

CLAIMS

1. A modified pluripotent stem cell engineered to eliminate endogenous TCR or HLA expression.
2. The cell of claim 1, comprising a deficient TCR α constant (TRAC) gene, a deficient TCR β constant (TRBC) gene or a deficient beta 2 microglobulin (b2m) gene, optionally wherein the deficient gene is created by knockout.
3. The cell of claim 2, wherein the deficient gene is edited using CRISPR/Cas9, a zinc finger nuclease (ZFN), a TALEN, a MegaTAL, a meganuclease, Cpf1, homologous recombination, or a single stranded oligodeoxynucleotide (ssODN).

4. The cell of any one of claims 1-3, comprising

an exogenous construct encoding a single chain HLA trimer, a single chain HLA trimer comprising an HLA linked to beta-2-microglobulin linked to a stabilizing peptide, optionally, wherein the HLA trimer is HLA-E, HLA-G, or a combination of HLA-E and HLA-G;

an exogenous construct encoding a chimeric antigen receptor (CAR) that targets a tumor antigen, optionally, wherein the tumor antigen is selected from a tumor-associated surface antigen, such as 5T4, alphafetoprotein (AFP), B7-1 (CD80), B7-2 (CD86), BCMA, B-human chorionic gonadotropin, CA-125, carcinoembryonic antigen (CEA), carcinoembryonic antigen (CEA), CD123, CD133, CD138, CD19, CD20, CD22, CD23, CD24, CD25, CD30, CD33, CD34, CD4, CD40, CD44, CD56, CD70, CD8, CLL-1, c-Met, CMV-specific antigen, CS-1, CSPG4, CTLA-4, DLL3, disialoganglioside GD2, ductal-epithelial mucine, EBV-specific antigen, EGFR variant III (EGFRvIII), ELF2M, endoglin, ephrin B2, epidermal growth factor receptor (EGFR), epithelial cell adhesion molecule (EpCAM), epithelial tumor antigen, ErbB2 (HER2/neu), fibroblast associated protein (fap), FLT3, folate binding protein, GD2, GD3, glioma-associated antigen, glycosphingolipids, gp36, HBV-specific antigen, HCV-specific antigen, HER1-HER2, HER2-HER3 in combination, HERV-K, high molecular weight-melanoma associated antigen (HMW-MAA), HIV-1 envelope glycoprotein gp41, HPV-specific antigen, human telomerase reverse transcriptase, IGF1 receptor, IGF-II, IL-11R α , IL-13R α 2, Influenza Virus-specific antigen; CD38, insulin growth factor (IGF)-I, intestinal carboxyl esterase, kappa chain, LAGA-1a, lambda chain, Lassa Virus-specific antigen, lectin-reactive AFP, lineage-specific

or tissue specific antigen such as CD3, MAGE, MAGE-A1, major histocompatibility complex (MHC) molecule, major histocompatibility complex (MHC) molecule presenting a tumor-specific peptide epitope, M-CSF, melanoma-associated antigen, mesothelin, mesothelin, MN-CA IX, MUC-1, mut hsp70-2, mutated p53, mutated p53, mutated ras, neutrophil elastase, NKG2D, Nkp30, NY-ESO-1, p53, PAP, prostase, prostate specific antigen (PSA), prostate-carcinoma tumor antigen-1 (PCTA-1), prostate-specific antigen protein, STEAP1, STEAP2, PSMA, RAGE-1, ROR1, RU1, RU2 (AS), surface adhesion molecule, surviving and telomerase, TAG-72, the extra domain A (EDA) and extra domain B (EDB) of fibronectin and the A1 domain of tenascin-C (TnC A1), thyroglobulin, tumor stromal antigens, vascular endothelial growth factor receptor-2 (VEGFR2), virus-specific surface antigen such as an HIV-specific antigen (such as HIV gpl20), as well as any derivate or variant of these surface markers;

an exogenous construct encoding a TCR, optionally, wherein the TCR is an alpha/beta TCR, gamma/delta TCR, a cancer or cancer associated antigen reactive TCR, a TCR that is reactive against murine or other non-human MHC, a class I or class II restricted TCR, an HPV recognizing TCR, a viral reactive TCR, an EBV TCR, a CMV TCR, or an influenza TCR, an HPV-16 E6 TCR, HPV-16 E7 TCR, or MAGEA3/A6 TCR or engineered variant, or TCR is derived from CD8, CD4, CD4/8 double positive, immature or developing T cell, Treg, NKT, or NK T cell; and/or

an exogenous construct encoding a suicide gene, wherein the suicide gene allows for the elimination of gene modified cells or is used as a PET reporter gene for non-invasive imaging, optionally, wherein the suicide gene is sr39TK, is a chemically induced caspase, dimerization induced by a small molecule / chemically induced dimerizer (CID), a selectable surface marker, or a selectable surface marker selected from CD19, CD20, CD34, EGFR or LNGFR.

5. A method of generating a modified pluripotent stem cell comprising:
 - (a) editing a gene locus to eliminate expression of endogenous TCR or block expression of donor HLA; and
 - (b) introducing an exogenous construct encoding a CAR, TCR, or HLA gene.
6. The method of claim 5, wherein the method further comprises a step of isolating a hematopoietic stem cell, an embryonic stem, or an induced pluripotent stem cell.

7. A method of generating a T cell lineage of interest comprising:
 - (a) providing a modified pluripotent stem cell of any one of claims 1-5; and
 - (b) inducing T cell or T cell-like differentiation.
8. The method of claim 7, wherein T cell differentiation is induced using an artificial thymic organoid (ATO) system, notch agonist, OP9-DLL1, OP9-DLL4, fetal thymic organoid culture (FTOC), chemical induction, bone marrow / liver / thymus or other humanized mouse, embryoid body (EB).
9. The method of claim 7 or 8, wherein the T cell lineage is selected by detecting expression of one or more biomarkers, optionally, wherein the T cell lineage of interest is a CD8 single positive T cell, a CD4 single positive T cell, a CD4 CD8 double positive T cell, a double negative T cell, a CD3 positive cell, an NK cell, a proT cell, a pre-proT cell, a mesodermal progenitor, a B cell, a common lymphoid progenitor, a hematopoietic progenitor, a hematopoietic stem cell.
10. A method of generating a T cell lineage of interest, comprising:
 - (a) providing a modified pluripotent stem cell of any one of claims 1-5;
 - (b) editing a gene encoding a cell fate regulator to promote, impair or eliminate the generation of a specific cell lineage; and
 - (c) inducing T cell differentiation.
11. The method of claim 10, wherein the cell fate regulator is a transcription factor, T-BET, STAT1, STAT4, STAT, RUNX3, GATA3, Stat5, Stat6, DEC2, MAF, THPOK, GATA3, Smads, Stat6, PU.1, RORgt, RORa, Stat3, AHR, Bcl-6, MAF, FoxP3, Smad3, Stat5, FOXO1, FOXO3, GRAIL, or PLZF.
12. The method of claim 10 or 11, wherein the specific lineage is Th1, Th2, Th9, Th17, Th22, Tfh, Treg, ILC, NK, or NKT.
13. A modified pluripotent stem cell with enriched or enhanced pairing between a pre-TCR α (pTa) protein and a TCR β protein as compared to an unmodified control cell.
14. The modified pluripotent stem cell of claim 13, wherein the modified pluripotent stem cell comprises an exogenous construct encoding the pre-TCR α (pTa) protein, optionally, wherein the exogenous construct is a viral construct, an AAV construct, lentiviral construct, or retroviral construct.

15. The modified pluripotent stem cell of claim 13 or 14, wherein the modified pluripotent stem cell comprises a deficient TCR α gene, optionally, wherein the deficient TCR α gene is created by knockout using an engineered nuclease, TALEN, megaTAL, CRISPR, ZFN, knockout using homologous recombination, or antisense RNA.
16. The modified pluripotent stem cell of any one of claims 13-15, wherein the modified pluripotent stem cell is substantially free of TCR α and TCR β pairing.
17. The modified pluripotent stem cell of any one of the preceding claims, wherein the modified pluripotent stem cell further comprises a chimeric antigen receptor (CAR), an exogenous TCR, and/or an antigen receptor.
18. A method of generating a modified pluripotent stem cell comprising a step of introducing an exogenous pre-TCR α (pTa) protein and/or creating a deficient TCR α gene.
19. A method of generating a T cell lineage of interest, comprising steps of providing a modified pluripotent stem cell of any one of claims 13-17, and inducing T cell differentiation in an artificial thymic organoid.
20. A method of generating a T cell lineage of interest, comprising steps of providing a modified pluripotent stem cell of any one of claims 13-17, and inducing T cell differentiation in the presence or absence of peptide:MHC, optionally, wherein the T cell lineage of interest is cytotoxic CD8 $^{+}$ T cells, helper CD4 $^{+}$ T cells, helper CD4 $^{+}$ T cells that are Th1/Th2/Th17 cells, regulatory T cells, intra epithelial lymphocyte (IEL), or mature alpha-beta or gamma-delta T cell.