

Several peptides of the WT1 protein are known to be tumor-associated antigen peptides that are HLA-A*0201-restricted antigens.

There is a clear need for alternative highly WT1 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 provides a T cell receptor (TCR) comprising: a TCR α -chain variable (V_α) domain, and a TCR β -chain variable (V_β) domain, wherein the V_α domain comprises the CDR1 α , CDR2 α , and CDR3 α amino acid sequences set forth in SEQ ID NOs.:194, 195, and 196 or 12, respectively, wherein the V_β domain comprises the CDR1 β , CDR2 β , and CDR3 β amino acid sequences set forth in SEQ ID NOs.:197, 198, and 199 or 1, respectively, and wherein the TCR binds to a VLDFAPPGA (SEQ ID NO.:59):human leukocyte antigen (HLA) complex.

In another aspect, the present disclosure provides an isolated polynucleotide encoding the TCR according to the disclosure.

In another aspect, the present disclosure provides an expression vector, comprising the polynucleotide of the disclosure operably linked to an expression control sequence.

In another aspect, the present disclosure provides a host cell, comprising the polynucleotide according to the disclosure or the expression vector according to the disclosure, wherein the host cell expresses on its cell surface the TCR encoded by the polynucleotide.

In another aspect, the present disclosure provides a composition, comprising the host cell of the disclosure, and a pharmaceutically acceptable carrier, diluent, or excipient.

In another aspect, the present disclosure provides a host cell of the disclosure or the composition of the disclosure for use in a method of treating a hyperproliferative or proliferative disorder in a subject, wherein the host cell or composition is to be

administered to the subject, wherein the hyperproliferative or proliferative disorder is a hematological malignancy or a solid cancer.

In another aspect, the present disclosure provides a unit dose form comprising the host cell according to the disclosure or the composition of the disclosure, wherein the host cell is at a dose of about 10^7 cells/m² to about 10^{11} cells/m².

In another aspect, the present disclosure provides a TCR of the disclosure, the polynucleotide of the disclosure, the expression vector of the disclosure, the host cell of the disclosure, or the composition of the disclosure, or any combination thereof, for use in therapy.

In another aspect, the present disclosure provides a use of the TCR of the disclosure, the polynucleotide of the disclosure, the expression vector of the disclosure, the host cell of the disclosure, or the composition of the disclosure, or any combination thereof, in the manufacture of a medicament for treating of a proliferative or hyperproliferative disorder associated with Wilms tumor protein 1 (WT1) expression or overexpression, wherein the proliferative or hyperproliferative disorder associated with Wilms tumor protein 1 (WT1) expression or overexpression is a hematological malignancy or a solid cancer.

BRIEF DESCRIPTION OF THE FIGURES

Figures 1A and 1B show how WT1₃₇-specific TCRs were identified by a high-throughput sequencing-based strategy. (A) Schematic of initial sequencing-based strategy for identifying TCR clonotypes associated with high WT1₃₇₋₄₅ peptide/MHC tetramer-binding. (B) Enrichment in sort populations versus percentage of total population is shown, with selected TCR highlighted. All TCRs indicated by black circles were synthesized and evaluated for antigen-specificity (27 total).

Figure 2 shows results of functional evaluation of TCRs that bind high levels of CD8 independent (CD8i) tetramer. TCR constructs were expressed in Jurkat cells that lack endogenous TCR α/β chains. Tetramer staining versus CD3 expression for each TCR is shown (CD3 expression directly correlates with transgenic TCR surface expression).

Figures 3A – 3C show additional WT1₃₇-specific TCRs were identified by a modified high-throughput sequencing-based strategy using a CD8 independent (CD8i) tetramer. (A) Schematic of modified sequencing-based strategy for identifying TCR clonotypes associated with high CD8-independent WT1₃₇ peptide/MHC tetramer-binding. (B) Enrichment in original sort populations versus percentage of total population as compared with (C) a similar analysis when CD8i tetramer is used is shown. An additional 14 TCRs were selected based on decreased surface CD3 levels and CD8i tetramer binding. All TCRs indicated by shaded (diagonal line pattern) circles were synthesized and evaluated for antigen-specificity.

Figure 4 shows CD8i tetramer binding of selected WT1₃₇ TCRs. TCR constructs were expressed in Jurkat cells that lack endogenous TCR α/β chains.

What is claimed is:

1. A T cell receptor (TCR) comprising:
a TCR α -chain variable (V_α) domain, and a TCR β -chain variable (V_β) domain, wherein the V_α domain comprises the CDR1 α , CDR2 α , and CDR3 α amino acid sequences set forth in SEQ ID NOs.:194, 195, and 196 or 12, respectively, wherein the V_β domain comprises the CDR1 β , CDR2 β , and CDR3 β amino acid sequences set forth in SEQ ID NOs.:197, 198, and 199 or 1, respectively, and
wherein the TCR binds to a VLDFAPPGA (SEQ ID NO.:59):human leukocyte antigen (HLA) complex.
2. The TCR of claim 1, which specifically binds to a VLDFAPPGA (SEQ ID NO.:59):HLA complex.
3. The TCR of claim 1 or 2, which specifically binds to a SEQ ID NO.:59 HLA complex with an IFN γ production pEC₅₀ of 8.5 or higher.
4. The TCR of any one of claims 1-3, wherein the TCR further specifically binds to the VLDFAPPGA (SEQ ID NO.:59):human leukocyte antigen (HLA) complex on a cell surface independent of CD8 or in the absence of CD8.
5. The TCR of any one of claims 1-4, wherein the HLA comprises HLA-A*201.
6. The TCR according to any one of claims 1-5, wherein:
 - (a) the V_α domain comprises an amino acid sequence that has at least about 90% sequence identity to the amino acid sequence set forth in SEQ ID NO.:34; and/or
 - (b) the V_β domain comprises an amino acid sequence that has at least about 90% sequence identity to the amino acid sequence set forth in SEQ ID NO.:23.

7. The TCR according to any one of claims 1-6, wherein the V_α domain comprises or consists of the amino acid sequence set forth in SEQ ID NO.:253 or 34, and/or

wherein the V_β domain comprises or consists of the amino acid sequence set forth in SEQ ID NO.:242 or 23.

8. The TCR according to any one of claims 1-7, wherein:

(i) the V_α domain comprises or consists of the amino acid sequence set forth in SEQ ID NO.:253; and

(ii) the V_β domain comprises or consists of the amino acid sequence set forth in SEQ ID NO.:242.

9. The TCR according to any one of claims 1-8, wherein:

(i) the V_α domain comprises or consists of the amino acid sequence set forth in SEQ ID NO.:34; and

(ii) the V_β domain comprises or consists of the amino acid sequence set forth in SEQ ID NO.:23.

10. The TCR according to any one of claims 1-9, wherein the TCR further comprises:

(i) a TCR α -chain constant domain having at least about 90% sequence identity to the amino acid sequence of SEQ ID NO.:47; and/or

(ii) a TCR β -chain constant domain having at least about 90% sequence identity to the amino acid sequence of SEQ ID NO.:45 or 46.

11. The TCR according to any one of claims 1-10, wherein the TCR comprises a TCR α -chain comprising a V_α domain and an α -chain constant domain, wherein:

(a) the V_α domain has at least about 90% sequence identity to the amino acid sequence set forth in SEQ ID NO.:34, and the α -chain constant domain has at least about 98% sequence identity to the amino acid sequence of SEQ ID NO.:47;

(b) the V_α domain comprises the amino acid sequence set forth in SEQ ID NO.: 253, and the α -chain constant domain comprises the amino acid sequence of SEQ ID NO.:47; or

(c) the V_α domain consists of the amino acid sequence set forth in SEQ ID NO.: 253, and the α -chain constant domain consists the amino acid sequence of SEQ ID NO.:47.

12. The TCR according to any one of claims 1-11, wherein the TCR comprises a TCR β -chain comprising a V_β domain and a β -chain constant domain, wherein the V_β domain has at least about 90% sequence identity to the amino acid sequence set forth in SEQ ID NO.:23, and the β -chain constant domain comprises the amino acid sequence of SEQ ID NO.:45 or has at least about 95% sequence identity to the amino acid sequence of SEQ ID NO.:46.

13. The TCR according to any one of claims 1-12, wherein the TCR comprises a TCR β -chain comprising a V_β domain and an β -chain constant domain, wherein:

(a) the V_β domain comprises the amino acid sequence set forth in SEQ ID NO.:242, and the β -chain constant domain comprises the amino acid sequence of SEQ ID NO.:45 or 46;

(b) the V_β domain consists of the amino acid sequence set forth in SEQ ID NO.: 242, and the β -chain constant domain consists of the amino acid sequence of SEQ ID NO.:45 or 46;

or(c) the V_β domain comprises or consists of the amino acid sequence set forth in SEQ ID NO.:23, and the β -chain constant domain comprises or consists of the amino acid sequence of SEQ ID NO.:46.

14. The TCR of any one of claims 1-13, comprising:

(i) a TCR α -chain comprising a V_α comprising SEQ NO.:253 and a C_α comprising SEQ ID NO.:47; and

(ii) a TCR β -chain comprising a V_β comprising SEQ NO.:242 and a C_β comprising SEQ ID NO.:46.

15. The TCR of any one of claims 1-14, wherein the TCR comprises a scTCR or a CAR.

16. An isolated polynucleotide encoding the TCR according to any one of claims 1-15.
17. The isolated polynucleotide of claim 16, wherein:
- (i) the polynucleotide is codon optimized for a host cell of interest;
 - (ii) the polynucleotide has at least 75% identity to, comprises, or consists of the nucleotide sequence set forth in any one of SEQ ID NOs.:64, 75, 86, 97, 108, 109, 110, 111, 122, 133, and 144;
 - (iii) the polynucleotide has at least 95% identity to, comprises, or consists of the polynucleotide sequence set forth in SEQ ID NO.: 155; or
 - (iv) the polynucleotide encodes an amino acid sequence having at least 95% identity to, comprising, or consisting of the amino acid sequence set forth in SEQ ID NO.:48.
18. The polynucleotide according to claim 16 or 17, further comprising:
- (i) a polynucleotide encoding a polypeptide that comprises an extracellular portion of a CD8 co-receptor α chain, wherein, optionally, the encoded polypeptide is or comprises a CD8 co-receptor α chain;
 - (ii) a polynucleotide encoding a polypeptide that comprises an extracellular portion of a CD8 co-receptor β chain, wherein, optionally, the encoded polypeptide is or comprises a CD8 co-receptor β chain; and/or
 - (iii) a polynucleotide of (i) and a polynucleotide of (ii), and optionally further comprising a polynucleotide encoding a self-cleaving peptide disposed between the polynucleotide of (i) and the polynucleotide of (ii).
19. The polynucleotide of claim 18, further comprising a polynucleotide that encodes a self-cleaving peptide and is disposed between:
- (1) the polynucleotide encoding a binding protein and the polynucleotide encoding a polypeptide comprising an extracellular portion of a CD8 co-receptor α chain; and/or
 - (2) the polynucleotide encoding a binding protein and the polynucleotide encoding a polypeptide comprising an extracellular portion of a CD8 co-receptor β chain.

20. The polynucleotide of claim 18 or 19, comprising, operably linked in-frame:

- (i) (pnCD8 α)-(pnSCP₁)-(pnCD8 β)-(pnSCP₂)-(pnTCR);
- (ii) (pnCD8 β)-(pnSCP₁)-(pnCD8 α)-(pnSCP₂)-(pnTCR);
- (iii) (pnTCR)-(pnSCP₁)-(pnCD8 α)-(pnSCP₂)-(pnCD8 β);
- (iv) (pnTCR)-(pnSCP₁)-(pnCD8 β)-(pnSCP₂)-(pnCD8 α);
- (v) (pnCD8 α)-(pnSCP₁)-(pnTCR)-(pnSCP₂)-(pnCD8 β); or
- (vi) (pnCD8 β)-(pnSCP₁)-(pnTCR)-(pnSCP₂)-(pnCD8 α),

wherein pnCD8 α is the polynucleotide encoding a polypeptide that comprises an extracellular portion of a CD8 co-receptor α chain,

wherein pnCD8 β is the polynucleotide encoding a polypeptide that comprises an extracellular portion of a CD8 co-receptor α chain,

wherein pnTCR is the polynucleotide encoding a TCR,

and wherein pnSCP₁ and pnSCP₂ are each independently a polynucleotide encoding a self-cleaving peptide, wherein the polynucleotides and/or the encoded self-cleaving peptides are optionally the same or different.

21. The polynucleotide of any one of claims 18-20, wherein the encoded TCR comprises a TCR α chain and a TCR β chain, wherein the polynucleotide comprises a polynucleotide encoding a self-cleaving peptide disposed between the polynucleotide encoding a TCR α chain and the polynucleotide encoding a TCR β chain.

22. The polynucleotide of claim 21, comprising, operably linked in-frame:

- (i) (pnCD8 α)-(pnSCP₁)-(pnCD8 β)-(pnSCP₂)-(pnTCR β)-(pnSCP₃)-(pnTCR α);
- (ii) (pnCD8 β)-(pnSCP₁)-(pnCD8 α)-(pnSCP₂)-(pnTCR β)-(pnSCP₃)-(pnTCR α);
- (iii) (pnCD8 α)-(pnSCP₁)-(pnCD8 β)-(pnSCP₂)-(pnTCR α)-(pnSCP₃)-(pnTCR β);
- (iv) (pnCD8 β)-(pnSCP₁)-(pnCD8 α)-(pnSCP₂)-(pnTCR α)-(pnSCP₃)-(pnTCR β);
- (v) (pnTCR β)-(pnSCP₁)-(pnTCR α)-(pnSCP₂)-(pnCD8 α)-(pnSCP₃)-(pnCD8 β);
- (vi) (pnTCR β)-(pnSCP₁)-(pnTCR α)-(pnSCP₂)-(pnCD8 β)-(pnSCP₃)-(pnCD8 α);
- (vii) (pnTCR α)-(pnSCP₁)-(pnTCR β)-(pnSCP₂)-(pnCD8 α)-(pnSCP₃)-(pnCD8 β); or
- (viii) (pnTCR α)-(pnSCP₁)-(pnTCR β)-(pnSCP₂)-(pnCD8 β)-(pnSCP₃)-(pnCD8 α),

wherein pnCD8 α is the polynucleotide encoding a polypeptide that comprises an extracellular portion of a CD8 co-receptor α chain,

wherein pnCD8 β is the polynucleotide encoding a polypeptide that comprises an extracellular portion of a CD8 co-receptor β chain,

wherein pnTCR α is the polynucleotide encoding a TCR α chain,

wherein pnTCR β is the polynucleotide encoding a TCR β chain,

and wherein pnSCP₁, pnSCP₂, and pnSCP₃ are each independently a polynucleotide encoding a self-cleaving peptide, wherein the polynucleotides and/or the encoded self-cleaving peptides are optionally the same or different.

23. An expression vector, comprising the polynucleotide of any one of claims 16-22 operably linked to an expression control sequence.

24. The expression vector according to claim 23, wherein the vector is capable of delivering the polynucleotide to a host cell, wherein, optionally:

- (a) the host cell is a hematopoietic progenitor cell; or
- (b) the host cell is a human immune system cell, wherein, further optionally, the 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, wherein, still further optionally, the T cell is a naïve T cell, a central memory T cell, an effector memory T cell, or any combination thereof.

25. The expression vector according to claim 23 or 24, wherein the vector is a viral vector, wherein, optionally, the viral vector is an adenoviral vector, a lentiviral vector, or a γ -retroviral vector.

26. A host cell, comprising the polynucleotide according to any one of claims 16-22 or the expression vector according to any one of claims 23-25, wherein the host cell expresses on its cell surface the TCR encoded by the polynucleotide.

27. The host cell according to claim 26, wherein the host cell is a hematopoietic progenitor cell or a human immune system cell.

28. The host cell according to claim 27, wherein:

(i) the 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 natural killer T cell, dendritic cell, or any combination thereof, wherein, optionally, the combination, if present, comprises a CD4⁺ T cell and a CD8⁺ T cell; or

(ii) the immune system cell is a T cell, wherein, optionally, the T cell is a naïve T cell, a central memory T cell, an effector memory T cell, or any combination thereof, wherein, further optionally, the TCR has higher surface expression on the T cell as compared to an endogenous TCR.

29. The host cell according to claim 26, wherein the host cell is a T cell.

30. The host cell according to claim 26, wherein the host cell is a CD4⁺ T cell, a CD8⁺ T cell, or both.

31. The host cell of claim 29 or 30, wherein the host cell is capable of killing:

- (i) a tumor cell of breast cancer cell line MDA-MB-468;
- (ii) a tumor cell of pancreatic adenocarcinoma cell line PANC-1;
- (iii) a tumor cell of breast cancer cell line MDA-MB-231;
- (iv) a tumor cell of myelogenous leukemia cell line K562 expressing an HLA-A2, wherein, optionally, the HLA-A2 comprises HLA-A*201;
- (v) a tumor cell of colon carcinoma cell line RKO expressing an HLA-A2, wherein, optionally, the HLA-A2 comprises HLA-A*201; or
- (vi) any combination of tumor cells of (i)-(v),

when the host cell and the tumor cell are both present in a sample, wherein, optionally, the host cell is capable of killing the tumor cell when the host cell and the tumor cell are present in the sample at a ratio of 32:1 host cell:tumor cell, 16:1, 8:1, 4:1, 2:1, or 1.5:1.

32. A composition, comprising the host cell of any one of claims 26-31 and a pharmaceutically acceptable carrier, diluent, or excipient.

33. The composition of claim 30, wherein the host cell is a T cell.

34. The composition of claim 30, wherein the composition comprises a host CD4+ T cell, a host CD8+ T cell, or both.

35. The host cell of any one of claims 29-31 or the composition of claim 33 or 34 for use in a method of treating a hyperproliferative or proliferative disorder in a subject, wherein the host cell or composition is to be administered to the subject,

wherein the hyperproliferative or proliferative disorder is a hematological malignancy or a solid cancer.

36. The host cell or composition for use according to claim 35, wherein the hematological malignancy is selected from acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), chronic myelogenous leukemia (CML), chronic eosinophilic leukemia (CEL), myelodysplastic syndrome (MDS), non-Hodgkin's lymphoma (NHL), or multiple myeloma (MM), and/or the solid cancer is selected from breast cancer, ovarian cancer, lung cancer, biliary cancer, bladder cancer, bone and soft tissue carcinoma, brain tumor, cervical cancer, colon cancer, colorectal adenocarcinoma, colorectal 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, mesothelioma, malignant melanoma, 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.

37. The host cell or composition for use according to claim 35 or 36, wherein:

(a) the subject is human; and/or

(b) the host cell is modified *ex vivo*, wherein, optionally, the host cell is an allogeneic cell, a syngeneic cell, or an autologous cell to the subject; and/or

(c) the host cell is formulated for parenteral administration, wherein, optionally, the host cell is formulated for administration to the subject at a dose of about 10^7 cells/m² to about 10^{11} cells/m²; and/or

(d) the host cell or composition is formulated for administering a plurality of doses of the host cell to the subject, wherein, optionally, the plurality of doses are formulated for administration at intervals between administrations of about two to about four weeks; and/or

(e) the subject is further receiving immunosuppressive therapy, wherein, optionally, the immunosuppressive therapy is selected from calcineurin inhibitors, corticosteroids, microtubule inhibitors, low dose of a mycophenolic acid prodrug, or any combination thereof; and/or

(f) the subject has received a non-myeloablative or a myeloablative hematopoietic cell transplant; wherein, optionally, the subject is to be administered the host cell at least three months after the non-myeloablative hematopoietic cell transplant; wherein, further optionally, the subject is to be administered the host cell at least two months after the myeloablative hematopoietic cell transplant; and/or

(g) the subject has received or is receiving an immune checkpoint inhibitor and/or an agonist of a stimulatory immune checkpoint agent.

38. A unit dose form comprising the host cell according to any one of claims 29-31 or the composition of claim 33 or 34, wherein the host cell is at a dose of about 10^7 cells/m² to about 10^{11} cells/m².

39. The TCR of any one of claims 1-15, the polynucleotide of any one of claims 16-22, the expression vector of any one of claims 23-25, the host cell of any one of claims 29-31, or the composition of claim 33 or 34, or any combination thereof, for use in therapy.

40. Use of the TCR of any one of claims 1-15, the polynucleotide of any one of claims 16-22, the expression vector of any one of claims 23-25, the host cell of any one of claims

29-31, or the composition of claim 33 or 34, or any combination thereof, in the manufacture of a medicament for treating of a proliferative or hyperproliferative disorder associated with Wilms tumor protein 1 (WT1) expression or overexpression, wherein the proliferative or hyperproliferative disorder associated with Wilms tumor protein 1 (WT1) expression or overexpression is a hematological malignancy or a solid cancer.

41. The use of claim 40, wherein:

(i) the hematological malignancy is selected from acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), chronic myelogenous leukemia (CML), chronic eosinophilic leukemia (CEL), myelodysplastic syndrome (MDS), non-Hodgkin's lymphoma (NHL), or multiple myeloma (MM); and/or

(ii) the solid cancer is selected from breast cancer, ovarian cancer, lung cancer, biliary cancer, bladder cancer, bone and soft tissue carcinoma, brain tumor, cervical cancer, colon cancer, colorectal adenocarcinoma, colorectal 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, mesothelioma, malignant melanoma, 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.