 22-39 or 50-56, wherein 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.

58. The method of claim 57, wherein 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.

59. The method of claim 57 or 58, wherein there is an absence of VOC for at least 12 months, starting six months after administration of the modified CD34+ hHSPCs.

60. The method of claim 59, wherein there is an absence of VOC for at least 24 months, starting six months after administration of the modified CD34+ hHSPCs.

61. The method of any one of claims 22-39 or 50-60, wherein 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).

62. The method of any one of claims 22-39 or 50-61, wherein 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.

63. The method of any one of claims 22-39 or 50-62, wherein in the subject there is a change in tricuspid regurgitant jet velocity (TRV) over time.

64. The method of any one of claims 22-39 or 50-63 further comprising administering red blood cells to the subject, wherein the red blood cells are administered before the step of administering the modified CD34+ hHSPCs.

65. The method of any one of claims 22-39 or 50-64, wherein the subject has received a red blood cell (RBC) transfusion before the step of administering the modified CD34+ hHSPCs.

66. The method of any one of claims 22-39 or 50-65, wherein the subject has a hemoglobin S (HbS) level of less than 30% of total Hb.

67. The method of any one of claims 22-39 or 50-66, wherein the subject has a total Hb concentration of 11 g/dL or less.

68. The method of any one of claims 22-67, 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.

69. The method of any one of claims 22-68, wherein in the subject there is a change in inflammatory and endothelial activation markers over a period of time.

70. The method of claim 69, wherein in the subject there is a change in the proportion of alleles with the genetic modification present in peripheral blood leukocytes over a period of time.

71. The method of any one of claims 22-70, wherein in the subject there is a change in the proportion of alleles with the genetic modification present in bone marrow cells over a period of time.

72. The method of any one of claims 68-71, wherein the period of time is at least three months following administration of the modified CD34+ hHSPCs.

73. The method of claim 72, wherein the period of time is at least six months following administration of the modified CD34+ hHSPCs.

74. The method of any one of claims 22-73, wherein 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.

75. The method of any one of claims 22-74, wherein the modified CD34+ hHSPCs exhibit a HbF mean percentage of HbF/(HbF+HbA) protein levels of 15% to 50%.

76. The method of any one of claims 22-75, wherein the modified CD34+ hHSPCs exhibit a ratio of $(\gamma+\beta)/\alpha$-globin mRNA that is at or above 0.4.

77. The method of any one of claims 22-76, wherein the modified CD34+ hHSPCs exhibit a mean allele editing frequency of 70% to 90%.

78. The method of any one of claims 22-77, wherein at least 50% of the modified CD34+ hHSPCs maintain multi-lineage potential for at least sixteen weeks after administration of the modified CD34+ hHSPCs.

79. The method of any one of claims 22-78, wherein the modified CD34+ hHSPCs exhibit an on-target indel rate of at least 40%.

80. The method of claim 79, wherein the modified CD34+ hHSPCs exhibit an on-target indel rate of at least 80%.

81. The method of any one of claims 22-80, wherein the subject does not exhibit neoplastic and/or myeloproliferative lesions resulting from administration of the modified CD34+ hHSPCs.

82. The method of any one of claims 22-81, wherein the dose is at least 2×10^6 modified CD34+ hHSPCs/kg.

83. The method of claim 82, wherein the dose is at least 3×10^6 to modified CD34+ hHSPCs/kg.

84. The method of any one of claims 22-83 further comprising administering plerixafor to the subject, wherein red blood cells are administered before the step of administering the modified CD34+ hHSPCs.

85. The method of any one of claims 22-84 further comprising administering a granulocyte colony stimulating factor to the subject.