

specific T cell lines representing thousands of individual T cell clones from the peripheral repertoires of greater than 75 normal donors, a very time and labor intensive process (Chapuis *et al.*, 2013; Schmitt *et al.*, *Hum. Gene Ther.* 20:1240, 2009; Ho *et al.*, *J. Immunol. Methods* 310:40, 2006).

There is a need for alternative antigen-specific TCR immunotherapies directed against various cancers, such as leukemia and tumors. Presently disclosed embodiments address these needs and provide other related advantages.

SUMMARY

In one aspect, the present disclosure refers to a modified cell comprising a heterologous polynucleotide encoding a binding protein, wherein the encoded binding protein comprises a T cell receptor (TCR) α -chain variable (V_α) domain having CDR1, CDR2, and CDR3 amino acid sequences according to SEQ ID NOS:48-50, respectively, and a TCR β -chain variable (V_β) domain having CDR1, CDR2, and CDR3 amino acid sequences according to SEQ ID NOS:45-47, respectively, wherein the binding protein is capable of specifically binding to a KVLEYVIKV (SEQ ID NO.:123):human leukocyte antigen (HLA)-A*0201 complex on a cell surface independent of CD8 or in the absence of CD8, and wherein the modified cell is a CD4+ T cell and further comprises a heterologous polynucleotide encoding at least an extracellular portion of a CD8 co-receptor.

In another aspect, the present disclosure refers to an isolated modified cell comprising a heterologous polynucleotide encoding a binding protein, wherein the encoded binding protein comprises a TCR V_α domain having CDR1, CDR2, and CDR3 amino acid sequences of SEQ ID NOS.:48-50, respectively, and a TCR V_β domain having CDR1, CDR2, and CDR3 amino acid sequences of SEQ ID NOS.:45-47,

respectively, wherein the binding protein is capable of specifically binding to a KVLEYVIKV (SEQ ID NO.: 123):human leukocyte antigen (HLA)-A *0201 complex on a cell surface independent of CD8 or in the absence of CD8, wherein (i) the encoded V α domain comprises an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO.: 19, and (ii) the encoded V β domain comprises an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO.: 17, and wherein the modified cell is a CD4+ T cell and further comprises a heterologous polynucleotide encoding at least an extracellular portion of a CD8 co-receptor.

In another aspect, the present disclosure refers to a composition comprising a modified cell as disclosed herein and a pharmaceutically acceptable carrier, diluent, or excipient.

In another aspect, the present disclosure refers to a combination comprising (i) the composition of claim 24, comprising at least about 30% modified CD4+ T cells, with (ii) a composition comprising at least about 30% CD8+ T cells modified to encode a binding protein capable of binding to a KVLEYVIKV (SEQ ID NO.:123):human leukocyte antigen (HLA)-A*0201 complex on a cell surface, wherein the modified CD4+ T cells and the modified CD8 + T cells are present in the combination in about a 1:1 ratio.

In another aspect, the present disclosure refers to an isolated polynucleotide encoding (1) a binding protein having a TCR V α domain and a TCR V β domain, wherein (i) the encoded V α domain comprises CDR1, CDR2, and CDR3 amino acid sequences of SEQ ID NOS.:48-50, respectively, and the encoded V β domain comprises CDR1, CDR2, and CDR3 amino acid sequences of SEQ ID NOS.:45-47, respectively; (ii) the encoded binding protein is capable of specifically binding to KVLEYVIKV (SEQ ID NO.:123): human leukocyte antigen (HLA)-A*0201 complex on a cell surface; and (2) at least an extracellular portion of a CD8 co-receptor.

In yet another aspect, the present disclosure refers to an expression vector, comprising a polynucleotide as disclosed herein operably linked to an expression control sequence.

In yet another aspect, the present disclosure refers to a use of a modified cell as disclosed herein, a composition as disclosed herein, or a combination as disclosed

herein in the manufacture of a medicament for treating a hyperproliferative disorder associated with MAGE-A1 expression in a human subject.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A and 1B show representative data illustrating that high-affinity T cells for viral antigens are found at higher frequencies (A) than high-affinity T cells for self-antigens, which are found at very low frequencies (B).

Figures 2A and 2B show, respectively, (A) a schematic of a T cell enrichment assay performed by the inventors of the present disclosure, (B) flow cytometry data from a series of sorting experiments used to enrich for antigen-specific CD8⁺ T cells.

Figure 3 shows exemplary data from a TCR β CDR3 enrichment scheme of the present disclosure using MAGE-A1:HLA tetramers.

Figures 4A and 4B show, respectively, (A) specific binding of MAGE-A1:HLA tetramers by TCRs identified using methods of the present disclosure and (B) enrichment of MAGE-A1-specific TCRs.

Figures 5A-5C provide, respectively, (A) flow cytometry data showing MAGE-A1-specific CD8⁺ T cells of the present disclosure binding MAGE-A1:HLA tetramers, (B) cytokine production by MAGE-A1-specific CD8⁺ T cells in the absence (left) or presence (right) of antigen-expressing U266 myeloma cells, and (C) specific lysis data showing that high-affinity MAGE-A1 TCR-transduced CD8⁺ T cells of this disclosure bind antigen:MHC tetramers and kill cells presenting MAGE-A1: MHC (A*0201). Data in (C) was from a standard Cr⁵¹-release assay in which the CD8⁺ T cells were co-cultured with U266 cells alone, with exogenous interferon-gamma (IFN γ) or with exogenous MAGE-A1 peptide.

Figure 6A illustrates an immunotherapy approach according to the present disclosure in which CD4⁺ T cells are transduced to express a TCR and a CD8 co-

CLAIMS

What is claimed is:

1. A modified cell comprising a heterologous polynucleotide encoding a binding protein, wherein the encoded binding protein comprises a T cell receptor (TCR) α -chain variable (V_α) domain having CDR1, CDR2, and CDR3 amino acid sequences according to SEQ ID NOS:48-50, respectively, and a TCR β -chain variable (V_β) domain having CDR1, CDR2, and CDR3 amino acid sequences according to SEQ ID NOS:45-47, respectively,
wherein the binding protein is capable of specifically binding to a KVLEYVIKV (SEQ ID NO.:123):human leukocyte antigen (HLA)-A*0201 complex on a cell surface independent of CD8 or in the absence of CD8, and wherein the modified cell is a CD4⁺ T cell and further comprises a heterologous polynucleotide encoding at least an extracellular portion of a CD8 co-receptor.
2. The modified cell of claim 1, wherein the encoded binding protein is capable of specifically binding to a KVLEYVIKV (SEQ ID NO.:123):HLA-A*0201 complex with a K_d less than or equal to about 10^{-8} M.
3. The modified cell of claim 1 or 2, wherein the V_β domain of the encoded binding protein is derived from a TRBV30 allele, a TRBV29 allele, or a TRBV9 allele.
4. The modified cell of any one of claims 1-3, wherein the V_α domain of the encoded binding protein is derived from a TRAV38-1 allele, a TRAV34 allele, a TRAV16 allele, or a TRAV5 allele.
5. An isolated modified cell comprising a heterologous polynucleotide encoding a binding protein, wherein the encoded binding protein comprises a TCR V_α domain having CDR1, CDR2, and CDR3 amino acid sequences of SEQ ID NOS.:48-50, respectively, and a TCR V_β domain having CDR1, CDR2, and CDR3 amino acid sequences of SEQ ID NOS.:45-47, respectively,

wherein the binding protein is capable of specifically binding to a KVLEYVIKV (SEQ ID NO.: 123):human leukocyte antigen (HLA)-A *0201 complex on a cell surface independent of CD8 or in the absence of CD8,

wherein

(i) the encoded $V\alpha$ domain comprises an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO.: 19, and

(ii) the encoded $V\beta$ domain comprises an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO.: 17, and

wherein the modified cell is a CD4⁺ T cell and further comprises a heterologous polynucleotide encoding at least an extracellular portion of a CD8 co-receptor.

6. The modified cell of any one of claims 1-5, wherein the encoded binding protein is a pre-binding protein and wherein the $V\alpha$ domain comprises or consists of the amino acid sequence according to SEQ ID NO.:19.

7. The modified cell of any one of claims 1-6, wherein the encoded binding protein is a pre-binding protein and wherein $V\beta$ domain comprises or consists of the amino acid sequence according to SEQ ID NO.:17.

8. The modified cell of any one of claims 1-7, wherein the encoded binding protein further comprises a TCR α -chain constant ($C\alpha$) domain having at least 90% sequence identity to an amino acid sequence according to SEQ ID NO.:20.

9. The modified cell of any one of claims 1-8, wherein the encoded binding protein further comprises a TCR β -chain constant ($C\beta$) domain comprising an amino acid sequence with at least 90% sequence identity to the amino acid sequence according to SEQ ID NO.:18.

10. The modified cell of any one of claims 1-9, wherein the encoded binding protein is a pre-binding protein and comprises a $V\alpha$ domain comprising or consisting of SEQ ID NO.:

19, a V_{β} domain comprising or consisting of SEQ ID NO.: 17, a C_{α} domain comprising or consisting of SEQ ID NO.: 20, and a C_{β} domain comprising or consisting of SEQ ID NO.: 18.

11. The modified cell of any one of claims 1-10, wherein the binding protein is a T cell receptor (TCR), an antigen-binding fragment of a TCR, or a chimeric antigen receptor.

12. The modified cell of claim 11, wherein the TCR, the chimeric antigen receptor, or the antigen-binding fragment of the TCR is chimeric, humanized or human.

13. The modified cell of claim 11 or 12, wherein the antigen-binding fragment of the TCR comprises a single chain TCR (scTCR).

14. The modified cell of any one of claims 11-13, wherein the binding protein is a chimeric antigen receptor, optionally a TCR-CAR.

15. The modified cell of claim 11 or 12, wherein the binding protein is a TCR.

16. The modified cell of any one of claims 1-15, wherein the modified cell is a human CD4⁺ cell.

17. The modified cell of claim 16, wherein the modified cell comprises a chromosomal gene knockout of a PD-1 gene; a LAG3 gene; a TIM3 gene; a CTLA4 gene; an HLA component gene; a TCR component gene, or any combination thereof.

18. The modified cell of claim 17, wherein the chromosomal gene knockout comprises a knockout of an HLA component gene selected from an $\alpha 1$ macroglobulin gene, an $\alpha 2$ macroglobulin gene, an $\alpha 3$ macroglobulin gene, a $\beta 1$ microglobulin gene, or a $\beta 2$ microglobulin gene.

19. The modified cell of claim 18, wherein the chromosomal gene knockout comprises a knockout of a TCR component gene selected from a TCR α variable region gene, a TCR β variable region gene, a TCR constant region gene, or a combination thereof.

20. The modified cell of any one of claims 1-19, wherein the polynucleotide encoding the binding protein and/or the polynucleotide encoding the at least an extracellular portion of a CD8 co-receptor is codon-optimized for expression by the modified cell.

21. The modified cell of any one of claims 1-20, wherein the encoded at least an extracellular portion of a CD8 co-receptor comprises a full-length CD8 co-receptor α -chain and a full-length CD8 co-receptor β -chain.

22. The modified cell of any one of claims 1-21, wherein the encoded at least an extracellular portion of a CD8 co-receptor comprises:

- (i) the CD8 co-receptor α -chain amino acid sequence of SEQ ID NO.:143; and
- (ii) the CD8 co-receptor β -chain amino acid sequence of any one of SEQ ID NOs.:144-145.

23. The modified cell of any one of claims 1-22, wherein the encoded at least an extracellular portion of a CD8 co-receptor comprises:

- (i) the CD8 co-receptor α -chain amino acid sequence of SEQ ID NO.:143; and
- (ii) the CD8 co-receptor β -chain amino acid sequence of SEQ ID NO.:144.

24. A composition comprising a modified cell of any one of claims 1-23 and a pharmaceutically acceptable carrier, diluent, or excipient.

25. A combination comprising (i) the composition of claim 24, comprising at least about 30% modified CD4⁺ T cells, with (ii) a composition comprising at least about 30% CD8⁺ T cells modified to encode a binding protein capable of binding to a KVLEYVIKV (SEQ ID NO.:123);human leukocyte antigen (HLA)-A*0201 complex on a cell surface, wherein the

modified CD4⁺ T cells and the modified CD8⁺ T cells are present in the combination in about a 1:1 ratio.

26. The combination of claim 25, wherein the combination contains substantially no naïve T cells.

27. An isolated polynucleotide encoding:

(1) a binding protein having a TCR V α domain and a TCR V β domain, wherein:

(i) the encoded V α domain comprises CDR1, CDR2, and CDR3 amino acid sequences of SEQ ID NOS.:48-50, respectively, and the encoded V β domain comprises CDR1, CDR2, and CDR3 amino acid sequences of SEQ ID NOS.:45-47, respectively;

(ii) the encoded binding protein is capable of specifically binding to a KVLEYVIKV (SEQ ID NO.:123): human leukocyte antigen (HLA)-A*0201 complex on a cell surface; and

(2) at least an extracellular portion of a CD8 co-receptor.

28. The isolated polynucleotide of claim 27, wherein:

(i) the encoded V α domain comprises an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO.: 19, and

(ii) the encoded V β domain comprises an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO.: 17.

29. The isolated polynucleotide of claim 28, wherein the encoded binding protein is a pre-binding protein, wherein the V α domain comprises or consists of the amino acid sequence of SEQ ID NO.: 19 and the encoded V β domain comprises or consists of the amino acid sequence of SEQ ID NO.: 17

30. The isolated polynucleotide of any one of claims 27-29, wherein:

the V $_{\alpha}$ -encoding polynucleotide comprises a nucleotide sequence having at least 80% identity to SEQ ID NO:90 and the V $_{\beta}$ -encoding polynucleotide comprises a nucleotide sequence having at least 80% identity to SEQ ID NO:88.

31. The isolated polynucleotide of any one of claims 27-30, wherein the encoded binding protein further comprises a TCR α -chain constant (C $_{\alpha}$) domain, a TCR β -chain constant (C $_{\beta}$) domain, or both.

32. The isolated polynucleotide of claim 31, comprising:

- (a) a C $_{\alpha}$ -domain-encoding polynucleotide having at least 80% identity to SEQ ID NO:91; and/or
- (b) a C $_{\beta}$ -domain-encoding polynucleotide having at least 80% identity to SEQ ID NO:89.

33. The isolated polynucleotide of any one of claims 27-32, wherein the encoded at least an extracellular portion of a CD8 co-receptor comprises a full-length CD8 co-receptor α -chain and a full-length CD8 co-receptor β -chain.

34. The isolated polynucleotide of any one of claims 27-33, wherein the encoded at least an extracellular portion of a CD8 co-receptor comprises:

- (i) the CD8 co-receptor α -chain amino acid sequence of SEQ ID NO.:143; and
- (ii) the CD8 co-receptor β -chain amino acid sequence of any one of SEQ ID NOs.:144-145.

35. The isolated polynucleotide of any one of claims 27-34, wherein the encoded at least an extracellular portion of a CD8 co-receptor comprises:

- (i) the CD8 co-receptor α -chain amino acid sequence of SEQ ID NO.:143; and
- (ii) the CD8 co-receptor β -chain amino acid sequence of SEQ ID NO.:144.

36. The isolated polynucleotide of any one of claims 27-35, further comprising a polynucleotide encoding a self-cleaving peptide disposed between a TCR α -chain- encoding polynucleotide and a TCR β -chain-encoding polynucleotide.

37. The isolated polynucleotide of claim 36, wherein the polynucleotide encoding a self-cleaving peptide comprises or consists of a nucleotide sequence of any one of SEQ ID NOS.:128-132.

38. The isolated polynucleotide of claim 36, wherein the polynucleotide encodes a self-cleaving peptide comprising or consisting of an amino acid sequence of any one of SEQ ID NOS.:124-127.

39. An expression vector, comprising a polynucleotide of any one of claims 27-38 operably linked to an expression control sequence.

40. The expression vector according to claim 39, wherein the vector is capable of delivering the polynucleotide to a host cell.

41. The expression vector of claim 40, wherein the host cell is a hematopoietic progenitor cell or a human immune system cell.

42. The expression vector of claim 41, wherein the human immune system cell is a CD4⁺ T cell, a CD8⁺ T cell, a CD4⁻ CD8⁻ double negative T cell, a $\gamma\delta$ T cell, a natural killer cell, a dendritic cell, or any combination thereof.

43. The expression vector of claim 42, wherein the T cell is a naïve T cell, a central memory T cell, an effector memory T cell, or any combination thereof.

44. The expression vector of any one of claims 39-43, wherein the vector is a viral vector.

45. The expression vector of claim 44, wherein the viral vector is a lentiviral vector or a γ -retroviral vector.

46. Use of a modified cell of any one of claims 1-23, a composition of claim 24, or a combination of any one of claims 25-26 in the manufacture of a medicament for treating a hyperproliferative disorder associated with MAGE-A1 expression in a human subject.

47. The use of claim 46, wherein the hyperproliferative disorder is a hematological malignancy or a solid cancer.

48. The use of claim 47, wherein the hematological malignancy is selected from acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), chronic myelogenous leukemia (CML), chronic eosinophilic leukemia (CEL), myelodysplastic syndrome (MDS), non-Hodgkin's lymphoma (NHL), or multiple myeloma (MM).

49. The use of claim 48, wherein the solid cancer is selected from non-small cell lung cancer (NSCLC), triple negative breast cancer (TNBC), ovarian cancer, malignant melanoma, colon cancer, colorectal adenocarcinoma, colorectal cancer, biliary cancer, bladder cancer, bone and soft tissue carcinoma, brain tumor, breast cancer, cervical cancer, desmoid tumor, embryonal cancer, endometrial cancer, esophageal cancer, gastric cancer, gastric adenocarcinoma, glioblastoma multiforme, gynecological tumor, head and neck squamous cell carcinoma, hepatic cancer, lung cancer, mesothelioma, osteosarcoma, pancreatic cancer, pancreatic ductal adenocarcinoma, primary astrocytic tumor, primary thyroid cancer, prostate cancer, renal cancer, renal cell carcinoma, rhabdomyosarcoma, skin cancer, soft tissue sarcoma, testicular germ-cell tumor, urothelial cancer, uterine sarcoma, or uterine cancer.

50. The use of any one of claims 46-49, wherein the modified cell is capable of promoting an antigen-specific T cell response against a MAGE-A1 in a class I HLA-restricted manner.

51. The use of claim 50, wherein the class I HLA-restricted response is transporter-associated with antigen processing (TAP)-independent.

52. The use of claim 51, wherein the antigen-specific T cell response comprises at least one of a CD4⁺ helper T lymphocyte (Th) response and a CD8⁺ cytotoxic T lymphocyte (CTL) response.

53. The use of claim 52, wherein the CTL response is directed against a cell having aberrant MAGE-A1 expression.

54. The use of any one of claims 46-53, wherein the modified cell is modified *ex vivo*.

55. The use of claim 54, wherein the modified cell is an allogeneic cell, a syngeneic cell, or an autologous cell.

56. The use of any one of claims 46-55, wherein the modified cell is a modified human CD4⁺ T cell.

57. The use of any one of claims 46-56, further comprising that a CD8⁺ T cell is to be administered to the subject that is capable of specifically binding to a MAGE-A1 peptide:HLA complex on a cell surface.

58. The use of any one of claims 46-57, wherein the modified cell is to be administered parenterally.

59. The use of any one of claims 46-58, wherein the method comprises that a plurality of doses of the modified cell is to be administered to the subject.

60. The use of claim 59, wherein the plurality of doses are to be administered at intervals between administrations of about two to about four weeks.

61. The use of any one of claims 46-60, wherein the modified cell is to be administered to the subject at a dose of about 10^7 cells/ m^2 to about 10^{11} cells/ m^2 .

62. The use of any one of claims 46-61, wherein the method further comprises that a cytokine is to be administered.

63. The use of claim 62, wherein the cytokine is IL-2, IL-15, IL-21, or any combination thereof.

64. The use of claim 63, wherein the cytokine is IL-2 and is to be administered concurrently or sequentially with the modified cell.

65. The use of claim 64, wherein the cytokine is to be administered sequentially, provided that the subject was administered the modified cell at least three or four times before cytokine administration.

66. The use of any one of claims 63-65, wherein the cytokine is IL-2 and is to be administered subcutaneously.

67. The use of any one of claims 46-66, wherein immunosuppressive therapy is to be further received by the subject.

68. The use of claim 67, wherein the immunosuppressive therapy is selected from calcineurin inhibitors, corticosteroids, microtubule inhibitors, low dose of a mycophenolic acid prodrug, an immune checkpoint inhibitor, or any combination thereof.

69. The use of any one of claims 46-68, wherein a stimulatory immune checkpoint molecule is to be administered to the subject.

70. The use of any one of claims 46-69, wherein the subject has received a non-myeloablative or a myeloablative hematopoietic cell transplant.

71. The use of claim 70, wherein the subject is to be administered the modified cell at least three months after the non-myeloablative hematopoietic cell transplant.

72. The use of claim 71, wherein the subject is to be administered the modified cell at least two months after the myeloablative hematopoietic cell transplant.

73. The use of any one of claims 46-72, wherein the subject has been administered one or more of a DNA hypomethylation agent and a HDAC inhibitor.

74. The use of any one of claims 46-73, wherein (i) an anti-PD-1 antibody or an anti-PD-L1 antibody is to be further received by the subject and/or (ii) the subject has received an anti-PD-1 antibody or an anti-PD-L1 antibody.

75. The use of claim wherein the anti-PD-L1 antibody comprises atezolizumab.