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.