

CLAIMS

What is claimed is:

1. An isolated T cell receptor (TCR) comprising a minimally murinized TCR α chain and a minimally murinized TCR β chain, and wherein the TCR α chain transmembrane domain comprises hydrophobic amino acid substitutions, wherein the TCR does not bind MAGEA4.
2. An isolated T cell receptor (TCR) comprising:
 - (a) a TCR α chain that comprises a constant domain comprising minimal murinization amino acid substitutions at positions 90, 91, 92, and 93, and hydrophobic amino acid substitutions at positions 115, 118, and 119; and
 - (b) a TCR β chain that comprises a constant domain comprising minimal murinization amino acid substitutions at positions 18, 22, 133, 136, and 139.
3. An isolated T cell receptor (TCR) comprising:
 - (a) a TCR α chain that comprises a constant domain comprising the amino acid substitutions, P90S, E91D, S92V, S93P, S115L, G118V, and F119L; and
 - (b) a TCR β chain that comprises a constant domain comprising the amino acid substitutions E18K, S22A, F133I, E/V136A, and Q139H.
4. An isolated T cell receptor (TCR) comprising:
 - (a) a TCR α chain that comprises a constant domain comprising at least 4 minimal murinization amino acid substitutions and at least 3 hydrophobic amino acid substitutions in the TCR α chain transmembrane domain, wherein the TCR α chain constant domain comprises an amino acid sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence set forth in SEQ ID NO: 4; and
 - (b) a TCR β chain that comprises a constant domain comprising at least 5 minimal murinization amino acid substitutions, wherein the TCR β chain constant domain comprises an

amino acid sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence set forth in SEQ ID NO: 5 or SEQ ID NO: 6.

5. An isolated T cell receptor (TCR) comprising:

(a) a TCR α chain comprising a constant domain comprising the amino acid sequence set forth in SEQ ID NO: 4; and

(b) a TCR β chain comprising a constant domain comprising the amino acid sequence set forth in SEQ ID NO: 5 or SEQ ID NO: 6.

6. The isolated TCR of any one of the preceding claims, wherein the TCR binds a target antigen selected from the group consisting of: α -fetoprotein (AFP), B Melanoma Antigen (BAGE) family members, Brother of the regulator of imprinted sites (BORIS), Cancer-testis antigens, Cancer-testis antigen 83 (CT-83), Carbonic anhydrase IX (CAIX), Carcinoembryonic antigen (CEA), Cytomegalovirus (CMV) antigens, Cytotoxic T cell (CTL)-recognized antigen on melanoma (CAMEL), Epstein-Barr virus (EBV) antigens, G antigen 1 (GAGE-1), GAGE-2, GAGE-3, GAGE-4, GAGE-5, GAGE-6, GAGE-7B, GAGE-8, Glycoprotein 100 (GP100), Hepatitis B virus (HBV) antigens, Hepatitis C virus (HCV) non-structure protein 3 (NS3), Human Epidermal Growth Factor Receptor 2 (HER-2), Human papillomavirus (HPV)-E6, HPV-E7, Human telomerase reverse transcriptase (hTERT), Latent membrane protein 2 (LMP2), Melanoma antigen family A, 1 (MAGE-A1), MAGE-A2, MAGE-A3, MAGE-A6, MAGE-A10, MAGE-A12, Melanoma antigen recognized by T cells (MART-1), Mesothelin (MSLN), Mucin 1 (MUC1), Mucin 16 (MUC16), New York esophageal squamous cell carcinoma-1 (NYESO-1), P53, P antigen (PAGE) family members, Placenta-specific 1 (PLAC1), Preferentially expressed antigen in melanoma (PRAME), Survivin, Synovial sarcoma X 1 (SSX1), Synovial sarcoma X 2 (SSX2), Synovial sarcoma X 3 (SSX3), Synovial sarcoma X 4 (SSX4), Synovial sarcoma X 5 (SSX5), Synovial sarcoma X 8 (SSX8), Thyroglobulin, Tyrosinase, Tyrosinase related protein (TRP)1, TRP2, Wilms tumor protein (WT-1), X Antigen Family Member 1 (XAGE1), and X Antigen Family Member 2 (XAGE2).

7. The isolated TCR of any one of claims 1 to 6, wherein the TCR expression and avidity is increased compared to a TCR that comprises a minimally murinized TCR α chain and a minimally murinized TCR β chain but wherein the TCR α chain transmembrane domain does not comprise hydrophobic amino acid substitutions.

8. The isolated TCR of any one of claims 1 to 6, wherein the TCR expression and avidity is increased compared to a TCR that does not comprise a minimally murinized TCR α chain and a minimally murinized TCR β chain but wherein the TCR α chain transmembrane domain comprises hydrophobic amino acid substitutions.

9. A fusion protein comprising the TCR α chain and the TCR β chain set forth in any one of the preceding claims.

10. A fusion protein comprising a minimally murinized TCR α chain wherein the TCR α chain transmembrane domain comprises hydrophobic amino acid substitutions; a polypeptide cleavage signal; and a minimally murinized TCR β chain, wherein the fusion protein does not bind MAGEA4.

11. A fusion protein comprising:

(a) a TCR α chain that comprises a constant domain comprising minimal murinization amino acid substitutions at positions 90, 91, 92, and 93, and hydrophobic amino acid substitutions at positions 115, 118, and 119;

(b) a polypeptide cleavage signal; and

(c) a TCR β chain that comprises a constant domain comprising minimal murinization amino acid substitutions at positions 18, 22, 133, 136, and 139, wherein the fusion protein does not bind MAGEA4.

12. A fusion protein comprising:

- (a) a TCR α chain that comprises a constant domain comprising the amino acid substitutions, P90S, E91D, S92V, S93P, S115L, G118V, and F119L;
 - (b) a polypeptide cleavage signal; and
 - (c) a TCR β chain that comprises a constant domain comprising the amino acid substitutions E18K, S22A, F133I, E/V136A, and Q139H,
- wherein the fusion protein does not bind MAGEA4.

13. A fusion protein comprising:

- (a) a TCR α chain that comprises a constant domain comprising at least 4 minimal murinization amino acid substitutions and at least 3 hydrophobic amino acid substitutions in the TCR α chain transmembrane domain, wherein the TCR α chain constant domain comprises an amino acid sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence set forth in SEQ ID NO: 4;
 - (b) a polypeptide cleavage signal; and
 - (c) a TCR β chain that comprises a constant domain comprising at least 5 minimal murinization amino acid substitutions, wherein the TCR β chain constant domain comprises an amino acid sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence set forth in SEQ ID NO: 5 or SEQ ID NO: 6,
- wherein the fusion protein does not bind MAGEA4.

14. A fusion protein comprising:

- (a) a TCR α chain comprising a constant domain comprising the amino acid sequence set forth in SEQ ID NO: 4;
 - (b) a polypeptide cleavage signal; and
 - (c) a TCR β chain comprising a constant domain comprising the amino acid sequence set forth in SEQ ID NO: 5 or SEQ ID NO: 6,
- wherein the fusion protein does not bind MAGEA4.

15. The fusion polypeptide of any one of claims 9 to 14, wherein the polypeptide cleavage signal is a viral self-cleaving peptide or ribosomal skipping sequence.

16. The fusion polypeptide of any one of claims 9 to 15, wherein the polypeptide cleavage signal is a viral 2A peptide.

17. The fusion polypeptide of any one of claims 9 to 16, wherein the polypeptide cleavage signal is an aphthovirus 2A peptide, a potyvirus 2A peptide, or a cardiovirus 2A peptide.

18. The fusion polypeptide of any one of claims 9 to 17, wherein the polypeptide cleavage signal is a viral 2A peptide selected from the group consisting of: a foot-and-mouth disease virus (FMDV) 2A peptide, an equine rhinitis A virus (ERAV) 2A peptide, a Thossea asigna virus (TaV) 2A peptide, a porcine teschovirus-1 (PTV-1) 2A peptide, a Theilovirus 2A peptide, and an encephalomyocarditis virus 2A peptide.

19. A nucleic acid encoding the TCR according to any one of claims 1 to 8 or the fusion protein of any one of claims 9 to 18.

20. A nucleic acid comprising a first polynucleotide encoding a minimally murinized TCR α chain wherein the TCR α chain transmembrane domain comprises hydrophobic amino acid substitutions; an internal ribosomal entry site (IRES); and a second polynucleotide encoding a minimally murinized TCR β chain, wherein the fusion protein does not bind MAGEA4.

21. A nucleic acid comprising:

(a) a first polynucleotide encoding a TCR α chain that comprises a constant domain comprising minimal murinization amino acid substitutions at positions 90, 91, 92, and 93, and hydrophobic amino acid substitutions at positions 115, 118, and 119;

(b) an IRES; and

(c) a second polynucleotide encoding a TCR β chain that comprises a constant domain comprising minimal murinization amino acid substitutions at positions 18, 22, 133, 136, and 139, wherein the fusion protein does not bind MAGEA4.

22. A nucleic acid comprising:

(a) a first polynucleotide encoding a TCR α chain that comprises a constant domain comprising the amino acid substitutions, P90S, E91D, S92V, S93P, S115L, G118V, and F119L;
(b) an IRES; and
(c) a second polynucleotide encoding a TCR β chain that comprises a constant domain comprising the amino acid substitutions E18K, S22A, F133I, E/V136A, and Q139H, wherein the fusion protein does not bind MAGEA4.

23. A nucleic acid comprising:

(a) a first polynucleotide encoding a TCR α chain that comprises a constant domain comprising at least 4 minimal murinization amino acid substitutions and at least 3 hydrophobic amino acid substitutions in the TCR α chain transmembrane domain, wherein the TCR α chain constant domain comprises an amino acid sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence set forth in SEQ ID NO: 4;
(b) an IRES; and
(c) a second polynucleotide encoding a TCR β chain that comprises a constant domain comprising at least 5 minimal murinization amino acid substitutions, wherein the TCR β chain constant domain comprises an amino acid sequence that is at least 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence set forth in SEQ ID NO: 5 or SEQ ID NO: 6, wherein the fusion protein does not bind MAGEA4.

24. A nucleic acid comprising:

(a) a first polynucleotide encoding a TCR α chain comprising a constant domain comprising the amino acid sequence set forth in SEQ ID NO: 4;
(b) an IRES; and

(c) a second polynucleotide encoding a TCR β chain comprising a constant domain comprising the amino acid sequence set forth in SEQ ID NO: 5 or SEQ ID NO: 6, wherein the fusion protein does not bind MAGEA4.

25. A vector comprising a nucleic acid encoding the TCR of any one of claims 1 to 8 or the fusion protein of any one of claims 9 to 18.

26. A vector comprising the nucleic acid of any one of claims 19 to 24, wherein the vector is preferably an expression vector, more preferably a retroviral vector or even more preferably a lentiviral vector.

27. A cell expressing the TCR of any one of claims 1 to 8.

28. A cell expressing the fusion protein of any one of claims 9 to 18.

29. A cell comprising the nucleic acid of any one of claims 19 to 24.

30. A cell comprising the vector of claim 25 or claim 26.

31. The cell of any one of claims 27 to 30, wherein the cell is an immune effector cell.

32. The cell of any one of claims 27 to 31, wherein the cell is an immune effector cell selected from the group consisting of: a T cell, a natural killer (NK) cell, or a natural killer T (NKT) cell.

33. A composition comprising the TCR of any one of claims 1 to 8, the fusion protein of any one of claims 9 to 18, the nucleic acid of any one of claims 19 to 24, the vector of claim 25 or claim 26, or the cell of any one of claims 27 to 32.

34. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and the TCR of any one of claims 1 to 8, the fusion protein of any one of claims 9 to 18, the nucleic acid of any one of claims 19 to 24, the vector of claim 25 or claim 26, or the cell of any one of claims 27 to 32.

35. The TCR of any one of claims 1 to 8, the fusion protein of any one of claims 9 to 18, the nucleic acid of any one of claims 19 to 24, the vector of claim 25 or claim 26, or the cell of any one of claims 27 to 32, the composition of claim 33, or the pharmaceutical composition of claim 34 for use as a medicament.

36. The TCR of any one of claims 1 to 8, the fusion protein of any one of claims 9 to 18, the nucleic acid of any one of claims 19 to 24, the vector of claim 25 or claim 26, or the cell of any one of claims 27 to 32, the composition of claim 33, or the pharmaceutical composition of claim 34 for use in the treatment of cancer, wherein the cancer is preferably a hematological cancer or a solid tumor, more preferably wherein the cancer is selected from the group consisting of sarcoma, prostate cancer, uterine cancer, thyroid cancer, testicular cancer, renal cancer, pancreatic cancer, ovarian cancer, esophageal cancer, non-small-cell lung cancer, non-Hodgkin's lymphoma, multiple myeloma, melanoma, hepatocellular carcinoma, head and neck cancer, gastric cancer, endometrial cancer, colorectal cancer, cholangiocarcinoma, breast cancer, bladder cancer, myeloid leukemia and acute lymphoblastic leukemia, most preferably wherein the cancer is selected from the group consisting of NSCLC, SCLC, breast, ovarian or colorectal cancer, sarcoma or osteosarcoma.