

CLAIMS

1. A genetically engineered mammalian cell comprising a heterologous sequence in the genome,
wherein the heterologous sequence comprises a transgene encoding a lineage commitment factor, and
wherein the lineage commitment factor promotes the differentiation of the cell to a CD4⁺ regulatory T cell (Treg) or promotes the maintenance of the cell as a CD4⁺ Treg.
2. The cell of claim 1, wherein the heterologous sequence is integrated into a T cell specific gene locus such that expression of the transgene is under the control of transcription-regulatory elements in the gene locus.
3. A method of making a genetically engineered mammalian cell, comprising:
contacting a mammalian cell with a nucleic acid construct comprising (i) a heterologous sequence and (ii) a first homologous region (HR) and a second HR flanking the heterologous sequence, wherein
the heterologous sequence comprises a transgene,
the first and second HRs are homologous to a first genomic region (GR) and a second GR, respectively, in a T cell specific gene locus or a genomic safe harbor locus in the mammalian cell; and
culturing the cell under conditions that allow integration of the heterologous sequence between the first and second GRs in the T cell specific gene locus or genomic safe harbor locus.
4. The method of claim 3, wherein the integration is facilitated by a zinc finger nuclease or nickase (ZFN), a transcription activator-like effector domain nuclease or nickase (TALEN), a meganuclease, an integrase, a recombinase, a transposase, or a CRISPR/Cas system.
5. The method of claim 3 or 4, wherein the nucleic acid construct is a lentiviral construct, an adenoviral construct, an adeno-associated viral construct, a plasmid, a DNA construct, or an RNA construct.

6. The cell or method of any one of the preceding claims, wherein the transgene comprises a coding sequence for an additional polypeptide, wherein the coding sequence for the lineage commitment factor and the coding sequence for the additional polypeptide are separated by an in-frame coding sequence for a self-cleaving peptide or by an internal ribosome entry site (IRES).
7. The cell or method of claim 6, wherein the additional polypeptide is another lineage commitment factor, a therapeutic protein, or a chimeric antigen receptor.
8. The cell or method of any one of the preceding claims, wherein the heterologous sequence is integrated into an exon in the T cell specific gene locus and comprises:
 - an internal ribosome entry site (IRES) immediately upstream of the transgene; or
 - a second coding sequence for a self-cleaving peptide immediately upstream of and in-frame with the transgene.
9. The cell or method of claim 8, wherein the heterologous sequence further comprises, immediately upstream of the IRES or the second coding sequence for a self-cleaving peptide, a nucleotide sequence comprising all the exonic sequences of the T cell specific gene locus that are downstream of the integration site, such that the T cell specific gene locus remains able to express an intact T cell specific gene product.
10. The cell or method of any one of the preceding claims, wherein the T cell specific gene locus is a T cell receptor alpha constant (*TRAC*) gene locus.
11. The cell or method of claim 10, wherein the heterologous sequence is integrated into exon 1, 2, or 3 of the *TRAC* gene locus.
12. The cell or method of any one of the preceding claims, wherein the transgene encodes FOXP3, Helios, or ThPOK.
13. The cell or method of claim 12, wherein the transgenes comprises a coding sequence for FOXP3 and a coding sequence of ThPOK, wherein these two coding sequences are in-frame and are separated by an in-frame coding sequence for a self-cleaving peptide.

14. The cell or method of any one of the preceding claims, wherein the cell is a human cell.
15. The cell or method of any one of claims 1-14, wherein the cell is a stem or progenitor cell, optionally selected from embryonic stem cell, induced pluripotent stem cell, mesodermal stem cell, mesenchymal stem cell, hematopoietic stem cell, a lymphoid progenitor cell, or a progenitor T cell.
16. The cell or method of claim 15, wherein the cell is reprogrammed from a T cell, optionally a Treg, a CD4⁺ T cell, or a CD8⁺ T cell.
17. The cell of any one of claims 1-14, wherein the cell is a Treg.
18. A method of producing the Treg of claim 17, the method comprising:
culturing the cell of claim 15 or 16 in a tissue culture medium that comprises (i) a low IL-2 dose, (ii) an inhibitor of IL-7Ra (CD27) signaling, (iii) an inhibitor of CCR7 signaling.
19. A method of producing the Treg of claim 17, the method comprising co-culturing the cell of claim 15 or 16 with MS5-DLL1/4 stromal cells; OP9 or OP9-DLL1 stromal cell; or EpCAM-CD56⁺ stromal cells.
20. The cell or method of any one of the preceding claims, wherein the cell comprises a null mutation in a gene selected from
a Class II major histocompatibility complex transactivator (CIITA) gene,
an HLA Class I or II gene,
a transporter associated with antigen processing,
a minor histocompatibility antigen gene, and
a β 2 microglobulin (B2M) gene.
21. The cell or method of any one of the preceding claims, wherein the cell comprises a suicide gene optionally selected from an HSV-TK gene, a cytosine deaminase gene, a nitroreductase gene, a cytochrome P450 gene, or a caspase-9 gene.

22. A genetically engineered mammalian regulatory T cell (Treg) produced by the process of claim 18 or 19.
23. A method of treating a patient in need of immunosuppression, comprising administering to the patient a cell of any one of claims 1, 2, 6-17, and 20-22.
24. Use of the cell of any one of claims 1, 2, 6-17, and 20-22 in the manufacture of a medicament in treating a patient in need of immunosuppression.
25. A cell of any one of claims 1, 2, 6-17, and 20-22 for use in treating a patient in need of immunosuppression.
26. The method, use, or cell for use of any one of claims 23-25, wherein the patient has an autoimmune disease.
27. The method, use, or cell for use of any one of claims 23-25, wherein the patient has received or will receive tissue transplantation.
28. The method, use, or cell for use of any one of claims 23-27, wherein the patient is a human.