# CLAIMS

WHAT IS CLAIMED IS:

1. A system comprising:

a deoxyribonucleic acid (DNA) endonuclease or nucleic acid encoding the DNA endonuclease; a guide RNA (gRNA) comprising a spacer sequence that is complementary to a sequence within a *FOXP3* gene, AAVS1 locus, or a *TRA* gene in a $CD34^+$ cell, or nucleic acid encoding the gRNA; and

a donor template comprising a nucleic acid sequence encoding a FOXP3 or a functional derivative thereof.

2. The system of claim 1, wherein the gRNA comprises:

i) a spacer sequence from any one of SEQ ID NOs: 1-7, 15-20, and 27-29 or a variant thereof having no more than 3 mismatches compared to any one of SEQ ID NOs: 1-7, 15-20, and 27-29;

ii) a spacer sequence from any one of SEQ ID NOs: 1-7 or a variant thereof having no more than 3 mismatches compared to any one of SEQ ID NOs: 1-7; or

iii) a spacer sequence from any one of SEQ ID NOs: 2, 3, and 5 or a variant thereof having no more than 3 mismatches compared to any one of SEQ ID NOs: 2, 3, and 5.

3. The system of claim 1 or 2, wherein the FOXP3 or functional derivative thereof is wild-type human FOXP3.

4. The system of any one of claims 1-3, wherein the DNA endonuclease is a Cas9.

5. The system of any one of claims 1-4, wherein the nucleic acid encoding the DNA endonuclease is an mRNA.

6. The system of any one of claims 1-5, wherein the donor template is encoded in an adeno-associated virus (AAV) vector.

7. The system of any one of claims 1-6, wherein the DNA endonuclease or nucleic acid encoding the DNA endonuclease is formulated in a liposome or lipid nanoparticle.

8. The system of any one of claims 1-7, wherein the liposome or lipid nanoparticle also comprises the gRNA.

9. A method of editing a genome in a $CD34^+$ cell, the method comprising providing the following to the cell:

(a) a gRNA comprising a spacer sequence that is complementary to a sequence within a *FOXP3* gene, AAVS1 locus, or a *TRA* gene in the cell, or nucleic acid encoding the gRNA;

(b) a DNA endonuclease or nucleic acid encoding the DNA endonuclease; and

(c) a donor template comprising a nucleic acid sequence encoding a FOXP3 or a functional derivative thereof.

10. The method of claim 9, wherein the gRNA comprises:

i) a spacer sequence from any one of SEQ ID NOs: 1-7, 15-20, and 27-29 or a variant thereof having no more than 3 mismatches compared to any one of SEQ ID NOs: 1-7, 15-20, and 27-29;

ii) a spacer sequence from any one of SEQ ID NOs: 1-7 or a variant thereof having no more than 3 mismatches compared to any one of SEQ ID NOs: 1-7; or

iii) a spacer sequence from any one of SEQ ID NOs: 2, 3, and 5 or a variant thereof having no more than 3 mismatches compared to any one of SEQ ID NOs: 2, 3, and 5.

11. The method of claim 9 or 10, wherein the FOXP3 or functional derivative thereof is wild-type human FOXP3.

12. The method of any one of claims 9-11, wherein the DNA endonuclease is a Cas9.

13. The method of any one of claims 9-12, wherein I) the nucleic acid encoding the DNA endonuclease is codon-optimized for expression in the cell; and/or II) the nucleic acid sequence

encoding a FOXP3 or a functional derivative thereof is codon-optimized for expression in the cell.

14. The method of any one of claims 9-13, wherein the nucleic acid encoding the DNA endonuclease is an mRNA.

15. The method of any one of claims 9-14, wherein the donor template is encoded in an Adeno Associated Virus (AAV) vector.

16. The method of any one of claims 9-15, wherein the DNA endonuclease or nucleic acid encoding the DNA endonuclease is formulated in a liposome or lipid nanoparticle.

17. The method of claim 16, wherein the liposome or lipid nanoparticle also comprises the gRNA.

18. The method of any one of claims 9-17, comprising providing to the cell the DNA endonuclease pre-complexed with the gRNA, forming a ribonucleoprotein (RNP) complex.

19. A genetically modified $CD34^+$ cell in which the genome of the cell is edited by the method of any one of claims 9-18.

20. A composition comprising the genetically modified $CD34^+$ cell of claim 19.

21. A method of treating or inhibiting a disease or condition associated with FOXP3 in a subject, comprising providing the following to a $CD34^+$ cell in the subject:

(a) a gRNA comprising a spacer sequence that is complementary to a sequence within a *FOXP3* gene, AAVS1 locus, or a *TRA* gene in the cell, or nucleic acid encoding the gRNA;

(b) a DNA endonuclease or nucleic acid encoding the DNA endonuclease; and

(c) a donor template comprising a nucleic acid sequence encoding a FOXP3 or a functional derivative thereof.

22. The method of claim 21, wherein the gRNA comprises:

i) a spacer sequence from any one of SEQ ID NOs: 1-7, 15-20, and 27-29 or a variant thereof having no more than 3 mismatches compared to any one of SEQ ID NOs: 1-7, 15-20, and 27-29;

ii) a spacer sequence from any one of SEQ ID NOs: 1-7 or a variant thereof having no more than 3 mismatches compared to any one of SEQ ID NOs: 1-7; or

iii) a spacer sequence from any one of SEQ ID NOs: 2, 3, and 5 or a variant thereof having no more than 3 mismatches compared to any one of SEQ ID NOs: 2, 3, and 5.

23. The method of claim 21 or 22, wherein the FOXP3 or functional derivative thereof is wild-type FOXP3.

24. The method of any one of claims 21-23, wherein the disease or condition is an inflammatory disease or an autoimmune disease.

25. The method of any one of claims 21-24, wherein the disease or condition is IPEX syndrome or Graft-versus-Host Disease.

26. A genetically modified $CD34^+$ cell in which the genome of the cell is edited by the method of any one of claims 9-18 for use in inhibiting or treating a disease or condition associated with FOXP3, such as an inflammatory disease or an autoimmune disease.

27. Use of a genetically modified $CD34^+$ cell in which the genome of the cell is edited by the method of any one of claims 9-18 as a medicament.

## CLAIMS

1. A method of making a genetically modified CD34+ cell, the method comprising delivering to a CD34+ cell a donor template comprising:

a) a first homology arm having homology to a sequence in a forkhead box protein P3 (FOXP3) gene, adeno-associated virus site 1 (AAVS1) locus, or T cell receptor alpha (TRA) gene in the CD34+ cell;

b) a second homology arm having homology to a sequence in the same gene or locus as the first homology arm;

c) a promoter; and

d) a FOXP3 cDNA sequence comprising at least 90% sequence identity to the nucleic acid sequence of SEQ ID NO: 110, wherein the FOXP3 cDNA sequence is codon-optimized for expression in the cell,

wherein the promoter and the FOXP3 cDNA sequence are located between the first homology arm and second homology arm.

2. The method of claim 1, further comprising delivering to the cell a DNA endonuclease or a nucleic acid encoding the DNA endonuclease.

3. The method of claim 2, further comprising delivering to the cell a gRNA comprising a spacer sequence that is complementary to the FOXP3 gene, AAVS1 locus, or TRA gene.

4. The method of claim 3, wherein the gRNA comprises:

i) the spacer sequence from any one of SEQ ID Nos: 1–7, 15–20, and 27–29 or a variant thereof having no more than 3 mismatches compared to any one of SEQ ID Nos: 1–7, 15–20, and 27–29;

ii) the spacer sequence from any one of SEQ ID Nos: 1–7 or a variant thereof having no more than 3 mismatches compared to any one of SEQ ID Nos: 1–7; or

iii) the spacer sequence from any one of SEQ ID Nos: 2, 3, and 5 or a variant thereof having no more than 3 mismatches compared to any one of SEQ ID Nos: 2, 3, and 5.

5. The method of claim 1, wherein the first homology arm has homology to a sequence in the FOXP3 gene, and the second homology arm has homology to another sequence in the FOXP3 gene.

6. The method of claim 1, wherein:

a) the donor template is encoded in an adeno-associated virus (AAV) vector; and/or

b) the promoter is a myeloproliferative sarcoma virus MPSV enhancer, negative control region NCR deletion, d1587rev primer binding site replacement (MND) promoter, phosphoglycerate kinase (PGK) promoter, elongation factor-1 alpha (EF-1α) promoter, or E2 factor (E2F) promoter.

7. The method of claim 1, wherein the donor template further comprises a sequence encoding a selectable marker, and the method further comprises separating cells expressing the selectable marker from cells that do not express the selectable marker.

8. A genetically modified CD34+ cell made by the method of claim 1, wherein the donor template is integrated within a FOXP3 gene, AAVS1 locus, or TRA gene in the genome of the CD34+ cell.

9. A pharmaceutical composition comprising the genetically modified CD34+ cell of claim 8 and a pharmaceutically acceptable excipient.

10. A nucleic acid comprising:

a) a first homology arm having homology to a sequence in a FOXP3 gene, AAVS1 locus, or TRA gene in a CD34+ cell;

b) a second homology arm having homology to a sequence in the same gene or locus as the first homology arm;

c) a promoter; and

d) a FOXP3 cDNA sequence comprising at least 90% sequence identity to the nucleic acid sequence of SEQ ID NO: 110, wherein the FOXP3 cDNA sequence is codon-optimized for expression in the cell,

wherein the promoter and the FOXP3 cDNA sequence are located between the first homology arm and second homology arm.