

Claims:

1. An *in vitro* or *ex vivo* method of producing a hematopoietic stem cell or progenitor thereof for the treatment of a hemoglobinopathy, hematologic cancer, or myeloproliferative disease, the method comprising:

(a) expressing in the hematopoietic stem cell or progenitor thereof a nucleobase editor polypeptide, wherein the nucleobase editor polypeptide comprises a nucleic acid programmable DNA binding protein (napDNAbp) and a deaminase; and

(b) contacting the hematopoietic stem cell or progenitor thereof with a guide RNA that targets a nucleic acid molecule encoding a CD117 protein, and introducing a T13A, I39V, H40R, D45G, D52G, E53G, I54V, R55G, T59A, K65E, K65R, T67A, E76G, N77G, N77S, N77Y, K78E, Q79R, N80G, E81G, E81D, N99G, K100G, H101R, L124P, Y125H, D129E, D129G, N130D, N130G, D131G, D131N, T132A, T144A, N145D, N145G, N145Y, N145S, Y146C, F162P, F162L, I163T, I163V, M171T, I172T, V195A, L196P, K199G, I201V, S220G, Y221C, Q256R, E257D, E257G, K258E, Y259C, T303A, T304A, M318V, T322A, V323A, F324L, K342G, K342R, Y350H, M351T, R353G, T354A, T354I, R381G, K383G, T385A, Y418C, D419G, R420G, I438M, D439G, Q460R, T461A, K484G, N486G, or T488A mutation in the CD117 protein.

2. Use of a hematopoietic stem cell or progenitor thereof in the preparation of a medicament for the treatment of a hemoglobinopathy, hematologic cancer, or myeloproliferative disease, wherein the hematopoietic stem cell or progenitor thereof is produced according to a method comprising:

(a) expressing in the hematopoietic stem cell or progenitor thereof a nucleobase editor polypeptide, wherein the nucleobase editor polypeptide comprises a nucleic acid programmable DNA binding protein (napDNAbp) and a deaminase; and

(b) contacting the hematopoietic stem cell or progenitor thereof with a guide RNA that targets a nucleic acid molecule encoding a CD117 protein, and introducing a T13A, I39V, H40R, D45G, D52G, E53G, I54V, R55G, T59A, K65E, K65R, T67A, E76G, N77G, N77S, N77Y, K78E, Q79R, N80G, E81G, E81D, N99G, K100G, H101R, L124P, Y125H, D129E, D129G, N130D, N130G, D131G, D131N, T132A, T144A, N145D, N145G, N145Y, N145S, Y146C, F162P, F162L, I163T, I163V, M171T, I172T, V195A, L196P, K199G, I201V, S220G, Y221C, Q256R, E257D, E257G, K258E, Y259C, T303A, T304A, M318V, T322A, V323A, F324L, K342G, K342R, Y350H, M351T, R353G, T354A, T354I, R381G,

K383G, T385A, Y418C, D419G, R420G, I438M, D439G, Q460R, T461A, K484G, N486G, or T488A mutation in the CD117 protein.

3. The method of claim 1 or the use of claim 2, wherein the hemoglobinopathy is selected from the group consisting of sickle cell anemia, sickle cell disease, thalassemia, Fanconi anemia, aplastic anemia, and Wiskott-Aldrich syndrome;

wherein the hematologic cancer is selected from the group consisting of acute myeloid leukemia, acute lymphoid leukemia, chronic myeloid leukemia, chronic lymphoid leukemia, multiple myeloma, diffuse large B-cell lymphoma, and non-Hodgkin's lymphoma; and

wherein the myeloproliferative disease is a myelodysplastic syndrome.

4. The method of claim 1, or the use of claim 2, wherein the cell is selected from a hematopoietic stem cell or a progenitor thereof, or a common myeloid progenitor, proerythroblast, or erythroblast; optionally, wherein the cell is a mammalian cell.

5. The method or use of any one of claims 1-4, wherein the method further comprises contacting the cell with one or more additional guide RNAs that target a protein selected from the group consisting of CXCR4, CD135, CD90, CD45, and CD34.

6. An *in vitro* or *ex vivo* method of base editing a gene encoding a CD117 protein expressed by a hematopoietic stem cell or a progenitor thereof, the method comprising:

(a) expressing in a hematopoietic stem cell or a progenitor thereof comprising a CD117 protein a nucleobase editor polypeptide, wherein the nucleobase editor polypeptide comprises a nucleic acid programmable DNA binding protein (napDNAbp) and a deaminase; and

(b) contacting the cell with a guide RNA capable of targeting a nucleic acid molecule encoding the CD117 protein, thereby base editing the gene encoding the CD117 protein and introducing a T13A, I39V, H40R, D45G, D52G, E53G, I54V, R55G, T59A, K65E, K65R, T67A, E76G, N77G, N77S, N77Y, K78E, Q79R, N80G, E81G, E81D, N99G, K100G, H101R, L124P, Y125H, D129E, D129G, N130D, N130G, D131G, D131N, T132A, T144A, N145D, N145G, N145Y, N145S, Y146C, F162P, F162L, I163T, I163V, M171T, I172T, V195A, L196P, K199G, I201V, S220G, Y221C, Q256R, E257D, E257G, K258E, Y259C,

T303A, T304A, M318V, T322A, V323A, F324L, K342G, K342R, Y350H, M351T, R353G, T354A, T354I, R381G, K383G, T385A, Y418C, D419G, R420G, I438M, D439G, Q460R, T461A, K484G, N486G, or T488A mutation in the CD117 protein.

7. The method of any one of claims 1-6, wherein the deaminase is an adenosine deaminase or a cytidine deaminase.

8. The method of claim 7, wherein the adenosine deaminase is a TadA*8.

9. The method of claim 8, wherein the adenosine deaminase is TadA*8.1, TadA*8.2, TadA*8.3, TadA*8.4, TadA*8.5, TadA*8.6, TadA*8.7, TadA*8.8, TadA*8.9, TadA*8.10, TadA*8.11, TadA*8.12, TadA*8.13, TadA*8.14, TadA*8.15, TadA*8.16, TadA*8.17, TadA*8.18, TadA*8.19, TadA*8.20, TadA*8.21, TadA*8.22, TadA*8.23, or TadA*8.24.

10. The method or use of any one of claims 1-9, wherein the deaminase is a monomer or heterodimer;

wherein the nucleobase editor polypeptide is an internal base editor comprising the deaminase inserted at an internal location of the napDNAbp;

wherein the nucleobase editor polypeptide further comprises one or more uracil glycosylase inhibitors; and/or

wherein the nucleobase editor polypeptide further comprises one or more nuclear localization sequences.

11. Use of a hematopoietic stem cell in the preparation of a medicament for administering a hematopoietic stem cell transplant to a subject, wherein the medicament comprises i) an edited hematopoietic stem cell and ii) an antibody, antibody drug conjugate, or chimeric antigen receptor expressing T cell (CAR-T) that selectively binds a wild-type version of a CD117 protein, that is to be administered to the subject concurrent with or subsequent to administration of the edited hematopoietic stem cell to the subject, wherein the edited hematopoietic stem cell is prepared according to a method comprising:

(a) expressing in a hematopoietic stem cell of the subject a nucleobase editor polypeptide comprising a nucleic acid programmable DNA binding protein (napDNAbp) and a deaminase;

(b) contacting the hematopoietic stem cell with a guide RNA capable of targeting a nucleic acid molecule encoding a CD117 protein, thereby introducing a T13A, I39V, H40R, D45G, D52G, E53G, I54V, R55G, T59A, K65E, K65R, T67A, E76G, N77G, N77S, N77Y, K78E, Q79R, N80G, E81G, E81D, N99G, K100G, H101R, L124P, Y125H, D129E, D129G, N130D, N130G, D131G, D131N, T132A, T144A, N145D, N145G, N145Y, N145S, Y146C, F162P, F162L, I163T, I163V, M171T, I172T, V195A, L196P, K199G, I201V, S220G, Y221C, Q256R, E257D, E257G, K258E, Y259C, T303A, T304A, M318V, T322A, V323A, F324L, K342G, K342R, Y350H, M351T, R353G, T354A, T354I, R381G, K383G, T385A, Y418C, D419G, R420G, I438M, D439G, Q460R, T461A, K484G, N486G, or T488A mutation in the CD117 protein and generating an edited hematopoietic stem cell; and
 wherein the edited hematopoietic stem cell is to be administered to the subject.=

12. The use of claim 11, wherein the deaminase is an adenosine deaminase or a cytidine deaminase.

13. The use of claim 12, wherein the adenosine deaminase is TadA*8.

14. The use of claim 13, wherein the adenosine deaminase is TadA*8.1, TadA*8.2, TadA*8.3, TadA*8.4, TadA*8.5, TadA*8.6, TadA*8.7, TadA*8.8, TadA*8.9, TadA*8.10, TadA*8.11, TadA*8.12, TadA*8.13, TadA*8.14, TadA*8.15, TadA*8.16, TadA*8.17, TadA*8.18, TadA*8.19, TadA*8.20, TadA*8.21, TadA*8.22, TadA*8.23, or TadA*8.24.

15. The use of any one of claims 11-14, wherein the deaminase is a monomer or heterodimer;

wherein the nucleobase editor polypeptide is an internal base editor comprising the deaminase inserted at an internal location of the napDNABp;

wherein the nucleobase editor polypeptide further comprises one or more uracil glycosylase inhibitors;

wherein the nucleobase editor polypeptide further comprises one or more nuclear localization sequences; and/or

wherein the mutation fails to alter the biological activity of the CD117 protein.

16. The method or use of any one of claims 1-15, wherein the CD117 protein comprising the mutation is capable of binding Stem Cell Factor (SCF); or is capable of SCF signaling.

17. The method or use of any one of claims 1-16, wherein the mutation results from the modification of one or more single target nucleobases.
18. The method or use of claim 17, wherein the single target nucleobase is a cytosine (C) and wherein the modification comprises conversion of the C to a thymine (T); or
wherein the single target nucleobase is an adenosine (A) and wherein the modification comprises conversion of the A to a guanine (G).
19. The method or use of any one of claims 1-18, wherein the cell is a CD34⁺ cell; and/or wherein the cell is from a subject having hemoglobinopathy, hematologic cancer, or a myeloproliferative disease.
20. The method or use of claim 19, wherein the cell is from a subject having sickle cell disease.
21. The method or use of any one of claims 1-20, wherein the deaminase is fused to the napDNAbp;
wherein the deaminase is inserted at an internal location of the napDNAbp;
wherein the napDNAbp is nuclease inactive or a nickase;
wherein the napDNAbp comprises a Cas9, Cas12a/Cpf1, Cas12b/C2c1, Cas12c/C2c3, Cas12d/CasY, Cas12e/CasX, Cas12g, Cas12h, Cas12i, or Cas12j/CasΦ polynucleotide or a portion thereof;
wherein the napDNAbp comprises a dead Cas9 or a Cas9 nickase;
wherein the napDNAbp is a modified *Staphylococcus aureus* Cas9, *Streptococcus thermophilus* 1 Cas9 (St1Cas9), or *Streptococcus pyogenes* Cas9 (SpCas9), or
wherein the napDNAbp comprises an SpCas9 having an altered protospacer-adjacent motif (PAM) specificity; optionally,
wherein the altered PAM has specificity for the nucleic acid sequence 5'-NGC-3';
22. The use of any one of claims 11-21, wherein the deaminase is a cytidine deaminase or an adenosine deaminase.

23. The use of claim 22, wherein the cytidine deaminase is an APOBEC deaminase ; or wherein the adenosine deaminase is a TadA deaminase, a TadA*8, or

TadA*8.1, TadA*8.2, TadA*8.3, TadA*8.4, TadA*8.5, TadA*8.6, TadA*8.7, TadA*8.8, TadA*8.9, TadA*8.10, TadA*8.11, TadA*8.12, TadA*8.13, TadA*8.14, TadA*8.15, TadA*8.16, TadA*8.17, TadA*8.18, TadA*8.19, TadA*8.20, TadA*8.21, TadA*8.22, TadA*8.23, or TadA*8.24.

24. The method or use of any one of claims 1-23, wherein the guide RNA comprises the nucleic acid sequence selected from Table 23.

25. The method or use of any one of claims 1-24, wherein the guide RNA comprises a nucleic acid sequence that hybridizes to the complement of a CD117 target sequence selected from the group consisting of:

CAAGCTATCTTCTTAGGGA;
GCAAGCTATCTTCTTAGGGAA;
ACTTACGACAGGCTCGTGAA;
TGACCAATTATTCCCTCAAG;
AGTGACCAATTATTCCCTCA;
ACTACAGTATTTGTAAACGA;
GTGACCAATTATTCCCTCAA;
TGTTTCATATAGATCCACTGC;
AAGTGACCAATTATTCCCTC;
TTCTGTTTCATATAGATCCAC;
ACAGGAGAAATATAATAGCT;
AAATATAATAGCTGGCATCA;
AAGACAACGACACGCTGGTC; and
GGCTGTTATGCACTGATCCG.

26. A base editor system comprising a fusion protein or a polynucleotide encoding the fusion protein, wherein the fusion protein comprises a nucleic acid programmable DNA binding protein (napDNAbp) and a deaminase, and a guide polynucleotide comprising a nucleic acid sequence selected from Table 23; or

a guide polynucleotide comprising a nucleic acid sequence that hybridizes to the complement of a target nucleic acid sequence selected from the group consisting of:

CAAGCTATCTTCTTAGGGA;
GCAAGCTATCTTCTTAGGGAA;
ACTTACGACAGGCTCGTGAA;
TGACCAATTATTCCCTCAAG;
AGTGACCAATTATTCCCTCA;
GTGACCAATTATTCCCTCAA;
TGTTTCATATAGATCCACTGC;
AAGTGACCAATTATTCCCTC;
TTCTGTTCATATAGATCCAC;
ACAGGAGAAATATAATAGCT;
AAATATAATAGCTGGCATCA;
ACTACAGTATTTGTAAACGA;
ACTACAGTATTTGTAAACGA;
AAGACAACGACACGCTGGTC; and
GGCTGTTATGCACTGATCCG.

27. The base editor system of claim 26, wherein the fusion protein further comprises one or more uracil glycosylase inhibitors ; and/or one or more nuclear localization sequences.

28. The base editor system of any one of claims 26 or 27, wherein the base editor system is capable of modifying one or more single target nucleobases to effect at least one amino acid substitution in a CD117 polypeptide.

29. The base editor system of claim 28, wherein the single target nucleobase is a cytosine (C) and wherein the modification comprises conversion of the C to a thymine (T); or wherein the single target nucleobase is an adenosine (A) and wherein the modification comprises conversion of the A to a guanine (G).

30. The base editor system of claim 29, wherein the at least one amino acid substitution is in domain 1, 2, 3, 4, or 5 of CD117; or wherein the at least one amino acid substitution is selected from the group consisting

of T13A, I39V, H40R, D45G, D52G, E53G, I54V, R55G, T59A, K65E, K65R, T67A, E76G, N77G, N77S, N77Y, K78E, Q79R, N80G, E81G, E81D, N99G, K100G, H101R, L124P, Y125H, D129E, D129G, N130D, N130G, D131G, D131N, T132A, T144A, N145D, N145G, N145Y, N145S, Y146C, F162P, F162L, I163T, I163V, M171T, I172T, V195A, L196P, K199G, I201V, S220G, Y221C, Q256R, E257D, E257G, K258E, Y259C, T303A, T304A, M318V, T322A, V323A, F324L, K342G, K342R, Y350H, M351T, R353G, T354A, T354I, R381G, K383G, T385A, Y418C, D419G, R420G, I438M, D439G, Q460R, T461A, K484G, N486G, and T488A.

31. The base editor system of any one of claims 26-30, wherein the napDNAbp is nuclease inactive or a nickase;

wherein the napDNAbp comprises a Cas9, Cas12a/Cpf1, Cas12b/C2c1, Cas12c/C2c3, Cas12d/CasY, Cas12e/CasX, Cas12g, Cas12h, Cas12i, or Cas12j/CasΦ polynucleotide or a portion thereof;

wherein the napDNAbp comprises a Cas9 polynucleotide or a portion thereof;

wherein the napDNAbp comprises a dead Cas9 or a Cas9 nickase;

wherein the napDNAbp is a modified *Staphylococcus aureus* Cas9, *Streptococcus thermophilus* 1 Cas9, or *Streptococcus pyogenes* Cas9 (SpCas9); or

wherein the napDNAbp comprises an SpCas9 having an altered protospacer-adjacent motif (PAM) specificity; optionally,

wherein the altered PAM has specificity for the nucleic acid sequence 5'-NGC-3'.

32. The base editor system of any one of claims 26-31, wherein the deaminase is capable of deaminating cytidine or adenine in DNA;

wherein the deaminase is a cytidine deaminase;

wherein the cytidine deaminase is an APOBEC deaminase;

wherein the deaminase is an adenosine deaminase;

wherein the adenosine deaminase is a TadA*8;

wherein the adenosine deaminase is TadA*8.1, TadA*8.2, TadA*8.3, TadA*8.4, TadA*8.5, TadA*8.6, TadA*8.7, TadA*8.8, TadA*8.9, TadA*8.10, TadA*8.11, TadA*8.12, TadA*8.13, TadA*8.14, TadA*8.15, TadA*8.16, TadA*8.17, TadA*8.18, TadA*8.19, TadA*8.20, TadA*8.21, TadA*8.22, TadA*8.23, or TadA*8.24; and/or

wherein the deaminase is a monomer or heterodimer.

33. A polynucleotide encoding the base editor system of any one of claims 26-32.
34. A cell produced by the method of claim 1 or 3.
35. A cell produced by introducing into a cell, or a progenitor thereof, the base editor system of any one of claims 26-32 or the polynucleotide of claim 33.
36. The cell of claim 34 or 35, wherein the cell is produced *ex vivo* or *in vitro*;
wherein the cell is a hematopoietic stem cell, a CD34⁺ cell, or a common myeloid progenitor, proerythroblast, or erythroblast; and/or
wherein the cell is a mammalian cell .
37. A pharmaceutical composition comprising an effective amount of the cell of any one of claims 34-36.
38. The pharmaceutical composition of claim 37 for use in treating a subject with a hemoglobinopathy, hematologic cancer, or myeloproliferative disease.
39. Use of the cell of any one of claims 34-36 in the preparation of a pharmaceutical composition for treating a subject with a hemoglobinopathy, hematologic cancer, or myeloproliferative disease.
40. The cell of any one of claims 34-36 for use in treating a subject with a hemoglobinopathy, hematologic cancer, or myeloproliferative disease, or the use of claim 39, wherein a conditioning regimen comprising an antibody, antibody drug conjugate or chimeric antigen receptor expressing T-cell that selectively binds a wild-type CD117 protein is to be administered to the subject.
41. The cell for use or the use of claim 40, wherein the antibody, antibody drug conjugate or chimeric antigen receptor expressing T-cell selectively binds domain 1, 2, 3, 4, or 5 of CD117; optionally,
wherein the antibody is a monoclonal antibody, which is to be administered sequentially or concurrently; and/or
wherein the hemoglobinopathy is sickle cell disease; and/or wherein the subject is a

mammal.

42. A kit comprising the cell of any one of claims 34-36, the base editor system of any one of claims 26-32, the polynucleotide of claim 33, or the pharmaceutical composition of claim 37 or 38.