

Claims:

1. A 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 cell surface protein selected from the group consisting of CD117, CXCR4, CD135, CD90, CD45, and CD34, and introducing a mutation in the cell surface 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 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 cell surface protein selected from the group consisting of CD117, CXCR4, CD135, CD90, CD45, and CD34, and introducing a mutation in the cell surface 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 (SCD), thalassemia, hereditary persistence of fetal hemoglobin (HPFH), 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. A method of identifying a mutation that alters antibody binding to a cell surface protein, the method comprising
- (a) expressing in a cell comprising a cell surface protein a nucleobase editor polypeptide, wherein the nucleobase editor polypeptide comprises a nucleic acid programmable DNA binding protein (napDNAbp) and a deaminase;
 - (b) contacting the cell with a guide RNA capable of targeting a nucleic acid molecule encoding the cell surface protein and introducing a mutation in the cell surface protein; and
 - (c) contacting the cell with an antibody that specifically binds a wild-type cell surface protein, but that exhibits reduced binding to the cell surface protein comprising the mutation, thereby identifying a mutation that alters antibody binding to the cell surface protein.
5. The method of claim 1 or 4, or the use of claim 2, wherein the cell surface protein is selected from the group consisting of CD117, CXCR4, CD135, CD90, CD45, and CD34; and/or 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 or a human cell.
6. The method or use of any one of claims 1-5, wherein the method further comprises contacting the cell with one or more additional guide RNAs that target a cell surface protein selected from the group consisting of CXCR4, CD135, CD90, CD45, and CD34.
7. A method of identifying a mutation that alters antibody binding to a cell surface protein, the method comprising
- (a) expressing in a 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;
 - (b) contacting the cell with a guide RNA capable of targeting a nucleic acid molecule encoding a cell surface protein selected from the group consisting of CD117, CXCR4, CD135, CD90, CD45, CD34, and introducing a mutation in the cell surface protein; and
 - (c) contacting the cell with an antibody that specifically binds a wild-type cell surface protein, but that exhibits reduced binding to the cell surface protein comprising a mutation, thereby identifying a mutation that alters antibody binding to the cell surface protein.
8. A method of base editing a gene encoding a cell surface 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 cell surface protein.

9. The method of any one of claims 1-8, wherein the deaminase domain is an adenosine deaminase or a cytidine deaminase.

10. The method of claim 9, wherein the adenosine deaminase is a TadA*8 variant.

11. The method of claim 10, 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.

12. The method or use of any one of claims 1-11, wherein the deaminase is a monomer or heterodimer;

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

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

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

13. Use of a hematopoietic stem cell in the preparation of a medicament for conditioning a subject concurrent with or subsequent to a hematopoietic stem cell transplant (HSCT), wherein the hematopoietic stem cell is prepared according to the method comprising

(a) expressing in an isolated hematopoietic stem cell of the subject or of a donor 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 cell surface protein selected from the group consisting of CD117, CXCR4, CD135, CD90, CD45, and CD34, thereby introducing a mutation in the cell surface protein and generating an edited hematopoietic stem cell;

and wherein

(c) the edited hematopoietic stem cell is to be administered to the subject; and

(d) an antibody, antibody drug conjugate, or chimeric antigen receptor expressing T cell (CAR-T) that selectively binds a wild-type version of the cell surface protein, is to be administered to the subject, wherein the administration step (d) is concurrent with or subsequent to the administration step (c).

14. Use of a hematopoietic stem cell in the preparation of a medicament for conditioning a subject concurrent with or subsequent to a hematopoietic stem cell transplant (HSCT), wherein the hematopoietic stem cell is prepared according to the 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 mutation in the CD117 protein and generating an edited hematopoietic stem cell;

and wherein

(c) the edited hematopoietic stem cell is to be administered to the subject; and

(d) an antibody, antibody drug conjugate, or chimeric antigen receptor expressing T cell (CAR-T) that selectively binds a wild-type version of CD117, is to be administered to the subject, wherein the administration step (d) is concurrent with or subsequent to administration step (c).

15. The use of claim 13 or 14, wherein the deaminase is an adenosine deaminase or a cytidine deaminase.

16. The use of claim 15, wherein the adenosine deaminase is a TadA*8 variant.

17. The use of claim 16, 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.

18. The use of any one of claims 13-17, wherein the deaminase is a monomer or heterodimer;

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

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

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

wherein the mutation is a missense mutation; optionally,

wherein the missense mutation fails to alter the biological activity of the cell surface protein.

19. The method or use of any one of claims 1-18, wherein the CD117 cell surface protein comprising the mutation is capable of binding Stem Cell Factor (SCF); or is capable of SCF signaling.

20. The method or use of any one of claims 1-19, wherein the mutation is at least one amino acid substitution resulting from the modification of one or more single target nucleobases.

21. The method or use of claim 20, 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).

22. The method or use of claim 20 or 21, wherein the at least one amino acid substitution in CD117 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.

23. The method or use of any one of claims 1-22, wherein the cell is a CD34⁺ cell; and/or wherein the cell is from a subject having hemoglobinopathy, hematologic cancer, or a myeloproliferative disease.

24. The method or use of claim 23, wherein the cell is from a subject having sickle cell disease (SCD); or wherein the cell is from a subject having hereditary persistence of fetal hemoglobin (HPFH).

25. The method or use of any one of claims 1-24, wherein the deaminase domain is fused to the napDNAbp;

wherein the deaminase domain is inserted at an internal location of the napDNAbp;

wherein the napDNAbp is a nuclease inactive or nickase variant;

wherein the napDNAbp comprises a Cas9, Cas12a/Cpfl, Cas12b/C2cl, Cas12c/C2c3, Cas12d/CasY, Cas12e/CasX, Cas12g, Cas12h, Cas12i, or Cas12j/CasΦ polynucleotide or a portion thereof;

wherein the napDNAbp comprises a dead Cas9 (dCas9) or a Cas9 nickase (nCas9);

wherein the napDNAbp is a modified *Staphylococcus aureus* Cas9 (SaCas9), *Streptococcus thermophilus* 1 Cas9 (St1Cas9), a modified *Streptococcus pyogenes* Cas9 (SpCas9), or variants thereof; or

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

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

26. The use of any one of claims 13-25, wherein the deaminase domain is a cytidine deaminase domain or an adenosine deaminase domain.

27. The use of claim 26, wherein the cytidine deaminase domain is an APOBEC deaminase domain or a derivative thereof; or

wherein the adenosine deaminase domain is a TadA deaminase domain,

a TadA*8 variant, 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.

28. The method or use of any one of claims 1-27, wherein the one or more guide polynucleotides comprises a nucleic acid sequence selected from Table 23.

29. The method or use of any one of claims 1-28, wherein the one or more guide polynucleotides comprise 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;
AAGACAACGACACGCTGGTC; and
GGCTGTTATGCACTGATCCG.

30. 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 domain, 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;
ACTACAGTATTTGTAAACGA;

AAGACAACGACACGCTGGTC; and
GGCTGTTATGCACTGATCCG.

31. The base editor system of claim 30, wherein the fusion protein further comprises one or more uracil glycosylase inhibitors (UGIs); and/or one or more nuclear localization sequences (NLS).
32. The base editor system of any one of claims 30 or 31, 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.
33. The base editor system of claim 32, 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).
34. The base editor system of claim 33, 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.
35. The base editor system of any one of claims 30-34, wherein the napDNAbp is a nuclease inactive or nickase variant; 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 (dCas9) or a Cas9 nickase (nCas9);
 wherein the napDNAbp is a modified *Staphylococcus aureus* Cas9 (SaCas9),
Streptococcus thermophilus 1 Cas9 (St1Cas9), a modified *Streptococcus pyogenes* Cas9
 (SpCas9), or variants thereof; or
 wherein the napDNAbp comprises a variant of SpCas9 having an altered protospacer-
 adjacent motif (PAM) specificity; optionally,
 wherein the altered PAM has specificity for the nucleic acid sequence 5'-NGC-3'.

36. The base editor system of any one of claims 30-35, wherein the deaminase domain is capable of deaminating cytidine or adenine in DNA;
 wherein the deaminase domain is a cytidine deaminase domain;
 wherein the cytidine deaminase is an APOBEC deaminase or a derivative thereof;
 wherein the deaminase domain is an adenosine deaminase domain;
 wherein the adenosine deaminase is a TadA*8 variant;
 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.
37. A polynucleotide encoding the base editor system of any one of claims 30-36.
38. A cell produced by the method of claim 1 or 3.
39. A cell produced by introducing into a cell, or a progenitor thereof, the base editor system of any one of claims 30-36 or the polynucleotide of claim 37.
40. The cell of claim 38 or 39, 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 or a human cell.
41. A pharmaceutical composition comprising an effective amount of the cell of any one of claims 38-40.

42. The pharmaceutical composition of claim 41 for use in treating a subject with a hemoglobinopathy, hematologic cancer, or myeloproliferative disease.
43. Use of the cell of any one of claims 38-40 in the preparation of a pharmaceutical composition for treating a subject with a hemoglobinopathy, hematologic cancer, or myeloproliferative disease.
44. The cell of any one of claims 38-40 for use in treating a subject with a hemoglobinopathy, hematologic cancer, or myeloproliferative disease, or the use of claim 43, wherein a conditioning regimen comprising an antibody, antibody drug conjugate or chimeric antigen receptor expressing T-cell that selectively binds a cell surface protein is to be administered to the subject.
45. The cell for use or the use of claim 44, 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 or hereditary persistence of fetal hemoglobin (HPFH); and/or wherein the subject is a mammal or a human.
46. A kit comprising the cell of any one of claims 38-40, the base editor system of any one of claims 30-36, the polynucleotide of claim 37, or the pharmaceutical composition of claim 41 or 42.