

CLAIMS

1. A modified immune cell wherein the SUV39H1 gene is inactivated, said cell comprising:
a T cell receptor (TCR) alpha constant region gene inactivated by the insertion of a nucleic acid sequence encoding an antigen-specific receptor that specifically binds to an antigen, optionally a chimeric antigen receptor (CAR) or a heterologous TCR

wherein optionally the antigen is orphan tyrosine kinase receptor ROR1, tEGFR, Her2, p95HER2, LI-CAM, CD19, CD20, CD22, mesothelin, CEA, hepatitis B surface antigen, anti-folate receptor, CD23, CD24, CD30, CD33, CD38, CD44, EGFR, EGP-2, EGP-4, EPHA2, ErbB2, 3, or 4, FBP, fetal acetylcholine e receptor, GD2, GD3, HMW-MAA, IL-22R-alpha, IL-13R-alpha2, kdr, kappa light chain, BCMA, Lewis Y, MAGE-A1, mesothelin, MUC1, MUC16, PSCA, NKG2D Ligands, NY-ESO-1, MART-1, gp100, oncofetal antigen, TAG72, VEGF-R2, carcinoembryonic antigen (CEA), prostate specific antigen (PSMA), estrogen receptor, progesterone receptor, ephrinB2, CD 123, CS-1, c-Met, GD-2, MAGE A3, CE7, or Wilms Tumor 1 (WT-1).

2. A modified immune cell comprising a nucleic acid encoding a SUV39H1 inhibitor, optionally a dominant negative SUV39H1 gene, said cell comprising:

a T cell receptor (TCR) alpha constant region gene inactivated by the insertion of a nucleic acid sequence encoding an antigen-specific receptor that specifically binds to an antigen, optionally a chimeric antigen receptor (CAR) or a heterologous TCR

wherein optionally the antigen is orphan tyrosine kinase receptor ROR1, tEGFR, Her2, p95HER2, LI-CAM, CD19, CD20, CD22, mesothelin, CEA, hepatitis B surface antigen, anti-folate receptor, CD23, CD24, CD30, CD33, CD38, CD44, EGFR, EGP-2, EGP-4, EPHA2, ErbB2, 3, or 4, FBP, fetal acetylcholine e receptor, GD2, GD3, HMW-MAA, IL-22R-alpha, IL-13R-alpha2, kdr, kappa light chain, BCMA, Lewis Y, MAGE-A1, mesothelin, MUC1, MUC16, PSCA, NKG2D Ligands, NY-ESO-1, MART-1, gp100, oncofetal antigen, TAG72, VEGF-R2, carcinoembryonic antigen (CEA), prostate specific antigen (PSMA), estrogen receptor, progesterone receptor, ephrinB2, CD 123, CS-1, c-Met, GD-2, MAGE A3, CE7, or Wilms Tumor 1 (WT-1).

3. A modified immune cell wherein the SUV39H1 gene is inactivated and that expresses a chimeric antigen receptor (CAR) comprising:

- a) an extracellular antigen-binding domain that specifically binds an antigen,
- b) a transmembrane domain,
- c) optionally one or more costimulatory domains
- d) an intracellular signaling domain comprising a modified CD3zeta intracellular signaling domain in which ITAM2 and ITAM3 have been inactivated,

wherein optionally the antigen is orphan tyrosine kinase receptor ROR1, tEGFR, Her2, p95HER2, LI-CAM, CD19, CD20, CD22, mesothelin, CEA, hepatitis B surface antigen, anti-folate receptor, CD23, CD24, CD30, CD33, CD38, CD44, EGFR, EGP-2, EGP-4, EPHA2, ErbB2, 3, or 4, FBP, fetal acetylcholine e receptor, GD2, GD3, HMW-MAA, IL-22R-alpha, IL-13R-alpha2, kdr, kappa light chain, BCMA, Lewis Y, MAGE-A1, mesothelin, MUC1, MUC16, PSCA, NKG2D Ligands, NY-ESO-1, MART-1, gp100, oncofetal antigen, TAG72, VEGF-R2, carcinoembryonic antigen (CEA), prostate specific antigen (PSMA), estrogen receptor, progesterone receptor, ephrinB2, CD 123, CS-1, c-Met, GD-2, MAGE A3, CE7, or Wilms Tumor 1 (WT-1).

4. A modified immune cell comprising a nucleic acid encoding a SUV39H1 inhibitor, optionally a dominant negative SUV39H1 gene, and that expresses a chimeric antigen receptor (CAR) comprising:

- a) an extracellular antigen-binding domain that specifically binds an antigen,
- b) a transmembrane domain,
- c) optionally one or more costimulatory domains
- d) an intracellular signaling domain comprising a modified CD3zeta intracellular signaling domain in which ITAM2 and ITAM3 have been inactivated,

wherein optionally the antigen is orphan tyrosine kinase receptor ROR1, tEGFR, Her2, p95HER2, LI-CAM, CD19, CD20, CD22, mesothelin, CEA, hepatitis B surface antigen, anti-folate receptor, CD23, CD24, CD30, CD33, CD38, CD44, EGFR, EGP-2, EGP-4, EPHA2, ErbB2, 3, or 4, FBP, fetal acetylcholine e receptor, GD2, GD3, HMW-MAA, IL-22R-alpha, IL-13R-alpha2, kdr, kappa light chain, BCMA, Lewis Y, MAGE-A1, mesothelin, MUC1, MUC16, PSCA, NKG2D Ligands, NY-ESO-1, MART-1, gp100, oncofetal antigen, TAG72, VEGF-R2, carcinoembryonic antigen (CEA), prostate specific antigen (PSMA), estrogen receptor, progesterone receptor, ephrinB2, CD 123, CS-1, c-Met, GD-2, MAGE A3, CE7, or Wilms Tumor 1 (WT-1).

5. The modified immune cell of any of claims 1-4, wherein the cell is a T cell, a T cell progenitor, a hematopoietic stem cell, an iPSC, a CD4⁺ T cell, a CD8⁺ T cell, a CD4⁺ and CD8⁺ T cell, or a NK cell, or a T_N cell, T_{SCM}, T_{CM} or T_{EM} cell.
6. The modified immune cell of any of claims 1-4 wherein the immune cell is a T regulatory cell.
7. The modified immune cell of any of claims 1-6, wherein the CAR comprises: (a) an extracellular antigen-binding domain; (b) a transmembrane domain, (c) optionally one or more costimulatory domains, and (d) an intracellular signaling domain.
8. The modified immune cell of claim 7, wherein the extracellular antigen-binding domain is an scFv, optionally an scFv that specifically binds a cancer antigen.
9. The modified immune cell of claim 7 or 8 wherein the transmembrane domain is from CD28, CD8 or CD3-zeta.
10. The modified immune cell of any of claims 7-9 wherein the one or more costimulatory domains are selected from the group consisting of: 4-1BB, CD28, ICOS, OX40 and DAP10.
11. The modified immune cell of any of claims 7-10 wherein the intracellular signaling domain comprises the intracellular signaling domain of a CD3-zeta polypeptide, or a fragment thereof, optionally a CD3-zeta polypeptide wherein immunoreceptor tyrosine-based activation motif 2 (ITAM2) and immunoreceptor tyrosine-based activation motif 3 (ITAM3) are inactivated.
12. The modified immune cell of any of the preceding claims, wherein the T cell further comprises a second antigen-specific receptor, optionally a TCR or CAR, that specifically binds to a second antigen.

13. The modified immune cell of any of the preceding claims, wherein SUV39H1 expression is reduced by at least about 50%, 60%, 70%, 75%, 80%, 85%, 90% or 95%.
14. The modified immune cell of any of the preceding claims, wherein endogenous TCR expression is reduced by at least about 75%, 80%, 85%, 90% or 95%.
15. The modified immune cell of any of the preceding claims, wherein the immune cell is autologous.
16. The modified immune cell of any of the preceding claims, wherein the immune cell is allogeneic.
17. The modified immune cell of any of the preceding claims, wherein the HLA-A locus is inactivated.
18. The modified immune cell of claim 17 wherein HLA class I expression is reduced by at least about 75%, 80%, 85%, 90% or 95%.
19. The modified immune cell of any of the preceding claims that expresses two CARs, a first CAR that binds a first antigen and a second CAR that binds a second antigen.
20. A sterile pharmaceutical composition comprising the modified immune cell of any of the preceding claims.
21. A kit comprising the modified immune cell of any of the preceding claims, and a delivery device or container.
22. A method of using the modified immune cell or pharmaceutical composition or kit of any of the preceding claims to treat a patient suffering from or at risk of disease associated

with the antigen, optionally cancer, by administering a therapeutically effective amount of said immune cell or pharmaceutical composition to the patient.

23. The method of claim 22 wherein the immune cell is a CAR T-cell and a dose of less than about 5×10^7 cells, optionally about 10^5 to about 10^7 cells, is administered to the patient.

24. The method of claim 22 or 23 wherein a second therapeutic agent, optionally one or more cancer chemotherapeutic agents, cytotoxic agents, hormones, anti-angiogens, radiolabelled compounds, immunotherapy, surgery, cryotherapy, and/or radiotherapy, is administered to the patient.

25. The method of claim 22 or 23 wherein an immune checkpoint modulator is administered to the patient.

26. The method of claim 25, wherein the immune checkpoint modulator is an antibody that specifically binds to, or other inhibitor of, PD1, PDL1, CTLA4, LAG3, BTLA, OX2R, TIM-3, TIGIT, LAIR-1, PGE2 receptor, EP2/4 adenosine receptor, or A2AR.