

CLAIMS

1. A T cell derived from a modified pluripotent stem cell, wherein the modified pluripotent stem cell comprises (i) a deficient endogenous TCR α constant (TRAC) gene and/or a deficient endogenous TCR β constant (TRBC); (ii) a deficient endogenous beta-2-microglobulin (b2m) gene; and (iii) an exogenous construct encoding a single chain HLA trimer.
2. The cell of claim 1, wherein the deficient TRAC gene, the deficient TRBC gene, or the deficient b2m 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, wherein the single chain HLA trimer is HLA-E, HLA-G, or a combination of HLA-E and HLA-G.
5. The cell of any one of claims 1-3, wherein the HLA trimer comprises an HLA linked to beta-2-microglobulin linked to a stabilizing peptide.
6. The cell of any one of claims 1-5, further comprising an exogenous construct encoding a chimeric antigen receptor (CAR) that targets a tumor antigen and/or an exogenous construct encoding a TCR.
7. The cell of claim 6, 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-11Ralpha, IL-13R-a2, Influenza Virus-specific antigen; CD38, insulin growth factor (IGF1)-1, 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 gp120), as well as any derivate or variant of these surface markers.

8. The cell of claim 6, 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 the TCR is derived from CD8, CD4, CD4/8 double positive, immature or developing T cell, Treg, NKT, or NK T cell.
9. The cell of any of the preceding claims, further comprising 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.
10. The cell of claim 9, wherein the suicide gene is sr39TK, is a chemically induced caspase, a 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.
11. A method of generating a T cell lineage of interest comprising:
 - (a) editing a pluripotent stem cell by (i) making an endogenous TCR α constant (TRAC) gene and/or an endogenous TCR β constant (TRBC); deficient (ii) making an endogenous beta-2-microglobulin (b2m) gene deficient; and (iii) introducing an exogenous construct encoding a single chain HLA trimer; and

- (b) inducing T cell or T cell-like differentiation.
12. The method of claim 11, wherein the method further comprises (c) introducing an exogenous construct encoding a CAR gene and/or a TCR gene into the pluripotent stem cell.
 13. The method of claim 11, wherein the method further comprises isolating a hematopoietic stem cell, an embryonic stem, or an induced pluripotent stem cell.
 14. The method of claim 11, 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).
 15. The method of claim 11 or 12, wherein the T cell lineage is selected by detecting expression of one or more biomarkers.
 16. The method of claim 15, 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.
 17. A method of generating a T cell lineage of interest, comprising:
 - (a) editing a pluripotent stem cell by (i) making an endogenous TCR α constant (TRAC) gene and/or an endogenous TCR β constant (TRBC); deficient (ii) making an endogenous beta-2-microglobulin (b2m) gene deficient; and (iii) introducing an exogenous construct encoding a single chain HLA trimer;
 - (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.
 18. The method of claim 17, 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.
 19. The method of claim 17 or 18, wherein the specific lineage is Th1, Th2, Th9, Th17, Th22, Tfh, Treg, ILC, NK, or NKT.