

ANTI-HPV T CELL RECEPTORS AND ENGINEERED CELLS

CLAIM OF PRIORITY

This application claims the benefit of international Application No. PCT/CN2020/074366,
5 filed on February 5, 2020. The entire contents of the foregoing application are incorporated
herein by reference.

TECHNICAL FIELD

The present disclosure relates to T cells receptors that recognize or bind to a cancer
10 antigen, engineered cells, and cell-based therapies.

BACKGROUND

Cancer is one of the most widespread cellular anomalies caused by biological and
environmental factors, such as age, gender, genetic mutations, environmental exposure such as
15 UV radiation, occupational risk factors, carcinogens, asbestos, radioactive materials, and viral
infections (e.g., HPV, EBV, HBV, HCV, HTLV-1 and KSHV) (Margaret E *et al.*, “Viruses
Associated With Human Cancer,” *Biochimica et Biophysica Acta*. 1782:127–150 (2008)).
Particularly, some cancers (e.g., cervical cancer) are primarily caused by virus (e.g., human
papilloma virus, HPV) infection (Stanley *et al.*, “HPV: From Infection To Cancer.” *Biochemical*
20 *Society Transactions*: 35: part 6 (2007)).

Despite advancement in treatments such as chemotherapy, the efficacy of various
treatments for cancers, including HPV associated cancers, is relatively poor. Accordingly, there
exists an unmet need for effective therapies for cancers.

25 SUMMARY

The present disclosure is related to T cells receptors that recognize or bind tumor antigen
human papilloma virus (HPV) E6, genetically engineered cells, and cell therapies for treating
HPV associated cancers.

In one aspect, the disclosure is related to a T cell receptor (TCR) or antigen-binding
30 fragment thereof, comprising an alpha chain comprising a variable alpha (Va) region and a beta
chain comprising a variable beta (Vb) region. In some embodiments, the Va region comprises a

complementarity determining region 1 (CDR-1), a complementarity determining region 2 (CDR-2), and a complementarity determining region 3 (CDR-3); in some embodiments, the Va region CDR-1 comprises an amino acid sequence that is identical to a selected Va region CDR-1 amino acid sequence, the Va region CDR-2 comprises an amino acid sequence that is identical to a
5 selected Va region CDR-2 amino acid sequence, and the Va region CDR-3 comprises an amino acid sequence that is identical to a selected Va region CDR-3 amino acid sequence; and in some embodiments, the Vb region comprises a complementarity determining region 1 (CDR-1), a complementarity determining region 2 (CDR-2), and a complementarity determining region 3 (CDR-3); in some embodiments, the Vb region CDR-1 comprises an amino acid sequence that is
10 identical to a selected Vb region CDR-1 amino acid sequence, the Vb region CDR-2 comprises an amino acid sequence that is identical to a selected Vb region CDR-2 amino acid sequence, and the Vb region CDR-3 comprises an amino acid sequence that is identical to a selected Vb region CDR-3 amino acid sequence. In some embodiments, the selected Va region CDR-1, CDR-2, and CDR-3 amino acid sequences and the selected Vb region CDR-1, CDR-2, and CDR-
15 3 amino acid sequences are one of the following:

- (1) the selected Va region CDR-1, CDR-2, and CDR-3 amino acid sequences are set forth in SEQ ID NOs: 5, 6, and 7, respectively, and the selected Vb region CDR-1, CDR-2, and CDR-3 amino acid sequences are set forth in SEQ ID NOs: 8, 9, and 10, respectively;
- (2) the selected Va region CDR-1, CDR-2, and CDR-3 amino acid sequences are set forth in
20 SEQ ID NOs: 27, 28, and 29, respectively, and the selected Vb region CDR-1, CDR-2, and CDR-3 amino acid sequences are set forth in SEQ ID NOs: 30, 31, and 32, respectively;
- (3) the selected Va region CDR-1, CDR-2, and CDR-3 amino acid sequences are set forth in SEQ ID NOs: 33, 34, and 35, respectively, and the selected Vb region CDR-1, CDR-2, and CDR-3 amino acid sequences are set forth in SEQ ID NOs: 36, 37, and 38, respectively;
- 25 (4) the selected Va region CDR-1, CDR-2, and CDR-3 amino acid sequences are set forth in SEQ ID NOs: 39, 40, and 41, respectively, and the selected Vb region CDR-1, CDR-2, and CDR-3 amino acid sequences are set forth in SEQ ID NOs: 42, 43, and 44, respectively.

In some embodiments, the Va region comprises CDR-1, CDR-2, and CDR-3 with the amino acid sequences set forth in SEQ ID NOs: 5, 6, and 7, respectively; and the Vb region
30 comprises CDR-1, CDR-2, and CDR-3 with the amino acid sequences set forth in SEQ ID NOs: 8, 9, and 10, respectively.

In some embodiments, the Va region comprises the amino acid sequence set forth in any of SEQ ID NO: 1, or an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto; and the Vb region comprises the amino acid sequence set forth in any of SEQ ID NO: 2, or an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.

In some embodiments, the Va region comprises CDR-1, CDR-2, and CDR-3 with the amino acid sequences set forth in SEQ ID NOs: 27, 28, and 29, respectively; and the Vb region comprises CDR-1, CDR-2, and CDR-3 with the amino acid sequences set forth in SEQ ID NOs: 30, 31, and 32, respectively.

In some embodiments, the Va region comprises the amino acid sequence set forth in any of SEQ ID NO: 45, or an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto; and the Vb region comprises the amino acid sequence set forth in any of SEQ ID NO: 46 or an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.

In some embodiments, the Va region comprises CDR-1, CDR-2, and CDR-3 with the amino acid sequences set forth in SEQ ID NOs: 33, 34 and 35, respectively; and the Vb region comprises CDR-1, CDR-2, and CDR-3 with the amino acid sequences set forth in SEQ ID NOs: 36, 37, and 38, respectively.

In some embodiments, the Va region comprises the amino acid sequence set forth in any of SEQ ID NO: 47, or an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto; and the Vb region comprises the amino acid sequence set forth in any of SEQ ID NO: 48 or an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.

In some embodiments, the Va region comprises CDR-1, CDR-2, and CDR-3 with the amino acid sequences set forth in SEQ ID NOs: 39, 40, and 41, respectively; and the Vb region comprises CDR-1, CDR-2, and CDR-3 with the amino acid sequences set forth in SEQ ID NOs: 42, 43, and 44, respectively.

In some embodiments, the Va region comprises the amino acid sequence set forth in any

of SEQ ID NO: 49, or an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto; and the Vb region comprises the amino acid sequence set forth in any of SEQ ID NO: 50 or an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.

In some embodiments, the alpha chain comprises a mouse alpha chain constant region, and the beta chain comprises a mouse beta chain constant region.

In some embodiments, the alpha chain comprises the amino acid sequence set forth in any of SEQ ID NO: 15, or an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto; and the beta chain comprises the amino acid sequence set forth in any of SEQ ID NO: 16, or an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.

In some embodiments, the alpha chain comprises the amino acid sequence set forth in any of SEQ ID NO: 51, or an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto; and the beta chain comprises the amino acid sequence set forth in any of SEQ ID NO: 52, or an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.

In some embodiments, the alpha chain comprises the amino acid sequence set forth in any of SEQ ID NO: 53, or an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto; and the beta chain comprises the amino acid sequence set forth in any of SEQ ID NO: 54, or an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.

In some embodiments, the alpha chain comprises the amino acid sequence set forth in any of SEQ ID NO: 55, or an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto; and the beta chain comprises the amino acid sequence set forth in any of SEQ ID NO: 56, or an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.

In some embodiments, the TCR or antigen-binding fragment thereof binds to or recognizes a peptide epitope of E6 (SEQ ID NO: 19) that is presented by a major histocompatibility complex (MHC) molecule.

In some embodiments, the MHC molecule is an HLA-A2 molecule.

5 In some embodiments,, the TCR or antigen-binding fragment thereof, when expressed on the surface of a T cell, stimulates cytotoxic activity against a target cancer cell.

In some embodiments, the target cancer cell comprises HPV DNA sequences or expresses E6.

10 In one aspect, the disclosure is related to a T cell receptor (TCR) or antigen-binding fragment thereof, comprising an alpha chain comprising a variable alpha (Va) region and a beta chain comprising a variable beta (Vb) region; In some embodiments, the Va region comprises a complementarity determining region 1 (CDR1), a complementarity determining region 2 (CDR2), and a complementarity determining region 3 (CDR3), and the Vb region comprises a CDR1, a CDR2, and a CDR3; in some embodiments,

15 (1) the Va region CDR1, CDR2, and CDR3 are identical to complementarity determining regions 1, 2, and 3 in SEQ ID NO: 1, respectively, and the Vb region CDR1, CDR2, and CDR3 are identical to complementarity determining regions 1, 2, and 3 in SEQ ID NO: 2, respectively;

(2) the Va region CDR1, CDR2, and CDR3 are identical to complementarity determining regions 1, 2, and 3 in SEQ ID NO: 45, respectively, and the Vb region CDR1, CDR2, and CDR3
20 are identical to complementarity determining regions 1, 2, and 3 in SEQ ID NO: 46, respectively;

(3) the Va region CDR1, CDR2, and CDR3 are identical to complementarity determining regions 1, 2, and 3 in SEQ ID NO: 47, respectively, and the Vb region CDR1, CDR2, and CDR3 are identical to complementarity determining regions 1, 2, and 3 in SEQ ID NO: 48, respectively;
or

25 (4) the Va region CDR1, CDR2, and CDR3 are identical to complementarity determining regions 1, 2, and 3 in SEQ ID NO: 49, respectively, and the Vb region CDR1, CDR2, and CDR3 are identical to complementarity determining regions 1, 2, and 3 in SEQ ID NO: 50, respectively.

In one aspect, the disclosure is related to a vector comprising a nucleic acid encoding the TCR or antigen-binding fragment thereof as described herein.

30 In some embodiments, the vector is an expression vector, a viral vector, a retroviral vector, or a lentiviral vector.

In one aspect, the disclosure is related to a vector including: a) a first nucleic acid sequence encoding a TCR alpha chain comprising an alpha chain variable region of a human anti-E6 TCR and an alpha chain constant region; and b) a second nucleic acid sequence encoding a TCR beta chain comprising a beta chain variable region of the human anti-E6 TCR and a beta chain constant region.

In some embodiments, the alpha chain constant region is a human TCR alpha chain constant region and the beta chain constant region is a human TCR beta chain constant region.

In some embodiments, the alpha chain constant region is a mouse TCR alpha chain constant region and the beta chain constant region is a mouse TCR beta chain constant region.

In some embodiments, the first nucleic acid sequence and the second nucleic acid sequence are linked by a linker sequence. In some embodiments, the linker sequence is a P2A sequence.

In some embodiments, the first nucleic acid sequence comprises a sequence set forth in SEQ ID NO: 17, or a nucleic acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto; and the second nucleic acid sequence comprises a sequence set forth in SEQ ID NO: 18, or a nucleic acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto. In some embodiments, the first nucleic acid sequence comprises a sequence set forth in SEQ ID NO: 57, or a nucleic acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto; and the second nucleic acid sequence comprises a sequence set forth in SEQ ID NO: 58, or a nucleic acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto; In some embodiments, the first nucleic acid sequence comprises a sequence set forth in SEQ ID NO: 59, or a nucleic acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto; and the second nucleic acid sequence comprises a sequence set forth in SEQ ID NO: 60, or a nucleic acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto. In some embodiments, the first nucleic acid sequence comprises a sequence set forth in SEQ ID NO: 61, or a nucleic acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto; and the second nucleic acid sequence comprises a sequence set forth in SEQ ID NO: 62, or a nucleic acid sequence that has

at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto.

In some embodiments, the vector as described herein further includes a third nucleic acid sequence encoding a checkpoint inhibitor.

5 In some embodiments, the checkpoint inhibitor is an antibody.

In some embodiments, the checkpoint inhibitor is an anti-PD-1 antibody scFv, or an anti-CTLA4 antibody scFv.

In some embodiments, the antibody comprises a heavy chain variable domain comprising an amino acid sequence that is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%,
10 98%, or 99% identical to SEQ ID NO: 11; and a light chain variable domain comprising an amino acid sequence that is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 12.

In some embodiments, the third nucleic acid sequence comprises a nucleic acid sequence that is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical
15 to SEQ ID NO: 13; and a nucleic acid sequence that is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 14.

In some embodiments, the vector is an expression vector, a viral vector, a retroviral vector, or a lentiviral vector. In some embodiments, the retroviral vector is pMP71.

In some embodiments, the vector comprises (1) a nucleic acid sequence that is at least
20 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 20; (2) a nucleic acid sequence that is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 63; (3) a nucleic acid sequence that is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 64; or
(4) a nucleic acid sequence that is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%,
25 97%, 98%, or 99% identical to SEQ ID NO: 65.

In one aspect, the disclosure is related to an engineered cell comprising the vector as described herein.

In one aspect, the disclosure is related to an engineered cell, comprising the TCR or antigen-binding fragment thereof as described herein.

30 In some embodiments, the TCR or antigen binding fragment thereof is heterologous to the cell.

In some embodiments, the engineered cell is a cell line.

In some embodiments, the engineered cell is a primary cell obtained from a subject (e.g., a human subject).

In some embodiments, the engineered cell is a T cell. In some embodiments, the T-cell is isolated from a human subject. In some embodiments, the T cell is CD8+. In some embodiments, the T cell is CD4+.

In one aspect, the disclosure is related to a method for producing the engineered cell, comprising introducing the vector as described herein into a cell *in vitro* or *ex vivo*.

In some embodiments, the vector is a viral vector and the introducing is carried out by transduction.

In one aspect, the disclosure is related to a method of treating a disease or a disorder, comprising administering the engineered cell as described herein to a subject having a disease or disorder associated with HPV.

In some embodiments, the disease or disorder associated with HPV is a cancer. In some embodiments, the cancer is a cancer of the head and neck, uterine cervix, oropharynx, anus, anal canal, anorectum, vagina, vulva, or penis.

In one aspect, the disclosure is related to a method of treating a tumor in a subject, the method comprising administering to the subject in need thereof (a) an engineered T cell, comprising: a nucleic acid encoding a TCR or antigen-binding fragment thereof that specifically binds to an HPV antigen; and (b) a checkpoint inhibitor.

In some embodiments, the tumor is an HPV-induced tumor.

In one aspect, the disclosure is related to a TCR or antigen-binding fragment thereof that cross competes with the TCR or antigen-binding fragment thereof as described herein.

In one aspect, the disclosure provides a method of administering to a patient an effective amount of genetically engineered anti-cancer human T cells to treat a disease, disorder or condition in the patient, wherein the genetically engineered anti-cancer human T cells express an anti-tumor T-cell receptor for HPV E6 antigen. In some embodiments, the alpha chain of an anti-tumor T-cell receptor is encoded by the nucleotide sequence of SEQ ID NO: 3, and the beta chain is encoded by the nucleotide sequence of SEQ ID NO: 4. In some embodiments, the alpha chain of an anti-tumor T-cell receptor is encoded by the nucleotide sequence of SEQ ID NO: 78, and the beta chain is encoded by the nucleotide sequence of SEQ ID NO: 79. In some

embodiments, the alpha chain of an anti-tumor T-cell receptor is encoded by the nucleotide sequence of SEQ ID NO: 80, and the beta chain is encoded by the nucleotide sequence of SEQ ID NO: 81. In some embodiments, the alpha chain of an anti-tumor T-cell receptor is encoded by the nucleotide sequence of SEQ ID NO: 82, and the beta chain is encoded by the nucleotide sequence of SEQ ID NO: 83. In some embodiments, the alpha chain of the anti-tumor T cell receptor has a variable alpha ($V\alpha$) region comprising an amino acid sequence of SEQ ID NO: 1 and beta chain of the anti-tumor human T cell receptor has a variable beta ($V\beta$) region comprising an amino acid sequence of SEQ ID NO: 2. In some embodiments, the alpha chain of the anti-tumor T cell receptor has a variable alpha ($V\alpha$) region comprising an amino acid sequence of SEQ ID NO: 45, and beta chain of the anti-tumor human T cell receptor has a variable beta ($V\beta$) region comprising an amino acid sequence of SEQ ID NO: 46. In some embodiments, the alpha chain of the anti-tumor T cell receptor has a variable alpha ($V\alpha$) region comprising an amino acid sequence of SEQ ID NO: 47, and beta chain of the anti-tumor human T cell receptor has a variable beta ($V\beta$) region comprising an amino acid sequence of SEQ ID NO: 48. In some embodiments, the alpha chain of the anti-tumor T cell receptor has a variable alpha ($V\alpha$) region comprising an amino acid sequence of SEQ ID NO: 49, and beta chain of the anti-tumor human T cell receptor has a variable beta ($V\beta$) region comprising an amino acid sequence of SEQ ID NO: 50. The disease, disorder or condition can be cancer-related, such as cervical cancer, head and neck cancer, oropharyngeal cancers, anal cancer, penile cancer, vaginal cancer and vulvar cancer.

In one aspect, the disclosure also provides a T cell receptor. In some instances, the alpha chain of the anti-tumor human T cell receptor has a sequence of a variable alpha ($V\alpha$) region (SEQ ID NO: 1) and the beta chain of the anti-tumor human T cell receptor has a sequence of variable beta ($V\beta$) region (SEQ ID NO: 2). In some instances, the alpha chain of the anti-tumor human T cell receptor has a sequence of a variable alpha ($V\alpha$) region (SEQ ID NO: 45) and the beta chain of the anti-tumor human T cell receptor has a sequence of variable beta ($V\beta$) region (SEQ ID NO: 46). In some instances, the alpha chain of the anti-tumor human T cell receptor has a sequence of a variable alpha ($V\alpha$) region (SEQ ID NO: 47) and the beta chain of the anti-tumor human T cell receptor has a sequence of variable beta ($V\beta$) region (SEQ ID NO: 48). In some instances, the alpha chain of the anti-tumor human T cell receptor has a sequence of a variable alpha ($V\alpha$) region (SEQ ID NO: 49) and the beta chain of the anti-tumor human T cell receptor

has a sequence of variable beta ($V\beta$) region (SEQ ID NO: 50). In some instances, the variable alpha ($V\alpha$) region of the anti-tumor human T cell receptor is fused to a constant region of a mouse T-cell receptor alpha chain. In some instances, the variable beta ($V\beta$) region of the anti-tumor human T cell receptor is fused to a constant region of a mouse T-cell receptor beta chain.

5 In one aspect, the disclosure provides an engineered T cell comprising a nucleic acid encoding a genetically engineered antigen receptor that specifically binds to human papilloma virus (HPV) antigen E6.

In one aspect, the disclosure further provides a method for patient-specific T-cell therapy, wherein a gene is engineered into patient-specific T cells and delivered back into the patient as a
10 therapeutic agent.

The present disclosure further provides a method of diagnosing a disease/condition, wherein the condition can include cancer, and wherein the disease can be diagnosed by analyzing the complex formed as a result of the contact between the T-cell receptors with the sample from the patient/mammal to be diagnosed, and wherein the complex can be detected by any of the
15 means well-known in the art. In some embodiments, the results can be used to determine whether the cell therapy will be effective.

The present disclosure further provides a pharmaceutical composition comprising an engineered T cell receptor (TCR) or an antigen-binding fragment thereof having antigenic specificity for human papillomavirus (HPV) antigen E6 and a pharmaceutically acceptable
20 carrier.

The present disclosure also provides a vector system for transfecting cells with a chimeric gene, wherein the vector system includes nucleic acid sequences encoding the variable region of the alpha chain of a human anti-E6 TCR, nucleic acid sequences encoding the variable region of the beta chain of same human anti-E6 TCR and a linker sequence.

25 As used herein, the term “about” refers to a measurable value such as an amount, a time duration, and the like, and encompasses variations of $\pm 20\%$, $\pm 10\%$, $\pm 5\%$, $\pm 1\%$, $\pm 0.5\%$ or $\pm 0.1\%$ from the specified value.

As used herein, the term “HPV antigen” refers to a polypeptide molecule derived from human papilloma virus (HPV). In some embodiments, the HPV is HPV1, HPV2, HPV3, HPV4, HPV6, HPV10, HPV11, HPV16, HPV18, HPV26, HPV27, HPV28, HPV29, HPV30, HPV31,
30 HPV33, HPV34, HPV35, HPV39, HPV40, HPV41, HPV42, HPV43, HPV45, HPV49, HPV51,

HPV52, HPV54, HPV55, HPV56, HPV57, HPV58, HPV59, HPV68, or HPV69. Particularly, the HPV can be a high risk HPV, for example, HPV16, HPV18, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, HPV59, HPV68, or HPV69. In some embodiments, the HPV polypeptide molecule is selected from E6.

5 As used herein, the term "peripheral blood cells" refers to cells normally found in the peripheral blood including, but is not limited to, eosinophils, neutrophils, T cells, monocytes, K cells, granulocytes, and B cells.

 As used herein, the term "genetically engineered cell" or "genetically modified cell" refers to a cell with a modification of a nucleic acid sequence in the cell, including, but not
10 limited to, a cell having a insertion, deletion, substitution, or modification of one or more nucleotides in its genome, and a cell with an exogenous nucleic acid sequence (e.g., a vector), wherein the exogenous nucleic acid sequence is not necessarily integrated into the genome.

 As used herein, the term "cancer" or "cancer cell" refers to the cells dividing in an uncontrolled manner. Examples of such cells include cells having an abnormal state or condition
15 characterized by rapidly proliferating cell growth. The term is meant to include cancerous growths, e.g., tumors; oncogenic processes, metastatic tissues, and malignantly transformed cells, tissues, or organs, irrespective of histopathologic type or stage of invasiveness. The cancer cells can form the solid tumors or the excessive tumor cells in blood (e.g., hematologic cancer). Alternatively or additionally it can include all types of cancerous growths or oncogenic processes,
20 metastatic tissues or malignantly transformed cells, tissues, or organs, irrespective of histopathologic type or stage of invasiveness. Examples of solid tumors include malignancies, e.g., sarcomas, adenocarcinomas, and carcinomas, of the various organ systems, such as those affecting liver, lung, breast, lymphoid, gastrointestinal (e.g., colon), genitourinary tract (e.g., renal, urothelial cells), prostate and pharynx. Adenocarcinomas include malignancies such as
25 most colon cancers, rectal cancer, renal-cell carcinoma, liver cancer, non-small cell carcinoma of the lung, cancer of the small intestine and cancer of the esophagus. Examples of cancers that can be treated by the methods described herein include e.g., bone cancer, pancreatic cancer, skin cancer (e.g., melanoma), cancer of the head or neck, cutaneous or intraocular malignant melanoma, uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region, stomach
30 cancer, testicular cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva,

Hodgkin Disease, non-Hodgkin lymphoma, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, chronic or acute leukemias including acute myeloid leukemia, chronic myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, lymphocytic lymphoma, cancer of the bladder, cancer of the kidney or ureter, carcinoma of the renal pelvis, neoplasm of the central nervous system (CNS), primary CNS lymphoma, tumor angiogenesis, spinal axis tumor, brain stem glioma, pituitary adenoma, Kaposi's sarcoma, epidermoid cancer, squamous cell cancer, and/or T-cell lymphoma.

10 As used herein, the term "HPV associated cancer" refers to cancers that are associated or caused by HPV infection.

As used herein, the term "vector" refers to a vehicle by which a polynucleotide sequence (e.g. a foreign gene) can be introduced into a host cell, in order to obtain the desired gene expression of the introduced nucleotide sequence. Cloning vectors can include e.g., plasmids, phages, viruses, etc. Most popular type of vector is a "plasmid", which refers to a closed circular double stranded DNA loop into which additional DNA segments comprising gene of interest can be ligated. Another type of vector is a viral vector, in which a nucleic acid construct to be transported is ligated into the viral genome. Viral vectors are capable of autonomous replication in a host cell into which they are introduced or may integrate themselves into the genome of a host cell and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "recombinant expression vectors" or simply "expression vectors". In some embodiments, the vectors are viral vectors (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses).

25 As used herein, a "subject" is a mammal, such as a human or a non-human animal. In some embodiments, the subject, e.g., patient, to whom the cells, cell populations, or compositions are administered is a mammal, typically a primate, such as a human. In some embodiments, the primate is a monkey or an ape. The subject can be male or female and can be any suitable age, including infant, juvenile, adolescent, adult, and geriatric subjects. In some 30 embodiments, the subject is a non-primate mammal, such as a dog, a cat, a horse, a rodent, a rat, or a mouse.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Methods and materials are described herein for use in the present invention; other, suitable methods and materials known in the art can also be used. The materials, methods, and examples are illustrative only and not intended to be limiting. All publications, patent applications, patents, sequences, database entries, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control.

Other features and advantages of the invention will be apparent from the following detailed description and figures, and from the claims.

DESCRIPTION OF THE DRAWINGS

Exemplary embodiments are illustrated in referenced figures. It is intended that the embodiments and figures disclosed herein are to be considered illustrative rather than restrictive.

FIG. 1 is a schematic diagram showing a pMP71 retroviral vector construct. P2A encodes a 2A self-cleaving peptide; Va encodes the variable region of the alpha chain of a human anti-E6 TCR; Vb encodes the variable region of the beta chain of the same human anti-E6 TCR; Ca encodes the constant region of the mouse TCR alpha chain; Cb encodes the constant region of the mouse TCR beta chain. Ψ indicates packaging sequences on viral RNA. 5'LTR and 3'LTR are long terminal repeats.

FIG. 2A shows the expression of TCR in non-transduced human primary T cells. NT is a non-transduced control. After 48 hours of culture, expression of the recombinant TCR was detected by staining mouse TCR beta chain. A viable CD3⁺ lymphocyte gating strategy was used.

FIG. 2B shows the expression of E202 TCR in human primary T cells transduced with the E202 construct. After 48 hours of culture, expression of the recombinant TCR was detected by staining mouse TCR beta chain. A viable CD3⁺ lymphocyte gating strategy was used.

FIG. 3A is a graph showing the intracellular IFN- γ expression of non-transduced human T cells upon antigen-specific stimulation. NT is a non-transduced control. The non-transduced human T cells were co-cultured overnight with target cells expressing the HPV E6 antigen at 1:1 effector-to-target ratio. The T cells were then collected and the intracellular IFN- γ expression was determined by flow cytometry.

FIG. 3B is a graph showing the intracellular IFN- γ expression of E202 TCR-T cells upon antigen-specific stimulation. TCR-T cells were co-cultured overnight with target cells expressing the HPV E6 antigen at 1:1 effector-to-target ratio. The T cells were then collected and the intracellular IFN- γ expression was determined by flow cytometry.

5 FIG. 4 is a graph showing the activation curve of TCR-T cells containing the E202 TCR. TCR-T cells were co-cultured overnight with different concentrations of HPV peptide-pulsed APCs at 1:1 effector-to-target ratio. The T cells was then collected and the intracellular IFN- γ expression was measured to determine the EC50.

10 FIG. 5 is a graph showing the relation of the specific killing percentage of target cells by E202 TCR-T cells and E:T ratios. Target cells expressing HPV E6 antigen were pre-stained with CFSE and then co-cultured overnight with TCR-T cells at 1:2, 1:1, 3:1 and 10:1 effector-to-target ratios. The cytotoxicity of T cells against target cells was measured by 7-AAD staining. NT is a non-transduced control.

15 FIG. 6A is a schematic diagram showing a pMP71 retroviral vector construct. P2A encodes a 2A self-cleaving peptide; Va encodes the variable region of the alpha chain of a human anti-HPV16 E6 TCR; Vb encodes the variable region of the beta chain of the same human anti-HPV16 E6 TCR; Ca encodes the constant region of the mouse TCR alpha chain; Cb encodes the constant region of the mouse TCR beta chain. Ψ indicates packaging sequences on viral RNA. 5'LTR and 3'LTR are long terminal repeats.

20 FIG. 6B is a schematic diagram showing a pMP71 retroviral vector construct (E202P03). P2A and T2A encodes 2A self-cleaving peptides; Va encodes the variable region of the alpha chain of a human anti-HPV16 E6 TCR; Vb encodes the variable region of the beta chain of the same human anti-HPV16 E6 TCR; Ca encodes the constant region of the mouse TCR alpha chain; Cb encodes the constant region of the mouse TCR beta chain; VH encodes the variable
25 region of the heavy chain of an immune checkpoint inhibitor (ICI); VL encodes the variable region of the light chain of the immune checkpoint inhibitor (ICI). VH and VL are linked with a GS linker. Ψ indicates packaging sequences on viral RNA. 5'LTR and 3'LTR are long terminal repeats.

30 FIG. 7A shows the expression of TCR in non-transduced human primary T cells. NT is a non-transduced control. After 13 days of culture, expression of the recombinant TCR was detected by staining mouse TCR beta chain. A viable CD3⁺ lymphocyte gating strategy was used.

FIG. 7B shows the expression of E202 TCR in human primary T cells transduced with the E202 construct. After 13 days of culture, expression of the recombinant TCR was detected by staining mouse TCR beta chain. A viable CD3⁺ lymphocyte gating strategy was used.

FIG. 7C shows the expression of E202P03 TCR in human primary T cells transduced
5 with the E202P03 construct. After 13 days of culture, expression of the recombinant TCR was detected by staining mouse TCR beta chain. A viable CD3⁺ lymphocyte gating strategy was used.

FIG. 8A is a graph showing the intracellular IFN- γ expression of non-transduced human T cells upon antigen-specific stimulation. NT is a non-transduced control. The non-transduced human T cells were co-cultured overnight with target cells expressing the HPV E6 antigen at 1:1
10 effector-to-target ratio. The T cells were then collected and the intracellular IFN- γ expression was determined by flow cytometry.

FIG. 8B is a graph showing the intracellular IFN- γ expression of E202 TCR-T cells upon antigen-specific stimulation. TCR-T cells were co-cultured overnight with target cells expressing the HPV E6 antigen at 1:1 effector-to-target ratio. The T cells were then collected and the
15 intracellular IFN- γ expression was determined by flow cytometry.

FIG. 8C is a graph showing the intracellular IFN- γ expression of E202P03 TCR-T cells upon antigen-specific stimulation. TCR-T cells were co-cultured overnight with target cells expressing the HPV E6 antigen at 1:1 effector-to-target ratio. The T cells were then collected and the intracellular IFN- γ expression was determined by flow cytometry.

FIG. 9 is a histogram showing the IFN- γ expression of E202 and E202P03 TCR-T cells upon antigen-specific stimulation in the cell culture supernatant. TCR-T cells were co-cultured overnight with target cells expressing the HPV E6 antigen at the indicated effector-to-target ratios. The cell culture supernatant was then collected and the IFN- γ expression in the supernatant was measured. NT is a non-transduced control.

FIG. 10A is a graph showing the CD107a expression of non-transduced human T cells upon antigen-specific stimulation. NT is a non-transduced control. The non-transduced human T cells were co-cultured overnight with target cells expressing the HPV E6 antigen at 1:1 effector-to-target ratio. The T cells were then collected and the CD107a expression was determined in the CD8 subpopulation by flow cytometry.

FIG. 10B is a graph showing the CD107a expression of E202 TCR-T cells upon antigen-specific stimulation. TCR-T cells were co-cultured overnight with target cells expressing the

HPV E6 antigen at 1:1 effector-to-target ratio. The T cells were then collected and the CD107a expression was determined in the CD8 subpopulation by flow cytometry.

FIG. 10C is a graph showing the CD107a expression of E202P03 TCR-T cells upon antigen-specific stimulation. TCR-T cells were co-cultured overnight with target cells expressing the HPV E6 antigen at 1:1 effector-to-target ratio. The T cells were then collected and the CD107a expression was determined in the CD8 subpopulation by flow cytometry.

FIG. 10D is a graph showing the CD107a expression of non-transduced human T cells upon antigen-specific stimulation. NT is a non-transduced control. The non-transduced human T cells were co-cultured overnight with target cells expressing the HPV E6 antigen at 1:1 effector-to-target ratio. The T cells were then collected and the CD107a expression was determined in the CD4 subpopulation by flow cytometry.

FIG. 10E is a graph showing the CD107a expression of E202 TCR-T cells upon antigen-specific stimulation. TCR-T cells were co-cultured overnight with target cells expressing the HPV E6 antigen at 1:1 effector-to-target ratio. The T cells were then collected and the CD107a expression was determined in the CD4 subpopulation by flow cytometry.

FIG. 10F is a graph showing the CD107a expression of E202P03 TCR-T cells upon antigen-specific stimulation. TCR-T cells were co-cultured overnight with target cells expressing the HPV E6 antigen at 1:1 effector-to-target ratio. The T cells were then collected and the CD107a expression was determined in the CD4 subpopulation by flow cytometry.

FIG. 11 is a graph showing the relation of the specific killing percentage of target cells by E202 or E202P03 TCR-T cells and E:T ratios. Target cells expressing HPV E6 antigen were pre-stained with CFSE and then co-cultured overnight with TCR-T cells at 1:1, 3:1, and 10:1 effector-to-target ratios. The cytotoxicity of T cells against target cells was measured by 7-AAD staining. NT is a non-transduced control.

FIG. 12 is a histogram showing the anti-PD-1 scFv expression in the cell culture supernatant. Either E202 or E202P03 TCR-T cells were seeded in a 24-well plate at 3×10^6 /ml for 48 hours. The cell culture supernatant was then collected and the anti-PD-1 expression in the supernatant was determined.

FIG. 13A shows the expression of TCR in untransduced (UT) human PBMCs. Expression of the recombinant TCR was detected by staining mouse TCR beta chain. A viable CD3+ lymphocyte gating strategy was used.

FIG. 13B shows the expression of E203 TCR in human PBMCs transduced with the E203 construct. 5 days post transduction, expression of the recombinant TCR was detected by staining mouse TCR beta chain. A viable CD3+ lymphocyte gating strategy was used.

FIG. 13C shows the expression of E204 TCR in human PBMCs transduced with the E204 construct. 5 days post transduction, expression of the recombinant TCR was detected by staining mouse TCR beta chain. A viable CD3+ lymphocyte gating strategy was used.

FIG. 13D shows the expression of E205 TCR in human PBMCs transduced with the E205 construct. 5 days post transduction, expression of the recombinant TCR was detected by staining mouse TCR beta chain. A viable CD3+ lymphocyte gating strategy was used.

FIG. 14A shows the intracellular IFN- γ expression of non-transduced (UT) human CD4+ T cells without antigen-specific stimulation.

FIG. 14B shows the intracellular IFN- γ expression of non-transduced (UT) human CD4+ T cells that were co-cultured overnight with Ca Ski E6/E7 cells at 1: 2 effector-to-target cell ratio. The intracellular IFN- γ expression was determined by flow cytometry.

FIG. 14C shows the intracellular IFN- γ expression of non-transduced (UT) human CD4+ T cells that were co-cultured overnight with Ca Ski cells at 1: 2 effector-to-target cell ratio. The intracellular IFN- γ expression was determined by flow cytometry.

FIG. 14D shows the intracellular IFN- γ expression of non-transduced (UT) human CD4+ T cells that were co-cultured overnight with 293T cells at 1: 2 effector-to-target cell ratio. The intracellular IFN- γ expression was determined by flow cytometry.

FIG. 14E shows the intracellular IFN- γ expression of human CD4+ E203 TCR-T cells without antigen-specific stimulation.

FIG. 14F shows the intracellular IFN- γ expression of human CD4+ E203 TCR-T cells that were co-cultured overnight with Ca Ski E6/E7 cells at 1: 2 effector-to-target cell ratio. The intracellular IFN- γ expression was determined by flow cytometry.

FIG. 14G shows the intracellular IFN- γ expression of human CD4+ E203 TCR-T cells that were co-cultured overnight with Ca Ski cells at 1: 2 effector-to-target cell ratio. The intracellular IFN- γ expression was determined by flow cytometry.

FIG. 14H shows the intracellular IFN- γ expression of human CD4+ E203 TCR-T cells that were co-cultured overnight with 293T cells at 1: 2 effector-to-target cell ratio. The intracellular IFN- γ expression was determined by flow cytometry.

FIG. 14I shows the intracellular IFN- γ expression of human CD4⁺ E204 TCR-T cells without antigen-specific stimulation.

FIG. 14J shows the intracellular IFN- γ expression of human CD4⁺ E204 TCR-T cells that were co-cultured overnight with Ca Ski E6/E7 cells at 1: 2 effector-to-target cell ratio. The intracellular IFN- γ expression was determined by flow cytometry.

FIG. 14K shows the intracellular IFN- γ expression of human CD4⁺ E204 TCR-T cells that were co-cultured overnight with Ca Ski cells at 1: 2 effector-to-target cell ratio. The intracellular IFN- γ expression was determined by flow cytometry.

FIG. 14L shows the intracellular IFN- γ expression of human CD4⁺ E204 TCR-T cells that were co-cultured overnight with 293T cells at 1: 2 effector-to-target cell ratio. The intracellular IFN- γ expression was determined by flow cytometry.

FIG. 14M shows the intracellular IFN- γ expression of human CD4⁺ E205 TCR-T cells without antigen-specific stimulation.

FIG. 14N shows the intracellular IFN- γ expression of human CD4⁺ E205 TCR-T cells that were co-cultured overnight with Ca Ski E6/E7 cells at 1: 2 effector-to-target cell ratio. The intracellular IFN- γ expression was determined by flow cytometry.

FIG. 14O shows the intracellular IFN- γ expression of human CD4⁺ E205 TCR-T cells that were co-cultured overnight with Ca Ski cells at 1: 2 effector-to-target cell ratio. The intracellular IFN- γ expression was determined by flow cytometry.

FIG. 14P shows the intracellular IFN- γ expression of human CD4⁺ E205 TCR-T cells that were co-cultured overnight with 293T cells at 1: 2 effector-to-target cell ratio. The intracellular IFN- γ expression was determined by flow cytometry.

FIG. 15A shows the intracellular IFN- γ expression of non-transduced (UT) human CD8⁺ T cells without antigen-specific stimulation.

FIG. 15B shows the intracellular IFN- γ expression of non-transduced (UT) human CD8⁺ T cells that were co-cultured overnight with Ca Ski E6/E7 cells at 1: 2 effector-to-target cell ratio. The intracellular IFN- γ expression was determined by flow cytometry.

FIG. 15C shows the intracellular IFN- γ expression of non-transduced (UT) human CD8⁺ T cells that were co-cultured overnight with Ca Ski cells at 1: 2 effector-to-target cell ratio. The intracellular IFN- γ expression was determined by flow cytometry.

FIG. 15D shows the intracellular IFN- γ expression of non-transduced (UT) human CD8+ T cells that were co-cultured overnight with 293T cells at 1: 2 effector-to-target cell ratio. The intracellular IFN- γ expression was determined by flow cytometry.

FIG. 15E shows the intracellular IFN- γ expression of human CD8+ E203 TCR-T cells
5 without antigen-specific stimulation.

FIG. 15F shows the intracellular IFN- γ expression of human CD8+ E203 TCR-T cells that were co-cultured overnight with Ca Ski E6/E7 cells at 1: 2 effector-to-target cell ratio. The intracellular IFN- γ expression was determined by flow cytometry.

FIG. 15G shows the intracellular IFN- γ expression of human CD8+ E203 TCR-T cells
10 that were co-cultured overnight with Ca Ski cells at 1: 2 effector-to-target cell ratio. The intracellular IFN- γ expression was determined by flow cytometry.

FIG. 15H shows the intracellular IFN- γ expression of human CD8+ E203 TCR-T cells that were co-cultured overnight with 293T cells at 1: 2 effector-to-target cell ratio. The intracellular IFN- γ expression was determined by flow cytometry.

FIG. 15I shows the intracellular IFN- γ expression of human CD8+ E204 TCR-T cells
15 without antigen-specific stimulation.

FIG. 15J shows the intracellular IFN- γ expression of human CD8+ E204 TCR-T cells that were co-cultured overnight with Ca Ski E6/E7 cells at 1: 2 effector-to-target cell ratio. The intracellular IFN- γ expression was determined by flow cytometry.

FIG. 15K shows the intracellular IFN- γ expression of human CD8+ E204 TCR-T cells
20 that were co-cultured overnight with Ca Ski cells at 1: 2 effector-to-target cell ratio. The intracellular IFN- γ expression was determined by flow cytometry.

FIG. 15L shows the intracellular IFN- γ expression of human CD8+ E204 TCR-T cells that were co-cultured overnight with 293T cells at 1: 2 effector-to-target cell ratio. The
25 intracellular IFN- γ expression was determined by flow cytometry.

FIG. 15M shows the intracellular IFN- γ expression of human CD8+ E205 TCR-T cells without antigen-specific stimulation.

FIG. 15N shows the intracellular IFN- γ expression of human CD8+ E205 TCR-T cells that were co-cultured overnight with Ca Ski E6/E7 cells at 1: 2 effector-to-target cell ratio. The
30 intracellular IFN- γ expression was determined by flow cytometry.

FIG. 15O shows the intracellular IFN- γ expression of human CD8⁺ E205 TCR-T cells that were co-cultured overnight with Ca Ski cells at 1: 2 effector-to-target cell ratio. The intracellular IFN- γ expression was determined by flow cytometry.

FIG. 15P shows the intracellular IFN- γ expression of human CD8⁺ E205 TCR-T cells that were co-cultured overnight with 293T cells at 1: 2 effector-to-target cell ratio. The intracellular IFN- γ expression was determined by flow cytometry.

FIG. 16A shows the absolute killing efficacy of Ca Ski E6/E7 cells by untransduced (UT), E203, E204, and E205 TCR-T cells. CellTraceTM CFSE-labeled Ca Ski E6/E7 cells and CellTraceTM Violet-labeled 293T cells were mixed and co-cultured overnight with TCR-T cells at a 0:1, 0.4: 1, 2:1, or 10:1 effector-to-target cell ratio. Beads were added as a reference for flow cytometry analysis.

FIG. 16B shows the competitive killing efficacy of Ca Ski E6/E7 cells by untransduced (UT), E203, E204, and E205 TCR-T cells. CellTraceTM CFSE-labeled Ca Ski E6/E7 cells and CellTraceTM Violet-labeled 293T cells were mixed and co-cultured overnight with TCR-T cells at a 0:1, 0.4: 1, 2:1, or 10:1 effector-to-target cell ratio.

FIG. 17A shows the activation curve of CD8⁺ TCR-T cells containing the E203 TCR. The intracellular IFN- γ expression was measured to determine the EC50.

FIG. 17B shows the activation curve of CD4⁺ TCR-T cells containing the E203 TCR. The intracellular IFN- γ expression was measured to determine the EC50.

FIG. 18A shows the activation curve of CD8⁺ TCR-T cells containing the E204 TCR. The intracellular IFN- γ expression was measured to determine the EC50.

FIG. 18B shows the activation curve of CD4⁺ TCR-T cells containing the E204 TCR. The intracellular IFN- γ expression was measured to determine the EC50.

FIG. 19A shows the activation curve of CD8⁺ TCR-T cells containing the E205 TCR. The intracellular IFN- γ expression was measured to determine the EC50.

FIG. 19B shows the activation curve of CD4⁺ TCR-T cells containing the E205 TCR. The intracellular IFN- γ expression was measured to determine the EC50.

FIG. 20 is table showing sequences of E202, E203, E204, and E205 TCR. CDR1 α , CDR2 α , and CDR3 α are CDR1, CDR2 and CDR3 of the TCR alpha chain variable domain, respectively. CDR1 β , CDR2 β , and CDR3 β are CDR1, CDR2 and CDR3 of the TCR beta chain variable domain, respectively. TRA_VJ are the rearranged V and J segments encoding the alpha

chain variable domain of the TCR. TRB_VDJ are the rearranged V, D, and J segments encoding the beta chain variable domain of the TCR.

FIG. 21 provides several sequences as described in the disclosure.

5

DETAILED DESCRIPTION

Human papilloma viruses (HPVs) are small (approximately 8000 pairs of bases) double-stranded DNA viruses which infect squamous epithelia and induce proliferative lesions such as skin warts (Xavier, "Natural History And Epidemiology Of Hpv Infection And Cervical Cancer". Gynecologic Oncology 110 (2008) S4–S7; Hausen *et al.*, "Human Papilloma Viruses." Annu. Rev. Microbial. 1994. 48;427-47). HPV has a well conserved genetic organization and all the potential open reading frames (ORFs) are located in one DNA strand, the reading frames of which are designated as early (E) or late (L) genes. While the early genes (E1-E8) are activated immediately after infection, the late genes encode structural proteins expressed in the granular layer of the epithelium. The gene products of the early genes are involved in controlling replication and expression of viral DNA (Mannarini *et al.* "Human Papilloma Virus (HPV) In Head And Neck Region: Review Of Literature". Acta Otorhinolaryngol Ital2009;29:119-126).

Chimeric Antigen Receptor (CARs) T-cell are engineered cells having an extracellular antigen recognition domain fused with intracellular T cell signaling and costimulatory domains. CARs can directly and selectively recognize cell surface tumor associated antigens (TAAs) in a major histocompatibility class (MHC)-independent manner. Despite the documented success of CAR T cell therapy in patients with hematologic malignancies, only modest responses have been observed in solid tumors. This can be attributed, in part, to the establishment of an immunosuppressive microenvironment in solid tumors. Such milieu involves the upregulation of several intrinsic inhibitory pathways mediated by increased expression of inhibitory receptors (IRs) in T cells reacting with their cognate ligands within the tumor (Ping *et al.*, "T-cell receptor-engineered T cells for cancer treatment: current status and future directions." Protein & cell 9.3 (2018): 254-266.).

Adoptive cell transfer (ACT) is a modality of cancer immunotherapy which has demonstrated remarkable success in treating hematologic malignancies and malignant melanoma. An especially effective form of ACT, which uses gene-modified T cells expressing a chimeric antigen receptor (CAR) to specifically target tumor-associated-antigen (TAA), such as CD19 and

GD2, has displayed encouraging results in clinical trials for treating such diseases as B cell malignancies and neuroblastoma (Simon *et al.*, "CAR-T cell therapy in melanoma: A future success story?." *Experimental dermatology* 27.12 (2018): 1315-1321). The use of modified TCRs for the treatment of different diseases has achieved significant results over the years and has been the focus area of a number of studies.

The present disclosure provides T-cell receptor (TCR)-engineered T cells, which can be used in cell therapy. The engineered T-cell receptors are capable of recognizing the surface antigen on the cell receptor which are otherwise not recognized by normal T-cells. The engineered T cells can be employed against multiple targets such as cancer cells expressing appropriate antigens.

Theoretically, a T cell receptor can have antigenic specificity for any HPV antigen. The E6 and E7 onco-proteins in HPV are necessary for malignant conversion of the cells. The HPV E7 protein mainly contributes to cancer development via inactivation of the Retinoblastoma protein, which results in constitutive cancer cell cycle activation. In some embodiments, the modified T cells are capable of recognizing an epitope of HPV in a MHC dependent manner (e.g., the HLA-A 02:01-restricted epitope of a high-risk serotype of HPV such as HPV-16). In this setting, HPV antigen positive tumor cells can be killed by engineered TCR-T cells.

T CELL RECEPTORS AND BINDING MOLECULES

T cells are a type of lymphocyte which typically develops in the thymus gland and plays a central role in the immune response. It plays an important role in the "adaptive immune response." T cells can be distinguished from other lymphocytes by the presence of a T-cell receptor on the cell surface. Differentiated T cells have an important role in controlling the immune response. CD8+ T cells, also known as "killer cells", are cytotoxic. Once they recognize a target cell, they are able to directly kill the target cell (e.g., virus-infected cells or cancer cells). CD8+ T cells can also produce cytokines and recruit other cells (e.g., macrophages and natural killer (NK) cells) to mount an immune response. CD4+ T cells, also known as "helper cells", can indirectly kill target cells, e.g., by facilitating maturation of B cells into plasma cells and memory B cells, and activation of cytotoxic T cells and macrophages. Helper T cells become activated when they are presented with peptide antigens by MHC class II molecules, which are expressed on the surface of antigen-presenting cells (APCs). Once activated, they divide rapidly and secrete

cytokines that regulate or assist the immune response. Regulatory T cells are important for tolerance, thereby preventing or inhibiting autoimmune response. The major role of regulatory T cells is to shut down T cell-mediated immunity toward the end of an immune reaction and to suppress autoreactive T cells that escaped the process of negative selection in the thymus.

5 T cells play an important role in cancer immunity where antigens from the cancer cells are taken up and presented on the cell surface of special immune cells called antigen-presenting cells (APCs) so that other immune cells can recognize the antigens of interest. In the lymph nodes, the APCs activate the T-cells and activate them to recognize the tumor cells. The activated T-cells can then travel via the blood vessels to reach the tumor, infiltrate it, recognize the cancer cells
10 and kill them.

The activation of T cells requires T cell receptors. A “T cell receptor” or “TCR” is a molecule that contains a variable α (or alpha) and β (or beta) chains (also known as TCR α and TCR β , respectively) or a variable γ (or gamma) and δ (or delta) chains (also known as TCR γ and TCR δ , respectively), or antigen-binding portions thereof, and which is capable of specifically
15 binding to an antigen, e.g., a peptide antigen or peptide epitope bound to a major histocompatibility complex (MHC) molecule.

The present disclosure provides a T cell receptor (TCR) or antigen-binding fragment thereof, and binding molecules derived from TCR. In some embodiments, the TCR is in the $\alpha\beta$ form. TCRs that exist in $\alpha\beta$ and $\gamma\delta$ forms are generally structurally similar, but T cells
20 expressing them may have distinct anatomical locations or functions. Generally, a TCR is found on the surface of T cells (or T lymphocytes) where it is generally responsible for recognizing antigens, such as peptides bound to major histocompatibility complex (MHC) molecules.

In some embodiments, the TCR is an intact or full-length TCR, such as a TCR containing the α chain and β chain. In some embodiments, the TCR is an antigen-binding portion that is less
25 than a full-length TCR but that binds to a specific peptide bound in an MHC molecule, such as binds to an MHC-peptide complex. In some cases, an antigen-binding portion or fragment of a TCR can contain only a portion of the structural domains of a full-length or intact TCR, but yet is able to bind the peptide epitope, such as MHC-peptide complex, to which the full TCR binds. In some cases, an antigen-binding portion contains the variable domains of a TCR, such as
30 variable α ($V\alpha$ or $V\alpha$) chain and variable β ($V\beta$ or $V\beta$) chain of a TCR, or antigen-binding fragments thereof sufficient to form a binding site for binding to a specific MHC-peptide

complex.

The variable domains of the TCR contain complementarity determining regions (CDRs), which generally are the primary contributors to antigen recognition and binding capabilities and specificity of the peptide, MHC and/or MHC-peptide complex. In some embodiments, a CDR of a TCR or combination thereof forms all or substantially all of the antigen-binding site of a given TCR molecule. The various CDRs within a variable region of a TCR chain generally are separated by framework regions (FRs), which generally display less variability among TCR molecules as compared to the CDRs. In some embodiments, CDR3 is the main CDR responsible for antigen binding or specificity, or is the most important among the three CDRs on a given TCR variable region for antigen recognition, and/or for interaction with the processed peptide portion of the peptide-MHC complex. In some contexts, the CDR1 of the alpha chain can interact with the N-terminal part of certain antigenic peptides. In some cases, CDR1 of the beta chain can interact with the C-terminal part of the peptide. In some contexts, CDR2 contributes most strongly to or is the primary CDR responsible for the interaction with or recognition of the MHC portion of the MHC-peptide complex.

The a-chain and/or b-chain of a TCR also can contain a constant domain, a transmembrane domain and/or a short cytoplasmic tail. In some aspects, each chain (e.g. alpha or beta) of the TCR can possess one N-terminal immunoglobulin variable domain, one immunoglobulin constant domain, a transmembrane region, and a short cytoplasmic tail at the C-terminal end. In some embodiments, a TCR, for example via the cytoplasmic tail, is associated with invariant proteins of the CD3 complex involved in mediating signal transduction. In some cases, the structure allows the TCR to associate with other molecules like CD3 and subunits thereof. For example, a TCR containing constant domains with a transmembrane region may anchor the protein in the cell membrane and associate with invariant subunits of the CD3 signaling apparatus or complex. The intracellular tails of CD3 signaling subunits (e.g. CD3 γ , CD3 δ , CD3 ϵ and CD3 ζ chains) contain one or more immunoreceptor tyrosine-based activation motif or ITAM and generally are involved in the signaling capacity of the TCR complex.

The exact locus of a domain or region can vary depending on the particular structural or homology modeling or other features used to describe a particular domain. It is understood that reference to amino acids, including to a specific sequence set forth as a SEQ ID NO used to describe domain organization of a TCR are for illustrative purposes and are not meant to limit

the scope of the embodiments provided. In some cases, the specific domain (e.g. variable or constant) can be several amino acids (such as one, two, three or four) longer or shorter. In some aspects, residues of a TCR are known or can be identified according to the International Immunogenetics Information System (IMGT) numbering system (see e.g. www.imgt.org;
5 Lefranc *et al.* "IMGT unique numbering for immunoglobulin and T cell receptor variable domains and Ig superfamily V-like domains." *Developmental & Comparative Immunology* 27.1 (2003): 55-77.). The structures and variations of TCR are known in the art, and are described, e.g., in WO 2019 /195486, which is incorporated herein by reference in its entirety.

In some embodiments, the a chain and b chain of a TCR each further contain a constant
10 domain. In some embodiments, the a chain constant domain (Ca) and b chain constant domain (Cb) individually are mammalian, such as is a human or a non-human constant domain (e.g., a mouse constant domain). In some embodiments, the constant domain is adjacent to the cell membrane. For example, in some cases, the extracellular portion of the TCR formed by the two chains contains two membrane-proximal constant domains, and two membrane-distal variable
15 domains, which variable domains each contain CDRs.

In some aspects, TCRs as described herein can contain a human constant region, such as an alpha chain containing a human Ca region and a beta chain containing a human Cb region. In some embodiments, the TCRs are fully human. In some embodiments, the expression and/or activity of TCRs, such as when expressed in human cells, e.g. human T cells, such as primary
20 human T cells, are not impacted by or are not substantially impacted by the presence of an endogenous human TCR.

In some embodiments, the engineered TCRs are expressed at similar or improved levels on the cell surface, exhibit the similar or greater functional activity (e.g. cytolytic activity) and/or exhibit similar or greater anti-tumor activity, when expressed by human cells that contain or
25 express an endogenous human TCR, such as human T cells, as compared to the level of expression, function activity and/or anti-tumor activity of the same TCR in similar human cells but in which expression of the endogenous TCR has been reduced or eliminated. In some examples an engineered TCR as described herein, when expressed in human T cells, is expressed on the cell surface at a level that is at least or at least about 80%, 85%, 90%, 95%, 100%, 105%,
30 110%, 115% or 120% of the level of expression of the same TCR when expressed in similar human T cells but in which expression of the endogenous TCR has been reduced or eliminated.

In some embodiments, each of the Ca and Cb domains is human. In some embodiments, the Ca is encoded by the TRAC gene (IMGT nomenclature) or is a variant thereof. In some embodiments, the variant of a Ca contains replacement of at least one non-native cysteine.

In some embodiments, the TCR can be a heterodimer of two chains a and b that are linked, such as by a disulfide bond or disulfide bonds. In some embodiments, the constant domain of the TCR can contain short connecting sequences in which a cysteine residue forms a disulfide bond, thereby linking the two chains of the TCR. In some embodiments, a TCR can have an additional cysteine residue in each of the a and b chains, such that the TCR contains two disulfide bonds in the constant domains. In some embodiments, each of the constant and variable domains contains disulfide bonds formed by cysteine residues.

In some embodiments, the TCR comprises CDRs, Va and/or Vb and constant region sequences as described herein.

In some embodiments, the TCR is a dimeric TCR (dTTCR). In some embodiments a dTTCR contains a first polypeptide wherein a sequence corresponding to a provided TCR a chain variable region sequence is fused to the N terminus of a sequence corresponding to a TCR a chain constant region extracellular sequence, and a second polypeptide wherein a sequence corresponding to a provided TCR b chain variable region sequence is fused to the N terminus a sequence corresponding to a TCR b chain constant region extracellular sequence, the first and second polypeptides being linked by a disulfide bond.

In some embodiments, a TCR can be cell-bound or in soluble form. In some embodiments, the TCR is in cell-bound form expressed on the surface of a cell.

In some embodiments, the TCR is a single chain TCR (scTCR). The scTCR is a single amino acid strand containing an a chain and a b chain that is able to bind to MHC-peptide complexes. Typically, a scTCR can be generated using methods known to those of skill in the art. These methods are described e.g., in WO 96/13593, WO 96/18105, W099/18129, WO 04/033685, W02006/037960, WO2011/044186; WO 2019 /195486; U.S. Patent No. 7,569,664; each of which is incorporated herein by reference in its entirety.

The TCR, antigen binding fragments thereof, and TCR-derived binding molecules can bind or recognize a peptide epitope associated with an antigen of interest (e.g., a cancer antigen). In some embodiments, the antigen can be a peptide epitope expressed on the surface of a cancer cell and/or a cell infected with a virus, e.g., HPV. In some embodiments, the antigen is presented

in the context of an MHC molecule. Such binding molecules include e.g., T cell receptors (TCRs) and antigen-binding fragments thereof, antibodies and antigen binding fragments thereof, and TCR-like CAR. They exhibit antigenic specificity for binding or recognizing such peptide epitopes. In some aspects, engineered cells that express a provided binding molecule, e.g. a TCR or antigen-binding fragment, exhibit cytotoxic activity against target cells expressing the peptide epitope, such as cancer cells or cells that are infected with HPV.

In some aspects, the TCR, antigen binding fragments thereof, and TCR-derived binding molecules recognize or bind to epitopes in the context of an MHC molecule, such as an MHC Class I molecule or an MHC class II molecule. Both MHC Class I molecules or MHC class II molecules are human leukocyte antigens (HLA). They play an important component of adaptive immune system. The HLA expression is controlled by genes located on chromosome 6. It encodes cell surface molecules specialized to present antigenic peptides to the T-cell receptor on T cells.

In some embodiments, the TCR, antigen binding fragments thereof, and TCR-derived binding molecules recognize or bind to epitopes in the context of an MHC Class I molecule. The MHC Class I molecule is a human leukocyte antigen (HLA)-A2 molecule, including any one or more subtypes thereof, e.g. HLA-A*0201, *0202, *0203, *0206, or *0207. The human leukocyte antigen A2 (HLA-A2) is among the most common human serotypes. In some cases, there can be differences in the frequency of subtypes between different populations. For example, more than 95% of the HLA-A2 positive Caucasian population is HLA-A*0201, whereas in the Chinese population the frequency has been reported to be approximately 23% for HLA-A*0201, 45% for HLA-A*0207, 8% for HLA-A*0206 and 23% for HLA-A*0203. In some embodiments, the MHC molecule is HLA-A*0201. In some embodiments, the present disclosure provides TCR or antigen-binding fragment thereof that bind an HPV-EB6/HLA-A2 complex.

In some embodiments, the binding molecule, e.g., TCR or antigen-binding fragment thereof or TCR-derived binding molecule, is isolated or purified, or is recombinant. In some aspects, the binding molecule, e.g., TCR or antigen-binding fragment thereof or TCR-derived binding molecule, is fully human. In some embodiments, the binding molecule is monoclonal. In some aspects, the binding molecule is a single chain. In other embodiments, the binding molecule contains two chains. In some embodiments, the binding molecule, e.g., TCR, antigen-binding fragment thereof or TCR-derived binding molecule, is expressed on the surface of a cell.

The TCR, antigen-binding fragment thereof, or TCR-derived binding molecules can have a Va and a Vb, or a region that is similar to Va and a region that is similar to Vb. In some embodiments, the Va region comprises the amino acid sequence set forth in any of SEQ ID NO: 1, 45, 47, 49, or an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 5 95%, 96%, 97%, 98%, or 99% sequence identity thereto. In some embodiments, the Vb region comprises the amino acid sequence set forth in any of SEQ ID NO: 2, 46, 48, 50, or an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto. In some embodiments, the Va region comprises one or more Va CDR sequences as described herein. In some embodiments, the Vb region comprises one or more 10 Vb CDR sequences as described herein.

In some embodiments, the TCR, TCR derived binding molecules, or antigen-binding fragment thereof, comprising an alpha chain comprising a variable alpha (Va) region and a beta chain comprising a variable beta (Vb) region, wherein the Va region can have complementarity determining regions (CDRs) 1, 2, 3, wherein the CDR1 region comprises or consists of an amino 15 acid sequence that is at least 80%, 85%, 90%, or 95% identical to a selected Va CDR1 amino acid sequence, the CDR2 region comprises or consists of an amino acid sequence that is at least 80%, 85%, 90%, or 95% identical to a selected Va CDR2 amino acid sequence, and the CDR3 region comprises or consists of an amino acid sequence that is at least 80%, 85%, 90%, or 95% identical to a selected Va CDR3 amino acid sequence, and a variable beta (Vb) region 20 comprising CDRs 1, 2, 3, wherein the CDR1 region comprises or consists of an amino acid sequence that is at least 80%, 85%, 90%, or 95% identical to a selected Vb CDR1 amino acid sequence, the CDR2 region comprises or consists of an amino acid sequence that is at least 80%, 85%, 90%, or 95% identical to a selected Vb CDR2 amino acid sequence, and the CDR3 region comprises or consists of an amino acid sequence that is at least 80%, 85%, 90%, or 95% identical 25 to a selected Vb CDR3 amino acid sequence. The selected Va CDRs 1, 2, 3 amino acid sequences and the selected Vb CDRs, 1, 2, 3 amino acid sequences are shown in **FIG. 20**.

In some embodiments, the TCR, antigen-binding fragment thereof, or TCR derived binding molecules described herein can contain a variable region (e.g., Va) containing one, two, or three of the CDRs of SEQ ID NO: 5 with zero, one or two amino acid insertions, deletions, or 30 substitutions; SEQ ID NO: 6 with zero, one or two amino acid insertions, deletions, or substitutions; SEQ ID NO: 7 with zero, one or two amino acid insertions, deletions, or

substitutions.

In some embodiments, the TCR, antigen-binding fragment thereof, or TCR derived binding molecules described herein can contain a variable region (e.g., Vb) containing one, two, or three of the CDRs of SEQ ID NO: 8 with zero, one or two amino acid insertions, deletions, or substitutions; SEQ ID NO: 9 with zero, one or two amino acid insertions, deletions, or substitutions; SEQ ID NO: 10 with zero, one or two amino acid insertions, deletions, or substitutions.

In some embodiments, the TCR, antigen-binding fragment thereof, or TCR derived binding molecules described herein can contain a variable region (e.g., Va) containing one, two, or three of the CDRs of SEQ ID NO: 27 with zero, one or two amino acid insertions, deletions, or substitutions; SEQ ID NO: 28 with zero, one or two amino acid insertions, deletions, or substitutions; SEQ ID NO: 29 with zero, one or two amino acid insertions, deletions, or substitutions.

In some embodiments, the TCR, antigen-binding fragment thereof, or TCR derived binding molecules described herein can contain a variable region (e.g., Vb) containing one, two, or three of the CDRs of SEQ ID NO: 30 with zero, one or two amino acid insertions, deletions, or substitutions; SEQ ID NO: 31 with zero, one or two amino acid insertions, deletions, or substitutions; SEQ ID NO: 32 with zero, one or two amino acid insertions, deletions, or substitutions.

In some embodiments, the TCR, antigen-binding fragment thereof, or TCR derived binding molecules described herein can contain a variable region (e.g., Va) containing one, two, or three of the CDRs of SEQ ID NO: 33 with zero, one or two amino acid insertions, deletions, or substitutions; SEQ ID NO: 34 with zero, one or two amino acid insertions, deletions, or substitutions; SEQ ID NO: 35 with zero, one or two amino acid insertions, deletions, or substitutions.

In some embodiments, the TCR, antigen-binding fragment thereof, or TCR derived binding molecules described herein can contain a variable region (e.g., Vb) containing one, two, or three of the CDRs of SEQ ID NO: 36 with zero, one or two amino acid insertions, deletions, or substitutions; SEQ ID NO: 37 with zero, one or two amino acid insertions, deletions, or substitutions; SEQ ID NO: 38 with zero, one or two amino acid insertions, deletions, or substitutions.

In some embodiments, the TCR, antigen-binding fragment thereof, or TCR derived binding molecules described herein can contain a variable region (e.g., Va) containing one, two, or three of the CDRs of SEQ ID NO: 39 with zero, one or two amino acid insertions, deletions, or substitutions; SEQ ID NO: 40 with zero, one or two amino acid insertions, deletions, or substitutions; SEQ ID NO: 41 with zero, one or two amino acid insertions, deletions, or substitutions.

In some embodiments, the TCR, antigen-binding fragment thereof, or TCR derived binding molecules described herein can contain a variable region (e.g., Vb) containing one, two, or three of the CDRs of SEQ ID NO: 42 with zero, one or two amino acid insertions, deletions, or substitutions; SEQ ID NO: 43 with zero, one or two amino acid insertions, deletions, or substitutions; SEQ ID NO: 44 with zero, one or two amino acid insertions, deletions, or substitutions.

The present disclosure also provides TCR a (alpha) and/or b (beta) chain as described herein. In some embodiments, the a chain comprises the amino acid sequence set forth in SEQ ID NO: 15, 51, 53, 55, or an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto. In some embodiments, the b chain comprises the amino acid sequence set forth in SEQ ID NO: 16, 52, 54, 56, or an amino acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto. In some embodiments, the a chain comprises one or more Va CDR sequences as described herein. In some embodiments, the b chain comprises one or more Vb CDR sequences as described herein.

In some embodiments, the TCR may be a heterodimer of two chains a and b that are linked, such as by a disulfide bond or disulfide bonds. In some embodiments, the constant domain of the TCR may contain short connecting sequences in which a cysteine residue forms a disulfide bond, thereby linking the two chains of the TCR. In some embodiments, a TCR may have an additional cysteine residue in each of the a and b chains, such that the TCR contains two disulfide bonds in the constant domains. In some embodiments, each of the constant and variable domains contains disulfide bonds formed by cysteine residues.

In some embodiments, the native disulfide bonds are not present. In some embodiments, the one or more of the native cysteines (e.g. in the constant domain of the a chain and b chain) that form a native interchain disulfide bond are substituted to another residue, such as to a serine

or alanine. In some embodiments, an introduced disulfide bond can be formed by mutating non cysteine residues on the alpha and beta chains, such as in the constant domain of the a chain and b chain, to cysteine. Opposing cysteines in the TCR a and b chains provide a disulfide bond that links the constant regions of TCR a and b chains of the substituted TCR to one another and
5 which is not present in a TCR comprising the unsubstituted constant region in which the native disulfide bonds are present, such as unsubstituted native human constant region or the unsubstituted native mouse constant region. In some embodiments, the presence of non-native cysteine residues (e.g. resulting in one or more non-native disulfide bonds) in a recombinant TCR can favor production of the desired recombinant TCR in a cell in which it is introduced
10 over expression of a mismatched TCR pair containing a native TCR chain.

In some embodiments, the nucleic acid encoding the alpha chain and the nucleic acid encoding the beta chain can be connected via a linker, such as any described elsewhere herein.

The disclosure also provides nucleic acid comprising a polynucleotide encoding a polypeptide comprising a TCR a chain variable region, a TCR b chain variable region, an
15 immunoglobulin heavy chain variable region or an immunoglobulin light chain variable region. The variable region comprises CDRs as shown in **FIG. 20**. When the polypeptides are paired with corresponding polypeptide (e.g., a corresponding a chain variable region or a corresponding b chain variable region), the paired polypeptides bind to the antigen of interest (e.g., HPV E6).

In some embodiments, by binding to the antigen of interest, the TCR or antigen-binding
20 fragment thereof, or TCR-derived binding molecules, can activate T cells (e.g., by activating TCR signaling pathway). In some embodiments, the activation can upregulate immune response, increase expression of cytokines (e.g., IFN γ) and/or CD107a, promote T-cell proliferation and T cell mediated killing.

In some embodiments, the TCR or antigen-binding fragment thereof, or TCR-derived
25 binding molecules as described herein can increase immune response, activity or number of T cells by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 2 folds, 3 folds, 5 folds, 10 folds, or 20 folds. In some embodiments, the TCR or antigen-binding fragment thereof, or TCR-derived binding molecules, when the antigen of interest is present, can increase serum concentrations of IFN- γ . In some embodiments, the activation can induce at least 10%, 20%,
30 30%, 40%, 50%, 60%, 70%, 80%, 90%, 1 fold, 2 folds, 5 folds, 10 folds, 100 folds, or 1000 folds increase of the serum concentrations of IFN- γ . In some embodiments, the activation can

induce at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 1 fold, 2 folds, 3 folds, 4 folds, or 5 folds increase of specific killing of target cells. In some embodiments, the specific killing of target cells is determined by absolute or competitive killing efficacy of target cells (e.g., APCs pulsed with a HPV peptide) using the methods described herein.

- 5 In some aspects, the provided recombinant TCRs include TCRs that are at least partially CD8-independent. In some aspects, the provided recombinant TCRs include TCRs that are at least partially CD8-dependent.

In some embodiments, the TCR or antigen-binding fragment thereof, or TCR-derived binding molecules as described herein specifically binds to HPV E6 epitope. In some
10 embodiments, the epitope has a sequence of SEQ ID NO: 19. In some embodiments, the epitope has a sequence of amino acids 29-38 of HPV E6 (SEQ ID NO: 75). Binding affinities can be deduced from the quotient of the kinetic rate constants ($KD=k_{off}/k_{on}$). In some embodiments, KD is less than $1 \times 10^{-6}M$, less than $1 \times 10^{-7}M$, less than $1 \times 10^{-8}M$, less than $1 \times 10^{-9}M$, or less than $1 \times 10^{-10}M$. In some embodiments, the KD is less than 50nM, 30 nM, 20 nM, 15 nM, 10 nM, 9
15 nM, 8 nM, 7 nM, 6 nM, 5 nM, 4 nM, 3 nM, 2 nM, or 1 nM. In some embodiments, KD is greater than $1 \times 10^{-7}M$, greater than $1 \times 10^{-8}M$, greater than $1 \times 10^{-9}M$, greater than $1 \times 10^{-10}M$, greater than $1 \times 10^{-11}M$, or greater than $1 \times 10^{-12}M$. General techniques for measuring the affinity of a binding molecule for an antigen include, e.g., ELISA, RIA, and surface plasmon resonance (SPR).

- 20 In some embodiments, the T cells expressing the TCR, antigen-binding fragment thereof, or TCR-derived binding molecules as described herein specifically binds to HPV peptide-pulsed APCs. In some embodiments, the T cells and the APCs are co-cultured at a 1: 1 effector-to-target cell ratio. In some embodiments, EC50 can be determined by measuring the intracellular IFN- γ expression. In some embodiments, the EC50 is determined in CD4+ T cell population. In some
25 embodiments, the EC50 is determined in CD8+ T cell population. In some embodiments, the EC50 is less than 50 ng/ml, less than 45 ng/ml, less than 40 ng/ml, less than 35 ng/ml, less than 30 ng/ml, less than 25 ng/ml, less than 20 ng/ml, less than 15 ng/ml, less than 10 ng/ml, less than 5 ng/ml, less than 4 ng/ml, less than 3 ng/ml, less than 2 ng/ml, or less than 1 ng/ml.

In some embodiments, the TCR or antigen-binding fragment thereof, or TCR-derived
30 binding molecules have a relatively high expression efficiency. For example, the expression efficiency for the TCR or antigen-binding fragment thereof, or TCR-derived binding molecules

described herein can be at least 10%, 20%, 30%, 40%, 50%, or 100% higher than an reference molecule (e.g., an endogenous TCR) under the same conditions.

In some embodiments, the binding molecule, e.g. TCR, does not exhibit cross-reactive or off-target binding, such as undesirable off-target binding, e.g. off-target binding to antigens present in healthy or normal tissues or cells.

In some embodiments, the CDRs of the Va region are encoded by sequences from a human TRAV gene segment and a human TRAJ gene segment. In some embodiments, the TRAV gene segment is TRAV38-2/DV8 (e.g., TRAV38-2/DV8*01), TRAV4 (e.g., TRAV4*01) or TRAV27 (TRAV27*01). In some embodiments, the TRAJ gene segment is TRAJ18 (e.g., TRAJ18*01), TRAJ12 (e.g., TRAJ12*01), TRAJ17 (e.g., TRAJ17*01), or TRAJ31 (e.g., TRAJ31*01). In some embodiments, the CDRs of the Vb region are encoded by sequences from a human TRBV gene segment, a human TRBD gene segment, and a human TRBJ gene segment. In some embodiments, the TRBV gene segment is TRBV12-4 (e.g., TRBV12-4*01) or TRBV29-1 (e.g., TRBV29-1*01). In some embodiments, the TRBD gene segment is TRBD2 (e.g., TRBD2*02). In some embodiments, the TRBJ gene segment is TRBJ1-1 (e.g., TRBJ1-1*01).

In some embodiments, the CDRs of the Va region of a TCR are encoded by sequences from human TRAV38-2/DV8 (e.g., TRAV38-2/DV8*01) gene segment and human TRAJ18 (e.g., TRAJ18*01) gene segment. In some embodiments, the CDRs of the Vb region of a TCR are encoded by sequences from human TRBV28 (e.g., TRBV28*01) gene segment, human TRBD1 (e.g., TRBD1*01) gene segment, and human TRBJ1-1 (e.g., TRBJ1-1*01) gene segment.

In some embodiments, the CDRs of the Va region of a TCR are encoded by sequences from human TRAV4 (e.g., TRAV4*01) gene segment and human TRAJ12 (e.g., TRAJ12*01) gene segment. In some embodiments, the CDRs of the Vb region of a TCR are encoded by sequences from human TRBV12-4 (e.g., TRBV12-4*01) gene segment, human TRBD2 (e.g., TRBD2*02) gene segment, and human TRBJ1-1 (e.g., TRBJ1-1*01) gene segment.

In some embodiments, the CDRs of the Va region of a TCR are encoded by sequences from human TRAV4 (e.g., TRAV4*01) gene segment and human TRAJ17 (e.g., TRAJ17*01) gene segment. In some embodiments, the CDRs of the Vb region of a TCR are encoded by sequences from human TRBV12-4 (e.g., TRBV12-4*01) gene segment, human TRBD2 (e.g.,

TRBD2*02) gene segment, and human TRBJ1-1 (e.g., TRAJ1-1*01) gene segment.

In some embodiments, the CDRs of the Va region of a TCR are encoded by sequences from human TRAV27 (e.g., TRAV27*01) gene segment and human TRAJ31 (e.g., TRAJ31*01) gene segment. In some embodiments, the CDRs of the Vb region of a TCR are encoded by sequences from human TRBV29-1 (e.g., TRAV29-1*01) gene segment, human TRBD2 (e.g., TRBD2*02) gene segment, and human TRBJ1-1 (e.g., TRAJ1-1*01) gene segment.

HPV infection and cancer

Human papilloma virus (HPV) infection is one of the most common type of sexually transmitted viral infection in humans. In most cases the symptoms of HPV infection are mild and regress naturally; however, prolonged infection can result in genital warts and cancer. Known cancer types associated with HPV include cervical cancer, head and neck cancer, oropharyngeal cancers, anal cancer, penile cancer, vaginal cancer and vulvar cancer.

HPV belongs to the Papillomaviridae family that consists of small, nonenveloped deoxyribonucleic acid (DNA) viruses. The HPV genome consists of double-stranded DNA and encodes DNA sequences for six early (E1, E2, E4, E5, E6, and E7) and two late proteins (L1 and L2). The E1 and E2 proteins are the early viral proteins required for replication and translation of virus, E2 also regulates the expression of E6 and E7, E4 and E5 participate in viral assembly and growth stimulation, whereas the late proteins L1 and L2 are the minor and major capsid proteins. There are more than 100 strains of HPV and based on their sequence they can be divided into alpha, beta, gamma, delta and mu. Most papillomaviruses that infect the cervix and oropharynx belong to the alphavirus genus. Further, these viruses can be classified into high-risk and low-risk HPV types depending on their oncogenic potential. Among them, HPV 16 is considered to have the highest ability to cause cancer.

The E6 and E7 gene products of HPV contribute to the pathogenesis of cancer. The HPV virus integrates into the host DNA within the nucleus and thereby dysregulates expression of the oncoproteins E6 and E7. Degradation of p53 is induced by E6, leading to loss of p53 activity. Its degradation is accomplished through the formation of a complex among p53, E6, and E6AP (Bernard *et al.* "Proteasomal degradation of p53 by human papillomavirus E6 oncoprotein relies on the structural integrity of p53 core domain." PloS one 6.10 (2011): e25981). In the physiological state, p53 functions to arrest cells in the G1 phase of the cell cycle to allow repair

of host DNA and, under conditions of severe DNA damage, p53 can also induce apoptosis. In addition to inhibiting p53, E7 also binds certain cyclin-dependent kinase inhibitors, resulting in further loss of cell cycle control.

HPV normally infects squamous epithelial cells, which have the capacity to proliferate, and also obtains access to basal cells during trauma or abrasion. In basal cells, HPV infection induces the expression of viral genes that assist in viral replication.

Although HPV is most commonly sexually transmitted, nonsexual transmission and occasional transmission through fomites has been known to occur. The risk factors that can contribute to HPV acquisition can be early onset of sexual activity, multiple sexual partners, and use of oral contraceptives. In addition, low socioeconomic status and smoking habits have been reported to increase the risks of acquiring infection. While in most cases infection is subclinical and is cleared by the immune system, persistent infection has been linked with oncogenesis.

Various methods of detecting HPV infection are known in the art. HPV infection can be detected by target amplification, signal amplification, and probe amplification. Target amplification is based on the duplication of HPV DNA fragments from a target gene sequence. Target amplification techniques include polymerase chain reaction (PCR) of viral genes (e.g., capsid L1 gene), amplicor human papilloma virus test, linear array human papilloma virus genotyping test, papillo check, real time polymerase chain reaction, and APTIMA human papilloma virus assays. Signal amplification techniques utilize DNA technology or hybrid capture to increase DNA signals to detectable levels. These include hybrid capture, Care human papilloma virus test, and Cervista, a FDA-approved genotyping test that can detect 14 high-risk HPV types. Probe amplification methods utilize a labeled molecular probe that can hybridize to a specified HPV DNA sequence.

The relationship between HPV and cancer and the various HPV detection methods are further described in Bansal *et al.*, "Human papillomavirus-associated cancers: A growing global problem," International Journal of Applied and Basic Medical Research 6.2 (2016): 84; Brianti *et al.*, "Review of HPV-related diseases and cancers," New Microbiol 40.2 (2017): 80-85; Chan *et al.*, "Human Papillomavirus Infection and Cervical Cancer: Epidemiology, Screening, and Vaccination—Review of Current Perspectives," Journal of oncology 2019 (2019); each of which is incorporated herein by reference in its entirety.

In some aspects, the present disclosure provides methods of preventing or reducing risk

of HPV infection and HPV associated cancer in subjects that are at risk for HPV infection. In some aspects, the present disclosure also provides methods of preventing or reducing risk of developing HPV associated cancer in subjects exhibiting HPV infection.

In some embodiments, the TCR, antigen binding fragments thereof, and TCR-derived
5 binding molecules can bind to antigens encoded by HPV. The HPV sub-type can be selected from HPV1, HPV2, HPV3, HPV4, HPV6, HPV10, HPV11, HPV16, HPV18, HPV26, HPV27, HPV28, HPV29, HPV30, HPV31, HPV33, HPV34, HPV35, HPV39, HPV40, HPV41, HPV42, HPV43, HPV45, HPV49, HPV51, HPV52, HPV54, HPV55, HPV56, HPV57, HPV58, HPV59, HPV68, and HPV69, among other variants. In some embodiments, the sub-type of HPV targeted
10 by the binding molecule is selected from at least one high-risk HPV: e.g., HPV16, HPV18, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, HPV59, HPV68, and HPV69.

In some embodiments, the HPV antigen includes but is not limited to, E1, E2, E3, E4, E6, E7, L1 and L2 proteins. In some embodiments, the antigen is an E6 antigen. In yet another
15 embodiment, the antigen is an E7 antigen. In some embodiments, the antigen is an HPV16 E6 antigen. In some embodiments, the recognized epitope is an E6 antigen peptide and has a sequence of SEQ ID NO: 19.

ENGINEERED CELLS

20 The present disclosure provides engineered cells (e.g., T cells) that comprise TCR or antigen-binding fragment thereof, or other similar antigen-binding molecules as described herein. These engineered cells can be used to treat various disorders or disease as described herein (e.g., virus infection, cancers, virus-induced disorders).

In various embodiments, the cell that is engineered can be obtained from e.g., humans
25 and non-human animals. In various embodiments, the cell that is engineered can be obtained from bacteria, fungi, humans, rats, mice, rabbits, monkeys, pig or any other species. Preferably, the cell is from humans, rats or mice. More preferably, the cell is obtained from humans. In various embodiments, the cell that is engineered is a blood cell. Preferably, the cell is a leukocyte (e.g., a T cell), lymphocyte or any other suitable blood cell type. In some embodiments, the cell
30 is a peripheral blood cell. In some embodiments, the cell is a T cell, B cell or NK cell.

In some embodiments, the cell is a T cell. In some embodiments, the T cells can express

a cell surface receptor that recognizes a specific antigenic moiety on the surface of a target cell. The cell surface receptor can be a wild type or recombinant T cell receptor (TCR), a chimeric antigen receptor (CAR), or any other surface receptor capable of recognizing an antigenic moiety that is associated with the target cell. T cells can be obtained by various methods known in the art, e.g., *in vitro* culture of T cells (e.g., tumor infiltrating lymphocytes) isolated from patients. TCR gene-modified T cells can be obtained by transducing T cells (e.g., isolated from the peripheral blood of patients), with a viral vector. In some embodiments, the T cell is a TCR gene-modified T cell. In some embodiments, the T cells are CD4⁺ T cells, CD8⁺ T cells, or regulatory T cells. In some embodiments, the T cells are T helper type 1 T cells and T helper type 2 T cells. In some embodiments, the T cell expressing this receptor is an $\alpha\beta$ -T cell. In alternate embodiments, the T cell expressing this receptor is a $\gamma\delta$ -T cell.

In some embodiments, the cell is an NK cell. In some embodiments, preparation of the engineered cells includes one or more culture and/or preparation steps. The cells for introduction of the binding molecule, e.g., TCR, can be isolated from a sample, such as a biological sample, e.g., one obtained from or derived from a subject. In some embodiments, the subject from which the cell is isolated is one having the disease or condition or in need of a cell therapy or to which cell therapy will be administered. The subject in some embodiments is a human in need of a particular therapeutic intervention, such as the adoptive cell therapy for which cells are being isolated, processed, and/or engineered.

In some embodiments, the cells are stem cells, such as multipotent and pluripotent stem cells, including induced pluripotent stem cells (iPSCs). The cells can be primary cells, such as those isolated directly from a subject and/or isolated from a subject and frozen. In some embodiments, the stem cells are cultured with additional differentiation factors to obtain desired cell types (e.g., T cells).

Different cell types can be obtained from appropriate isolation methods. The isolation methods include the separation of different cell types based on the expression or presence in the cell of one or more specific molecules, such as surface markers, e.g., surface proteins, intracellular markers, or nucleic acid. In some embodiments, any known method for separation based on such markers can be used. In some embodiments, the separation is affinity- or immunoaffinity-based separation. For example, the isolation in some aspects includes separation of cells and cell populations based on the cells' expression or expression level of one or more

markers, typically cell surface markers, for example, by incubation with an antibody or binding partner that specifically binds to such markers, followed generally by washing steps and separation of cells having bound the antibody or binding partner, from those cells having not bound to the antibody or binding partner.

5 Such separation steps can be based on positive selection, in which the cells having bound the reagents are retained for further use, and/or negative selection, in which the cells having not bound to the antibody or binding partner are retained. In some examples, both fractions are retained for further use. In some aspects, negative selection can be particularly useful where no antibody is available that specifically identifies a cell type in a heterogeneous population, such
10 that separation is best carried out based on markers expressed by cells other than the desired population.

 Also provided are methods, nucleic acids, compositions, and kits, for expressing the binding molecules, and for producing the genetically engineered cells expressing such binding molecules. The genetic engineering generally involves introduction of a nucleic acid encoding
15 the therapeutic molecule, e.g. TCR, CAR, e.g. TCR-like CAR, polypeptides, fusion proteins, into the cell, such as by retroviral transduction, transfection, or transformation. In some embodiments, gene transfer is accomplished by first stimulating the cell, such as by combining it with a stimulus that induces a response such as proliferation, survival, and/or activation, e.g., as measured by expression of a cytokine or activation marker, followed by transduction of the
20 activated cells, and expansion in culture to numbers sufficient for clinical application.

 In some embodiments, recombinant nucleic acids are transferred into cells using recombinant infectious virus particles, such as, e.g., vectors derived from simian virus 40 (SV40), adenoviruses, adeno-associated virus (AAV). In some embodiments, recombinant nucleic acids are transferred into T cells using recombinant lentiviral vectors or retroviral vectors, such as
25 gamma-retroviral vectors. In some embodiments, the retroviral vector has a long terminal repeat sequence (LTR), e.g., a retroviral vector derived from the Moloney murine leukemia virus (MoMLV), myeloproliferative sarcoma virus (MPSV), murine embryonic stem cell virus (MESV), murine stem cell virus (MSCV), or spleen focus forming virus (SFFV). Most retroviral vectors are derived from murine retroviruses. In some embodiments, the retroviruses include
30 those derived from any avian or mammalian cell source. The retroviruses typically are amphotropic, meaning that they are capable of infecting host cells of several species, including

humans. In some embodiments, the vector is a lentivirus vector. In some embodiments, recombinant nucleic acids are transferred into T cells via electroporation. In some embodiments, recombinant nucleic acids are transferred into T cells via transposition. Other methods of introducing and expressing genetic material in immune cells include calcium phosphate
5 transfection, protoplast fusion, cationic liposome-mediated transfection; tungsten particle-facilitated microparticle bombardment and strontium phosphate DNA co-precipitation. Many of these methods are described e.g., in WO2019195486, which is incorporated herein by reference in its entirety.

In some aspects, development of a humanized and/or fully human recombinant TCR
10 presents technical challenges. For example, in some aspects, a humanized and/or a fully human recombinant TCR receptor, when engineered into a human T cell, may compete with endogenous TCR complexes and/or can form mispairings with endogenous TCRA and/or TCRB chains, which may, in certain aspects, reduce recombinant TCR signaling, activity, and/or expression, and ultimately result in reduced activity of the engineered cells. The engineered cell can be
15 genetically modified. In some embodiments, the engineered cells can comprise a genetic disruption of a T cell receptor alpha constant (TRAC) gene and/or a T cell receptor beta constant (TRBC) gene. In some embodiments, the TRBC gene is one or both of a T cell receptor beta constant 1 (TRBC1) or T cell receptor beta constant 2 (TRBC2) gene. In some embodiments, the engineered cells do not express endogenous TCR a chain and/or TCR b chain. In some other
20 aspects, non-human constant domains are used, e.g., rodent (e.g., mouse) constant domains. The use of non-human constant domains can effectively reduce the likelihood of mispairing.

Also provided are populations of engineered cells, compositions containing such cells and/or enriched for such cells, such as in which cells expressing the binding molecule make up at least 15%, 20%, 25%, 30%, 35%, 40%, 50%, 60%, 70%, 80%, 90%, 91 %, 92%, 93%, 94%,
25 95%, 96%, 97%, 98%, 99%, or more percent of the total cells in the composition or cells of a certain type such as T cells, CD8⁺ or CD4⁺ cells.

RECOMBINANT VECTORS

The present disclosure also provides recombinant vectors (e.g., an expression vectors)
30 that include an isolated polynucleotide disclosed herein (e.g., a polynucleotide that encodes a polypeptide disclosed herein), host cells into which are introduced the recombinant vectors (i.e.,

such that the host cells contain the polynucleotide and/or a vector comprising the polynucleotide), and the production of recombinant polypeptides or fragments thereof by recombinant techniques.

As used herein, a “vector” is any construct capable of delivering one or more polynucleotide(s) of interest to a host cell when the vector is introduced to the host cell. An “expression vector” is capable of delivering and expressing the one or more polynucleotide(s) of interest as an encoded polypeptide in a host cell into which the expression vector has been introduced. Thus, in an expression vector, the polynucleotide of interest is positioned for expression in the vector by being operably linked with regulatory elements such as a promoter, enhancer, and/or a poly-A tail, either within the vector or in the genome of the host cell at or near or flanking the integration site of the polynucleotide of interest such that the polynucleotide of interest will be translated in the host cell introduced with the expression vector.

A vector can be introduced into the host cell by methods known in the art, e.g., electroporation, chemical transfection (e.g., DEAE-dextran), transformation, transfection, and infection and/or transduction (e.g., with recombinant virus). Thus, non-limiting examples of vectors include viral vectors (which can be used to generate recombinant virus), naked DNA or RNA, plasmids, cosmids, phage vectors, and DNA or RNA expression vectors associated with cationic condensing agents.

The present disclosure provides a recombinant vector comprising a nucleic acid construct suitable for genetically modifying a cell, which can be used for treatment of pathological disease or condition.

Any vector or vector type can be used to deliver genetic material to the cell. These vectors include but are not limited to plasmid vectors, viral vectors, bacterial artificial chromosomes (BACs), yeast artificial chromosomes (YACs), and human artificial chromosomes (HACs). Viral vectors can include but are not limited to recombinant retroviral vectors, recombinant lentiviral vectors, recombinant adenoviral vectors, foamy virus vectors, recombinant adeno-associated viral (AAV) vectors, hybrid vectors, and plasmid transposons (e.g., sleeping beauty transposon system, and PiggyBac transposon system) or integrase based vector systems. Other vectors that are known in the art can also be used in connection with the methods described herein.

In some embodiments, the vector is a viral vector. The viral vector can be grown in a culture medium specific for viral vector manufacturing. Any suitable growth media and/or

supplements for growing viral vectors can be used in accordance with the embodiments described herein.

In some embodiments, the vector used is a recombinant retroviral vector. A retroviral vector is capable of directing the expression of a nucleic acid molecule of interest. A retrovirus is present in the RNA form in its viral capsule and forms a double-stranded DNA intermediate when it replicates in the host cell. Similarly, retroviral vectors are present in both RNA and double-stranded DNA forms. The retroviral vector also includes the DNA form which contains a recombinant DNA fragment and the RNA form containing a recombinant RNA fragment. The vectors can include at least one transcriptional promoter/enhancer, or other elements which control gene expression. Such vectors can also include a packaging signal, long terminal repeats (LTRs) or portion thereof, and positive and negative strand primer binding sites appropriate to the retrovirus used. Long terminal repeats (LTRs) are identical sequences of DNA that repeat many times (e.g., hundreds or thousands of times) found at either end of retrotransposons or proviral DNA formed by reverse transcription of retroviral RNA. They are used by viruses to insert their genetic material into the host genomes. Optionally, the vectors can also include a signal which directs polyadenylation, selectable markers such as Ampicillin resistance, Neomycin resistance, TK, hygromycin resistance, phleomycin resistance histidinol resistance, or DHFR, as well as one or more restriction sites and a translation termination sequence. For example, such vectors can include a 5' LTR, a leading sequence, a tRNA binding site, a packaging signal, an origin of second strand DNA synthesis, and a 3' LTR or a portion thereof. Additionally, retroviral vector used herein can also refers to the recombinant vectors created by removal of the retroviral gag, pol, and env genes and replaced with the gene of interest.

In some embodiments, a MP71 vector is used. A MP71 retroviral vector construct is generated using standard molecular biology techniques. In some embodiments, the MP71 retroviral vector contains two genes linked by a P2A sequence: (1) the variable region of the alpha chain of a human anti-E6 TCR fused to the constant region of the mouse TCR alpha chain; (2) the variable region of the beta chain of same human anti-E6 TCR fused to the constant region of the mouse TCR beta chain. (**FIG. 1**)

In some embodiments, the vector can include an additional nucleic acid encoding an inhibitory protein (e.g., a checkpoint inhibitor). In various embodiments, the cell expresses the genetically engineered antigen receptor and the inhibitory protein. In various embodiments, the

inhibitory protein is constitutively expressed.

In some embodiments, the vector or construct can contain a single promoter that drives the expression of one or more nucleic acid molecules. In some embodiments, such promoters can be multicistronic (bicistronic or tricistronic). For example, in some embodiments, transcription units can be engineered as a bicistronic unit containing an IRES (internal ribosome entry site), which allows coexpression of gene products (e.g. encoding an alpha chain and/or beta chain of a TCR) by a message from a single promoter. Alternatively, in some cases, a single promoter may direct expression of an RNA that contains, in a single open reading frame (ORF), two or three genes (e.g. encoding an alpha chain and/or beta chain of a TCR) separated from one another by sequences encoding a self-cleavage peptide (e.g., P2A or T2A) or a protease recognition site (e.g., furin). The ORF thus encodes a single polypeptide, which, either during (in the case of 2A e.g., T2A) or after translation, is cleaved into the individual proteins. In some cases, the peptide, such as T2A, can cause the ribosome to skip (ribosome skipping) synthesis of a peptide bond at the C-terminus of a 2A element, leading to separation between the end of the 2A sequence and the next peptide downstream.

Various cell lines can be used in connection with the vectors as described herein. Exemplary eukaryotic cells that may be used to express polypeptides include, but are not limited to, COS cells, including COS 7 cells; 293 cells, including 293-6E cells; CHO cells, including CHO-S, DG44, Lec13 CHO cells, and FUT8 CHO cells; PER.C6® cells; and NSO cells. In some embodiments, a particular eukaryotic host cell is selected based on its ability to make desired post-translational modifications to the binding molecule. For example, in some embodiments, CHO cells produce polypeptides that have a higher level of sialylation than the same polypeptide produced in 293 cells.

In one aspect, the disclosure also relates to a nucleic acid comprising a polynucleotide encoding a polypeptide comprising:

- (1) a TCR a chain or a fragment thereof comprising an a chain variable region (Va) comprising complementarity determining regions (CDRs) 1, 2, and 3 comprising the amino acid sequences set forth in SEQ ID NOs: 5, 6, and 7, respectively, and wherein the Va, when paired with a b chain variable region (Vb) comprising the amino acid sequence set forth in SEQ ID NO: 2 binds to E6;

- (2) a TCR b chain or a fragment thereof comprising a b chain variable region (Vb) comprising complementarity determining regions (CDRs) 1, 2, and 3 comprising the amino acid sequences set forth in SEQ ID NOs: 8, 9, and 10, respectively, and wherein the Vb, when paired with a a chain variable region (Va) comprising the amino acid sequence set forth in SEQ ID NO: 1 binds to E6;
- (3) a TCR a chain or a fragment thereof comprising an a chain variable region (Va) comprising complementarity determining regions (CDRs) 1, 2, and 3 comprising the amino acid sequences set forth in SEQ ID NOs: 27, 28, and 29, respectively, and wherein the Va, when paired with a b chain variable region (Vb) comprising the amino acid sequence set forth in SEQ ID NO: 46 binds to E6;
- (4) a TCR b chain or a fragment thereof comprising a b chain variable region (Vb) comprising complementarity determining regions (CDRs) 1, 2, and 3 comprising the amino acid sequences set forth in SEQ ID NOs: 30, 31, or 32, respectively, and wherein the Vb, when paired with a a chain variable region (Va) comprising the amino acid sequence set forth in SEQ ID NO: 45 binds to E6;
- (5) a TCR a chain or a fragment thereof comprising an a chain variable region (Va) comprising complementarity determining regions (CDRs) 1, 2, and 3 comprising the amino acid sequences set forth in SEQ ID NOs: 33, 34, or 35, respectively, and wherein the Va, when paired with a b chain variable region (Vb) comprising the amino acid sequence set forth in SEQ ID NO: 48 binds to E6;
- (6) a TCR b chain or a fragment thereof comprising a b chain variable region (Vb) comprising complementarity determining regions (CDRs) 1, 2, and 3 comprising the amino acid sequences set forth in SEQ ID NOs: 36, 37, or 38, respectively, and wherein the Vb, when paired with a a chain variable region (Va) comprising the amino acid sequence set forth in SEQ ID NO: 47 binds to E6;
- (7) a TCR a chain or a fragment thereof comprising an a chain variable region (Va) comprising complementarity determining regions (CDRs) 1, 2, and 3 comprising the amino acid sequences set forth in SEQ ID NOs: 39, 40, or 41, respectively, and wherein the Va, when paired with a b chain variable region (Vb) comprising the amino acid sequence set forth in SEQ ID NO: 50 binds to E6; or

(8) a TCR b chain or a fragment thereof comprising a b chain variable region (Vb) comprising complementarity determining regions (CDRs) 1, 2, and 3 comprising the amino acid sequences set forth in SEQ ID NOs: 42, 43, or 44, respectively, and wherein the Vb, when paired with a a chain variable region (Va) comprising the amino acid sequence set forth in SEQ ID NO: 49 binds to E6.

In some embodiments, the VH when paired with a VL specifically binds to HPV E6, or the VL when paired with a VH specifically binds to HPV E6. In some embodiments, the nucleic acid is cDNA.

In one aspect, the disclosure relates to a vector comprising one or more of the nucleic acids as described herein. In one aspect, the disclosure also relates to a vector comprising two of the nucleic acids as described herein. In some embodiments, the vector encodes the Va region and the Vb region that together bind to an HPV antigen.

In one aspect, the disclosure relates to a pair of vectors, wherein each vector comprises one of the nucleic acids as described herein, wherein together the pair of vectors encodes the Va region and the Vb region that together bind to an HPV antigen.

In one aspect, the disclosure relates to a cell comprising the vector or the pair of vectors as described herein. In some embodiments, the cell is a T cell.

In some cases, certain TCRs, may exhibit poor expression or activity in part due to mispairing and/or competition with endogenous TCR chains and/or other factors. One method to address these challenges has been to design recombinant TCRs with mouse constant domains to prevent mispairings with endogenous human TCR a or b chains. However, the use of recombinant TCRs with mouse sequences may present a risk for immune response. In some embodiments, a genetic disruption is introduced, e.g., by gene editing, at an endogenous gene encoding one or more TCR chains.

As shown in **FIG. 1** the nucleic acid construct is cloned in a retroviral vector pMP71 containing two genes linked by a P2A sequence: (1) the variable region of the alpha chain of a human anti-E6 TCR fused to the constant region of the mouse TCR alpha chain; (2) the variable region of the beta chain of same human anti-E6 TCR fused to the constant region of the mouse TCR beta chain. In some embodiments, the nucleic acid construct further comprises a sequence encoding a signal peptide.

Referring to **FIG. 6B**, the nucleic acid construct comprises three sequences wherein the

three sequences include: (a) the variable region of the alpha chain of a human TCR fused to the constant region of a mouse TCR alpha chain identified as “*Va-Ca*”, wherein *Va* corresponds to the variable region of the alpha chain of a human TCR and *Ca* corresponds to the constant region of a mouse TCR alpha chain; (b) the variable region of the beta chain of same human TCR fused to the constant region of the mouse TCR beta chain identified as “*Vb-Cb*”, wherein *Vb* corresponds to the variable region of the beta chain of same human TCR and *Cb* corresponds to the constant region of the mouse TCR beta chain; and, (c) the variable regions of the heavy and light chain of an immune checkpoint inhibitor (ICI), linked with a GS linker (e.g., SEQ ID NO: 76). In some embodiments, the nucleic acid construct further comprises a sequence encoding a signal peptide. In some embodiments, the TCR is an anti-E6 TCR. In some embodiments, the immune checkpoint inhibitor is an anti-PD-1 antibody scFv. The nucleic acid construct can further include other sequences which can assist and/or enable in the transfection, transduction, integration, replication, transcription, translation, expression and/or stabilization of the construct. In some embodiments, the nucleic acid construct comprises a linker sequence, e.g., *P2A* and/or *T2A* sequences linking sequences (a), (b) and/or (c).

In some embodiments, the GS linker comprises at least or about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 30, 40, or 50 amino acid residues. In some embodiments, the GS linker comprises at least or about 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 20, 25, 30, or 40 glycine residues. In some embodiments, the GS linker comprises at least or about 1, 2, 3, 4, 5, 6, 7, or 8 serine residues. In some embodiments, the GS linker comprises or consists of both glycine and serine residues. In some embodiments, the GS linker comprises or consists of a sequence that is at least or about 70%, at least or about 75%, at least or about 80%, at least or about 85%, at least or about 90%, at least or about 95%, at least or about 99%, or 100% identical to GGGGSGGGGSGGGGS (SEQ ID NO: 76). In some embodiments, the GS linker comprises at least 1, 2, 3, 4, 5, 6, 7, or 8 repeats of GGGGS (SEQ ID NO: 77). In some embodiments, the GS linker has no more than 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 30, 40, or 50 amino acid residues.

The present disclosure also provides nucleic acids that encode TCR a and/or b chain as described herein. In some embodiments, the nucleic acid that encodes the a chain comprises the sequence set forth in SEQ ID NO: 15, 51, 53, 55, or a nucleic acid sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto. In

some embodiments, the nucleic acid that encodes the b chain comprises the sequence set forth in SEQ ID NO: 16, 52, 54, 56, or a sequence that has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity thereto. In some embodiments, the a chain comprises one or more Va CDR sequences as described herein. In some embodiments, the b chain comprises one or more Vb CDR sequences as described herein. In some embodiments, the vector comprises a sequence that is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 20, 63, 64, 65, 26, 66, 67, or 68.

In some embodiments, the inhibitory protein is an anti-PD-1 antibody (e.g., an anti-PD-1 scFV).

In some embodiments, the antibody comprises a heavy chain variable domain comprising an amino acid sequence that is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 11; and a light chain variable domain comprising an amino acid sequence that is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 12.

In some embodiments, the vector comprises a sequence that encodes an anti-PD-1 scFV. In some embodiments, the vector comprises a sequence that encodes an amino acid sequence that is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 24, 69, 70, or 71. In some embodiments, the vector comprises a sequence that is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 25, 72, 73, or 74.

The term “Linker” (L) or “linker domain” or “linker region” as used herein refer to an oligo- or polypeptide region from about 1 to 100 amino acids in length, which links together any of the domains/regions. Linkers can be composed of flexible residues like glycine and serine so that the adjacent protein domains are free to move relative to one another. Longer linkers can be used when it is desirable to ensure that two adjacent domains do not sterically interfere with one another. Linkers can be cleavable or non-cleavable. Examples of cleavable linkers include 2A linkers (for example P2A, T2A), 2A-like linkers or functional equivalents thereof and combinations thereof. In some embodiments, the linkers include the picornaviral 2A-like linker, CHYSEL sequences of porcine teschovirus (P2A), Thosea asigna virus (T2A) or combinations, variants and functional equivalents thereof. Other linkers will be apparent to those of skill in the art and can be used in the methods described herein.

The present disclosure also provides a nucleic acid sequence comprising a nucleotide sequence encoding any of the TCRs, antigen binding fragments thereof, and/or TCR-derived binding molecules (including e.g., functional portions and functional variants thereof, polypeptides, or proteins described herein). “Nucleic acid” as used herein can include
5 “polynucleotide,” “oligonucleotide,” and “nucleic acid molecule,” and generally means a polymer of DNA or RNA, which can be single-stranded or double-stranded, synthesized or obtained from natural sources, which can contain natural, non-natural or altered nucleotides. Furthermore, the nucleic acid comprises complementary DNA (cDNA). It is generally preferred that the nucleic acid does not comprise any insertions, deletions, inversions, and/or substitutions.
10 However, it can be suitable in some instances, as discussed herein, for the nucleic acid to comprise one or more insertions, deletions, inversions, and/or substitutions.

The nucleic acids as described herein can be constructed based on chemical synthesis and/or enzymatic ligation reactions using procedures known in the art. For example, a nucleic acid can be chemically synthesized using naturally occurring nucleotides or variously modified
15 nucleotides. In some of any such embodiments, the nucleotide sequence is codon-optimized.

The present disclosure also provides the nucleic acids comprising a nucleotide sequence complementary to the nucleotide sequence of any of the nucleic acids described herein or a nucleotide sequence which hybridizes under stringent conditions to the nucleotide sequence of any of the nucleic acids described herein.

20 In some embodiments, the nucleotide sequence encoding the alpha chain and the nucleotide sequence encoding the beta chain are separated by a peptide sequence that causes ribosome skipping. In some embodiments, the peptide that causes ribosome skipping is a P2A or T2A peptide. In some embodiments, the nucleic acid is synthetic. In some embodiments, the nucleic acid is cDNA.

25 In some embodiments, the vector can additionally include a nucleic acid sequence that encodes a checkpoint inhibitor (CPI) (e.g., an inhibitory protein). In some embodiments, the checkpoint inhibitor is e.g., any antibody or antigen binding fragment thereof as described herein. In some embodiments, the antibody or antigen binding fragments thereof can specifically bind to PD-1, PD-L1, PD-L2, 2B4 (CD244), 4-1BB, A2aR, B7.1, B7.2, B7-H2, B7-H3, B7-H4, B7-H6,
30 BTLA, butyrophilins, CD160, CD48, CTLA4, GITR, gp49B, HHLA2, HVEM, ICOS, ILT-2, ILT-4, KIR family receptors, LAG-3, OX-40, PIR-B, SIRPalpha (CD47), TFM-4, TIGIT, TIM-1,

TIM-3, TIM-4, or VISTA. In some embodiments, the inhibitory protein is a scFv (e.g., an anti-PD-1 scFv). In some embodiments, the anti-PD-1 scFv has a sequence that is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 22. The disclosure also provides a nucleic acid sequence that encodes the anti-PD-1 scFv. In some
5 embodiments, the nucleic acid has a sequence that is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 23.

In some embodiments, the vector can additionally include a nucleic acid sequence that encodes a bifunctional trap fusion protein. In some embodiments, the bifunctional trap protein targets both the PD-1 and TGF- β . In some embodiments, the bifunctional trap protein targets
10 both the PD-L1 and TGF- β . In some embodiments, the bifunctional fusion protein designed to block PD-L1 and sequester TGF- β . M7824 (MSB0011395C) comprises the extracellular domain of human TGF- β receptor II (TGF β RII) linked to the C-terminus of the human anti-PD-L1 scFv, based on the human IgG1 monoclonal antibody (mAb) avelumab. In some embodiments, the bifunctional fusion protein comprises the extracellular domain of human TGF- β receptor II
15 (TGF β RII) linked to the C-terminus of the human anti-PD-1 scFv.

In some of any such embodiments, the TCR or antigen-binding fragment thereof is encoded by a nucleotide sequence that has been codon-optimized. In certain embodiments, the alpha and/or beta chain further comprises a signal peptide. In particular embodiments, the TCR or antigen-binding fragment thereof is isolated or purified or is recombinant. In some of any such
20 embodiments, the TCR or antigen-binding fragment is recombinant. In some of any such embodiments, the TCR or antigen-binding fragment thereof is human.

The disclosure also provides a nucleic acid sequence that is at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identical to any
25 nucleotide sequence as described herein, and an amino acid sequence that is at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identical to any amino acid sequence as described herein. In some embodiments, the disclosure relates to nucleotide sequences encoding any peptides that are described herein, or any amino acid
30 sequences that are encoded by any nucleotide sequences as described herein.

In some embodiments, the nucleic acid sequence is at least or about 10, 20, 30, 40, 50, 60,

70, 80, 90, 100, 110, 120, 130, 150, 200, 250, 300, 350, 400, 500, or 600 nucleotides. In some embodiments, the amino acid sequence is at least or about 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, or 200 amino acid residues. In some embodiments, the nucleic acid sequence is less than 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 150, 200, 250, 300, 350, 400, 500, or 600 nucleotides. In some embodiments, the amino acid sequence is less than 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, or 200 amino acid residues.

To determine the percent identity of two amino acid sequences, or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-homologous sequences can be disregarded for comparison purposes. The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences.

20 Methods of generating T cell receptors and TCR-like molecules

The present disclosure also provides methods for identifying and generating T cell receptors that can recognize a target antigen. In some aspects, the methods involve subjecting biological samples containing T cells, such as primary T cells, including those derived from normal donors or patients having a disease or condition of interest, to multiple rounds of antigen exposure and assessment. In some aspects, the rounds involve the use of artificial or engineered antigen presenting cells, such as autologous dendritic cells or other APCs pulsed with a desired peptide antigen, to promote presentation on an MHC, such as a class I or II MHC.

In some aspects, multiple rounds of antigen exposure are carried out and in some aspects T cells are sorted following one or more of the rounds, e.g., based on ability to bind to the desired antigen (such as peptide-MHC tetramers).

Sorting can be carried out by methods known in the art, e.g., flow cytometry. Cells that

can bind to the desired antigen (positive fraction) and cells that cannot effectively bind to the desired antigen (negative fraction) are analyzed, e.g., by single-cell sequencing methods. In some embodiments, sequencing is performed to identify, at a single-cell level, TCR pairs present in each sample. In some aspects, the methods can quantify the number of copies of a given TCR pair present in a sample, and as such can assess the abundance of a given TCR in a given sample, and/or enrichment thereof over another sample, such as enrichment or abundance in the positive (antigen-binding) fraction, e.g., over one or more rounds, for example, as compared to the negative fraction. Such assays can be performed to generate antigen-specific T cell receptors (TCRs). In some aspects, clonal T cell lines are generated and the sequences of individual paired TCR alpha and beta chains and abundance thereof in various populations are determined on a single-cell basis, using high-throughput paired TCR sequencing.

The TCR or antigen-binding fragment thereof can be further modified. In some embodiments, the binding molecules, e.g., TCRs or antigen-binding fragments thereof, include one or more amino acid variations, e.g., substitutions, deletions, insertions, and/or mutations, compared to the sequence of a binding molecule, e.g., any TCR described herein. Exemplary variants include those designed to improve the binding affinity and/or other biological properties of the binding molecule. Amino acid sequence variants of a binding molecule can be prepared by introducing appropriate modifications into the nucleotide sequence encoding the binding molecule, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of residues within the amino acid sequences of the binding molecule. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics, e.g., specifically bind to the antigen.

Various binding molecules can be made from TCR. The binding molecules, e.g., TCRs or antigen-binding fragments thereof, can include one or more amino acid substitutions, e.g., as compared to a binding molecule, e.g., TCR, sequence described herein and/or compared to a sequence of a natural repertoire, e.g., human repertoire. Sites of interest for substitutional mutagenesis include the CDRs, FRs and /or constant regions. Amino acid substitutions can be introduced into a binding molecule of interest and the products screened for a desired activity, e.g., retained/improved antigen affinity or avidity, decreased immunogenicity, improved half-life, CD8-independent binding or activity, surface expression, promotion of TCR chain pairing and/or

other improved properties or functions.

In some embodiments, one or more residues within a CDR of a parent binding molecule, e.g., TCR, is/are substituted. In some embodiments, the substitution is made to revert a sequence or position in the sequence to a germline sequence, such as a binding molecule sequence found in the germline (e.g., human germline), for example, to reduce the likelihood of immunogenicity, e.g., upon administration to a human subject.

In some embodiments, a functional variant is made from a TCR or a TCR-derived binding molecule. The term "functional variant," as used herein, refers to a binding molecule having an adequate or significant sequence identity to a parent molecule. Further, the functional variant retains the same biological activity as of the parent protein. The functional variant encompasses those variants of the TCR protein described herein (the parent TCR, polypeptide, or protein) that retain the ability to specifically bind to HPV epitope for which the parent TCR has antigenic specificity or to which the parent polypeptide or protein specifically binds. Furthermore the binding region (e.g., variable domain) of the functional variant can be to a similar extent, the same extent, or to a higher extent, as the parent TCR protein. In reference to the parent TCR, polypeptide, or protein, the functional variant can, for instance, be at least about 30%, 50%, 75%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or more identical in amino acid sequence to the parent TCR, polypeptide, or protein.

Substitutions, insertions, or deletions can be made to one or more CDRs so long as such alterations do not substantially reduce the ability of the binding molecule, e.g., TCR or antigen-binding fragment thereof, to bind antigen. For example, conservative alterations (e.g., conservative substitutions as provided herein) that do not substantially reduce binding affinity can be made in CDRs. Such alterations can, for example, be outside of antigen contacting residues in the CDRs. In certain embodiments of the variable sequences provided herein, each CDR either is unaltered, or contains no more than one, two or three amino acid substitutions.

TCR-derived antibodies

The present disclosure also provides an antibody or antigen-binding fragment thereof that contains any one or more of the CDRs as described above. In some embodiments, the antibody or antigen-binding fragment contains variable heavy and light chain containing a CDR1, a CDR2 and/or a CDR3 contained in the alpha chain and a CDR1, a CDR2 and/or a CDR3 contained in

the beta chain. In some embodiments, the antibody or antigen-binding fragment contains one or more CDRs that are at least at or about 80%, 85%, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identical to CDR sequences in **FIG. 20**.

In some embodiments, the antibodies and antigen binding fragments thereof, e.g. TCR-like antibodies, specifically recognize a peptide epitope (e.g., HPV antigen) in the context of an MHC molecule, such as an MHC class I. In some cases, the MHC class I molecule is an HLA-A2 molecule, e.g. HLA-A2*01.

In some embodiments, the antibodies and antigen binding fragments thereof can specifically recognize a peptide epitope (e.g., HPV antigen) in an MHC molecule independent manner.

In general, antibodies (also called immunoglobulins) are made up of two classes of polypeptide chains, light chains and heavy chains. A non-limiting antibody of the present disclosure can be an intact, four immunoglobulin chain antibody comprising two heavy chains and two light chains. The heavy chain of the antibody can be of any isotype including IgM, IgG, IgE, IgA, or IgD or sub-isotype including IgG1, IgG2, IgG2a, IgG2b, IgG3, IgG4, IgE1, IgE2, etc. The light chain can be a kappa light chain or a lambda light chain. An antibody can comprise two identical copies of a light chain and two identical copies of a heavy chain. The heavy chains, which each contain one variable domain (or variable region, VH) and multiple constant domains (or constant regions), bind to one another via disulfide bonding within their constant domains to form the "stem" of the antibody. The light chains, which each contain one variable domain (or variable region, VL) and one constant domain (or constant region), each bind to one heavy chain via disulfide binding. The variable region of each light chain is aligned with the variable region of the heavy chain to which it is bound. The variable regions of both the light chains and heavy chains contain three hypervariable regions sandwiched between more conserved framework regions (FR).

In some embodiments, the antibody is an intact immunoglobulin molecule (e.g., IgG1, IgG2a, IgG2b, IgG3, IgM, IgD, IgE, IgA). The IgG subclasses (IgG1, IgG2, IgG3, and IgG4) are highly conserved, differ in their constant region, particularly in their hinges and upper CH2 domains. The sequences and differences of the IgG subclasses are known in the art, and are described, e.g., in Vidarsson, *et al*, "IgG subclasses and allotypes: from structure to effector functions." *Frontiers in immunology* 5 (2014); Irani, *et al*. "Molecular properties of human IgG

subclasses and their implications for designing therapeutic monoclonal antibodies against infectious diseases." *Molecular immunology* 67.2 (2015): 171-182; Shakib, Farouk, ed. *The human IgG subclasses: molecular analysis of structure, function and regulation*. Elsevier, 2016; each of which is incorporated herein by reference in its entirety.

5 The antibody can also be an immunoglobulin molecule that is derived from any species (e.g., human, rodent, mouse, camelid). Antibodies disclosed herein also include, but are not limited to, polyclonal, monoclonal, monospecific, polyspecific antibodies, and chimeric antibodies that include an immunoglobulin binding domain fused to another polypeptide. The term "antigen binding domain" or "antigen binding fragment" is a portion of an antibody that
10 retains specific binding activity of the intact antibody, i.e., any portion of an antibody that is capable of specific binding to an epitope on the intact antibody's target molecule. It includes, e.g., Fab, Fab', F(ab')₂, and variants of these fragments. Thus, in some embodiments, an antibody or an antigen binding fragment thereof can be, e.g., a scFv, a Fv, a Fd, a dAb, a bispecific antibody, a bispecific scFv, a diabody, a linear antibody, a single-chain antibody molecule, a multi-specific
15 antibody formed from antibody fragments, and any polypeptide that includes a binding domain which is, or is homologous to, an antibody binding domain. Non-limiting examples of antigen binding domains include, e.g., the heavy chain and/or light chain CDRs of an intact antibody, the heavy and/or light chain variable regions of an intact antibody, full length heavy or light chains of an intact antibody, or an individual CDR from either the heavy chain or the light chain of an
20 intact antibody.

 In some embodiments, the antigen binding fragment can form a part of a chimeric antigen receptor (CAR). In some embodiments, the chimeric antigen receptor are fusions of single-chain variable fragments (scFv) as described herein, fused to CD3-zeta transmembrane- and endodomain. In some embodiments, the chimeric antigen receptor also comprises intracellular
25 signaling domains from various costimulatory protein receptors (e.g., CD28, 41BB, ICOS). In some embodiments, the chimeric antigen receptor comprises multiple signaling domains, e.g., CD3z-CD28-41BB or CD3z-CD28-OX40, to increase potency. Thus, in one aspect, the disclosure further provides cells (e.g., T cells) that express the chimeric antigen receptors as described herein.

30 In some embodiments, the scFV comprises one heavy chain variable domain, and one light chain variable domain. In some embodiments, the scFV comprises two heavy chain variable

domains, and two light chain variable domains.

TCR-derived CAR

The antibody or antigen-binding portion thereof can be expressed on cells as part of a recombinant receptor, such as an antigen receptor. Among the antigen receptors are functional non-TCR antigen receptors, such as chimeric antigen receptors (CARs). Generally, a CAR containing an antibody or antigen-binding fragment that exhibits TCR-like specificity directed against a peptide in the context of an MHC molecule can also be referred to as a TCR-like CAR. Thus, among the provided binding molecules, e.g., HPV binding molecules, are antigen receptors, such as those that include one of the provided antibodies, e.g., TCR-like antibodies. In some embodiments, the antigen receptors and other chimeric receptors specifically bind to a region or epitope of an antigen, e.g. TCR-like antibodies. Among the antigen receptors are functional non-TCR antigen receptors, such as chimeric antigen receptors (CARs). Also provided are cells expressing the CARs and uses thereof in adoptive cell therapy, such as treatment of diseases and disorders associated with HPV antigen expression.

TCR-like CARs that contain a non-TCR molecule that exhibits T cell receptor specificity, such as for a T cell epitope or peptide epitope when displayed or presented in the context of an MHC molecule. In some embodiments, a TCR-like CAR can contain an antibody or antigen-binding portion thereof, e.g., TCR-like antibody, such as described herein. In some embodiments, the antibody or antibody-binding portion thereof is reactive against specific peptide epitope in the context of an MHC molecule, wherein the antibody or antibody fragment can differentiate the specific peptide in the context of the MHC molecule from the MHC molecule alone, the specific peptide alone, and, in some cases, an irrelevant peptide in the context of an MHC molecule. In some embodiments, an antibody or antigen-binding portion thereof can exhibit a higher binding affinity than a T cell receptor.

Exemplary antigen receptors, including CARs, and methods for engineering and introducing such receptors into cells, include those described, for example, in US2002/131960, US2013/287748, US2013/0149337, U.S. 6,451,995, U.S. 7,446,190, U.S. 8,252,592; each of which is incorporated herein by reference in its entirety.

In some embodiments, the CARs generally include an extracellular antigen (or ligand) binding domain, including e.g., an antibody or antigen-binding fragment thereof specific for a

peptide, linked to one or more intracellular signaling components, in some aspects via linkers and/or transmembrane domain(s). In some embodiments, such molecules can typically mimic or approximate a signal through a natural antigen receptor, such as a TCR, and, optionally, a signal through such a receptor in combination with a co-stimulatory receptor.

5 In some embodiments, the CAR typically includes in its extracellular portion one or more antigen binding molecules, such as one or more antigen-binding fragment, domain, or portion, or one or more antibody variable domains, and/or antibody molecules. In some embodiments, the CAR includes an antigen-binding portion or portions of an antibody molecule, such as a single-chain antibody fragment (scFv) derived from the variable heavy (VH) and variable light (VL)
10 chains of a monoclonal antibody (mAb). In some embodiments, the CAR contains a TCR-like antibody, such as an antibody or an antigen-binding fragment (e.g., scFv) that specifically recognizes a peptide epitope presented on the cell surface in the context of an MHC molecule.

In certain embodiments, the intracellular signaling domain comprises a CD28 transmembrane and signaling domain linked to a CD3 (e.g., CD3-zeta) intracellular domain. In
15 some embodiments, the intracellular signaling domain comprises a chimeric CD28 and CD 137 (4-1BB, TNFRSF9) co-stimulatory domains, linked to a CD3 zeta intracellular domain.

In some embodiments, the binding molecule can also be a genetically engineered T cell receptor (TCR), killer-cell immunoglobulin-like receptor (KIR), C-type lectin receptor, leukocyte immunoglobulin-like receptor (LILR), Type 1 cytokine receptor, Type 2 cytokine
20 receptor, tumor necrosis factor family, TGF β receptor, chemokine receptor, or a member of immunoglobulins superfamily (IgSF).

In some embodiments, the engineered cells are further modified in any number of ways, such that their therapeutic or prophylactic efficacy is increased. For example, the engineered TCR or other binding molecules expressed by the population can be conjugated either directly or
25 indirectly through a linker to a targeting moiety. The practice of conjugating binding molecules, e.g., the CAR or TCR, to targeting moieties is known in the art, and are described e.g., in Wadhwa *et al.* "Receptor mediated glycotargeting." Journal of drug targeting 3.2 (1995): 111-127., and U.S. Pat. No. 5,087,616; which are incorporated herein by reference in the entirety.

30 **METHOD FOR PREPARATION OF ENGINEERED CELLS**

The present disclosure provides a method or process for preparing, manufacturing

and/or using the engineered cells for treatment of pathological diseases or conditions.

The cells for introduction of the binding molecule, e.g., TCR, can be isolated from a sample, such as a biological sample, e.g., one obtained from or derived from a subject. In some embodiments, the subject from which the cell is isolated is one having the disease or condition or
5 in need of a cell therapy or to which cell therapy will be administered. The subject in some embodiments is a human in need of a particular therapeutic intervention, such as the adoptive cell therapy for which cells are being isolated, processed, and/or engineered.

Accordingly, the cells in some embodiments are primary cells, e.g., primary human cells. The samples include tissue, fluid, and other samples taken directly from the subject, as well as
10 samples resulting from one or more processing steps, such as separation, centrifugation, genetic engineering (e.g. transduction with viral vector), washing, and/or incubation. The biological sample can be a sample obtained directly from a biological source or a sample that is processed. Biological samples include, but are not limited to, body fluids, such as blood, plasma, serum, cerebrospinal fluid, synovial fluid, urine and sweat, tissue and organ samples, including
15 processed samples derived therefrom.

In some aspects, the sample from which the cells are derived or isolated is blood or a blood-derived sample, or is or is derived from an apheresis or leukapheresis product. Exemplary samples include whole blood, peripheral blood mononuclear cells (PBMCs), leukocytes, bone marrow, thymus, tissue biopsy, tumor, leukemia, lymphoma, lymph node, gut associated
20 lymphoid tissue, mucosa associated lymphoid tissue, spleen, other lymphoid tissues, liver, lung, stomach, intestine, colon, kidney, pancreas, breast, bone, prostate, cervix, testes, ovaries, tonsil, or other organ, and/or cells derived therefrom. Samples include, in the context of cell therapy, e.g., adoptive cell therapy, samples from autologous and allogeneic sources.

In some embodiments, the cells are derived from cell lines, e.g., T cell lines. The cells in
25 some embodiments are obtained from a xenogeneic source, for example, from mouse, rat, or non-human primate.

In some embodiments, the blood cells collected from the subject are washed, e.g., to remove the plasma fraction and to place the cells in an appropriate buffer or media for subsequent processing steps. In some embodiments, the cells are washed with phosphate
30 buffered saline (PBS). In some embodiments, the wash solution lacks calcium and/or magnesium and/or many or all divalent cations. In some aspects, a washing step is accomplished a semi-

automated "flow-through" centrifuge. In some aspects, a washing step is accomplished by tangential flow filtration (TFF). In some embodiments, the cells are resuspended in a variety of biocompatible buffers after washing, such as, for example, $\text{Ca}^{2+}/\text{Mg}^{2+}$ free PBS. In certain
5 in culture media. In some embodiments, the methods include density-based cell separation methods, such as the preparation of white blood cells from peripheral blood by lysing the red blood cells and centrifugation through a Percoll or Ficoll gradient.

In some embodiments, the method comprises one or more steps of: e.g., isolating the T cells from a patient's blood; transducing the population T cells with a viral vector including the
10 nucleic acid construct encoding a genetically engineered antigen receptor; expanding the transduced cells *in vitro*; and/or infusing the expanded cells into the patient, where the engineered T cells will seek and destroy antigen positive tumor cells. In some embodiments, the nucleic acid construct further includes a sequence encoding an inhibitory protein. In some
15 embodiments, these engineered T cells can block PD-1/PD-L1 immunosuppression and strengthen the antitumor immune response. The method further comprises: transfection of T cells with the viral vector containing the nucleic acid construct.

In some embodiments, the methods involve introducing any vectors described herein into a cell *in vitro* or *ex vivo*. In some embodiments, the vector is a viral vector and the introducing is carried out by transduction. In some embodiments, the methods further involve introducing into
20 the cell one or more agent, wherein each of the one or more agent is independently capable of inducing a genetic disruption of a T cell receptor alpha constant (TRAC) gene and/or a T cell receptor beta constant (TRBC) gene. In some embodiments, the one or more agent is an inhibitory nucleic acid (e.g., siRNA). In some embodiments, the one or more agent is a fusion protein comprising a DNA-targeting protein and a nuclease or an RNA-guided nuclease (e.g., a
25 clustered regularly interspaced short palindromic nucleic acid (CRISPR)-associated nuclease).

The transfection of T cells can be achieved by using any standard method such as calcium phosphate, electroporation, liposomal mediated transfer, microinjection, biolistic particle delivery system, or any other known methods by skilled artisan. In some embodiments, transfection of T cells is performed using the calcium phosphate method.

30 According to various embodiments described herein, the present disclosure provides an immunotherapy against tumors, particularly HPV associated cancers. The engineered T cells

recognize a tumor associated HPV antigen and simultaneously secrete a single-chain antibody (scFv) fusion protein that blocks Programmed Cell Death Protein 1 (PD-1). These engineered T cells demonstrate a stronger antitumor response and reduced T cell exhaustion. It has been found experimentally that PD-1 checkpoint blockade is more effective in the methods described herein because anti-PD-1 agent delivery is localized to the tumor site, thus has a higher concentration at the tumor site. Also, toxicity due to non-specific inflammation is reduced because anti-PD-1 drug delivery is localized to the tumor site. The present disclosure provides that combination of anti-HPV TCR and anti-PD-1 antibody improves T cell activation and/or prevents T cell exhaustion compared to existing alternatives.

The present disclosure provides a method to create a personalized anti-tumor immunotherapy. Genetically engineered T cells can be produced from a patient's blood cells. These engineered T cells are then reinfused into the patient as a cellular therapy product. This product can be applied to any patient who has an HPV associated tumor, including, but are not limited to cervical cancer, vulvar cancer, vaginal cancer, penile cancer, anal cancer, and oropharyngeal cancer.

Methods of preparing engineered cells and administering these engineered cells to a subject are known in the art, and are described e.g., in US Pat. No. 10,174,098 and Draper *et al.* "Targeting Of HPV-16+ Epithelial Cancer Cells By Tcr Gene Engineered t Cells Directed Against e6." Clinical Cancer Research 21.19 (2015): 4431-4439, both of which are incorporated by reference in their entirety.

METHODS OF TREATMENT

The methods disclosed herein can be used for various therapeutic purposes. In one aspect, the disclosure provides methods for treating a cancer in a subject, methods of reducing the rate of the increase of volume of a tumor in a subject over time, methods of reducing the risk of developing a metastasis, or methods of reducing the risk of developing an additional metastasis in a subject. In some embodiments, the treatment can halt, slow, retard, or inhibit progression of a cancer. In some embodiments, the treatment can result in the reduction of in the number, severity, and/or duration of one or more symptoms of the cancer in a subject.

In one aspect, the disclosure features methods that include administering a therapeutically effective amount of engineered cells expressing TCR, antigen binding fragments thereof, and

TCR-derived binding molecules to a subject in need thereof (e.g., a subject having, or identified or diagnosed as having, a cancer), e.g., an HPV-associated cancer. In some embodiments, the HPV-associated cancer is cervical cancer, head and neck cancer, oropharyngeal cancers, anal cancer, penile cancer, vaginal cancer or vulvar cancer.

5 In some embodiments, the subject has a solid tumor. In some embodiments, the subject has breast cancer (e.g., triple-negative breast cancer), carcinoid cancer, cervical cancer, endometrial cancer, glioma, head and neck cancer, liver cancer, lung cancer, small cell lung cancer, lymphoma, melanoma, ovarian cancer, pancreatic cancer, prostate cancer, renal cancer, colorectal cancer, gastric cancer, testicular cancer, thyroid cancer, bladder cancer, urethral cancer,
10 or hematologic malignancy. In some embodiments, the cancer is unresectable melanoma or metastatic melanoma, non-small cell lung carcinoma (NSCLC), small cell lung cancer (SCLC), bladder cancer, or metastatic hormone-refractory prostate cancer.

In some embodiments, the compositions and methods disclosed herein can be used for treatment of patients at risk for a cancer. Patients with cancer can be identified with various
15 methods known in the art.

Furthermore, the disclosure provides methods for treating infection or infection associated conditions in a subject. In some embodiments, the treatment can halt, slow, retard, or inhibit progression of the disease. These methods generally involve administering a therapeutically effective amount of genetic engineered cells disclosed herein to a subject in need
20 thereof. In some embodiments, the disease or condition treated is an infectious disease or condition, such as, but not limited to, viral, retroviral, bacterial, and protozoal infections, immunodeficiency, Human Papilloma Virus (HPV), Cytomegalovirus (CMV), Epstein-Barr virus (EBV), adenovirus, BK polyomavirus.

As used herein, by an "effective amount" is meant an amount or dosage sufficient to
25 effect beneficial or desired results including halting, slowing, retarding, or inhibiting progression of a disease, e.g., a cancer. An effective amount will vary depending upon, e.g., an age and a body weight of a subject to which the therapeutic agent and/or therapeutic compositions is to be administered, a severity of symptoms and a route of administration, and thus administration can be determined on an individual basis.

30 As used herein, the term "delaying development of a disease" refers to defer, hinder, slow, retard, stabilize, suppress and/or postpone development of the disease (such as cancer). This

delay can be of varying lengths of time, depending on the history of the disease and/or individual being treated. As is evident to one skilled in the art, a sufficient or significant delay can, in effect, encompass prevention, in that the individual does not develop the disease. For example, a late stage cancer, such as development of metastasis, can be delayed.

5 An effective amount can be administered in one or more administrations. By way of example, an effective amount of a composition is an amount sufficient to ameliorate, stop, stabilize, reverse, inhibit, slow and/or delay progression of a cancer in a patient or is an amount sufficient to ameliorate, stop, stabilize, reverse, slow and/or delay proliferation of a cell (e.g., a biopsied cell, any of the cancer cells described herein, or cell line (e.g., a cancer cell line)) *in*
10 *vitro*. As is understood in the art, an effective may vary, depending on, *inter alia*, patient history as well as other factors such as the type (and/or dosage) of compositions used.

Effective amounts and schedules for administrations may be determined empirically, and making such determinations is within the skill in the art. Those skilled in the art will understand that the dosage that must be administered will vary depending on, for example, the mammal that
15 will receive the treatment, the route of administration, the particular type of therapeutic agents and other drugs being administered to the mammal. Guidance in selecting appropriate doses can be found in the literature. In addition, a treatment does not necessarily result in the 100% or complete treatment or prevention of a disease or a condition. There are multiple treatment/prevention methods available with a varying degree of therapeutic effect which one of
20 ordinary skill in the art recognizes as a potentially advantageous therapeutic mean.

In some aspects, the present disclosure also provides methods of diagnosing a disease/condition in a mammal, wherein the TCRs, antigen binding fragments, TCR-derived binding molecules interact with the sample(s) obtained from a subject to form a complex, wherein the sample can comprise one more cells, polypeptides, proteins, nucleic acids,
25 antibodies, or antigen binding portions, blood, whole cells, lysates thereof, or a fraction of the whole cell lysates, e.g., a nuclear or cytoplasmic fraction, a whole protein fraction, or a nucleic acid fraction thereof, wherein the detection of the complex is the indicative of presence of a condition in the mammal, wherein the condition is cancer, HPV infection, or HPV-positive premalignancy. Further, the detection of the complex can be in any number of way known in the
30 art but not limited to, ELISA, Flow cytometry, Fluorescence in situ hybridization (FISH), Polymerase chain reaction (PCR), microarray, southern blotting, electrophoresis, Phage analysis,

chromatography and more. Thus, the treatment methods can further include determining whether a subject can benefit from a treatment as disclosed herein, e.g., by determining whether the subject has HPV infection or HPV associated cancer.

In any of the methods described herein, the engineered cells and, and/or at least one additional therapeutic agent can be administered to the subject at least once a week (e.g., once a week, twice a week, three times a week, four times a week, once a day, twice a day, or three times a day). In some embodiments, at least two different engineered cells (e.g., cells express different binding molecules) are administered in the same composition (e.g., a liquid composition). In some embodiments, engineered cells and at least one additional therapeutic agent are administered in the same composition (e.g., a liquid composition). In some embodiments, engineered cells and the at least one additional therapeutic agent are administered in two different compositions. In some embodiments, the at least one additional therapeutic agent is administered as a pill, tablet, or capsule. In some embodiments, the at least one additional therapeutic agent is administered in a sustained-release oral formulation.

In some embodiments, the one or more additional therapeutic agents can be administered to the subject prior to, concurrently with, or after administering the engineered cells to the subject.

In some embodiments, one or more additional therapeutic agents can be administered to the subject. The additional therapeutic agent can be a checkpoint inhibitor (CPI). In some embodiments, the checkpoint inhibitor is an inhibitory protein, e.g., an antibody or antigen binding fragment thereof. The checkpoint inhibitor can inhibit or block one or more immune checkpoints, including e.g., PD-1, PD-L1, PD-L2, 2B4 (CD244), 4-1BB, A2aR, B7.1, B7.2, B7-H2, B7-H3, B7-H4, B7-H6, BTLA, butyrophilins, CD160, CD48, CTLA4, GITR, gp49B, HHLA2, HVEM, ICOS, ILT-2, ILT-4, KIR family receptors, LAG-3, OX-40, PIR-B, SIRPalpha (CD47), TFM-4, TIGIT, TIM-1, TIM-3, TIM-4, VISTA and combinations thereof. In some embodiments, the inhibitory protein blocks PD-1 or PD-L1. In various embodiments, the inhibitory protein comprises an anti-PD-1 scFv. The inhibitory protein is capable of leading to reduced expression of PD-1 or PD-L1 and/or inhibiting upregulation of PD-1 or PD-L1 in T cells in the population and/or physically obstructing the formation of the PD-1/PD-L1 complex and subsequent signal transduction. In some embodiments, the inhibitory protein blocks PD-1. In some embodiments, the additional therapeutic agent is an anti-OX40 antibody, an anti-PD-L1

antibody, an anti-PD-L2 antibody, an anti-LAG-3 antibody, an anti-TIGIT antibody, an anti-BTLA antibody, an anti-CTLA-4 antibody, or an anti-GITR antibody. In some embodiments, the additional therapeutic agent is an anti-CTLA4 antibody (e.g., ipilimumab), an anti-CD20 antibody (e.g., rituximab), an anti-EGFR antibody (e.g., cetuximab), an anti-CD319 antibody (e.g., elotuzumab), or an anti-PD1 antibody (e.g., nivolumab).

In some embodiments, the additional therapeutic agent is a bifunctional trap fusion protein. Bifunctional trap proteins can target both immune checkpoints and TGF- β negative regulatory pathways. In addition to expression of immune checkpoints, the tumor microenvironment contains other immunosuppressive molecules. Of particular interest is the cytokine TGF- β (TGFB), which has multiple functions in cancer. TGF- β prevents proliferation and promotes differentiation and apoptosis of tumor cells early in tumor development. However, during tumor progression, tumor TGF- β insensitivity arises due to the loss of TGF- β receptor expression or mutation to downstream signaling elements. TGF- β then promotes tumor progression through its effects on angiogenesis, induction of epithelial-to-mesenchymal transition (EMT), and immune suppression. High TGF- β serum level and loss of TGF- β receptor (TGF β R) expression on tumors correlates with poor prognosis. TGF β -targeted therapies have demonstrated limited clinical activity. In some embodiments, the bifunctional trap protein targets both the PD-1 and TGF- β . In some embodiments, the bifunctional trap protein targets both the PD-L1 and TGF- β . In some embodiments, the bifunctional fusion protein designed to block PD-L1 and sequester TGF- β . M7824 (MSB0011395C) comprises the extracellular domain of human TGF- β receptor II (TGF β RII) linked to the C-terminus of the human anti-PD-L1 scFv, based on the human IgG1 monoclonal antibody (mAb) avelumab. In some embodiments, the bifunctional fusion protein comprises the extracellular domain of human TGF- β receptor II (TGF β RII) linked to the C-terminus of the human anti-PD-1 scFv. These bifunctional trap fusion proteins are described e.g., Knudson, *et al.* "M7824, a novel bifunctional anti-PD-L1/TGF β Trap fusion protein, promotes anti-tumor efficacy as monotherapy and in combination with vaccine." *Oncoimmunology* 7.5 (2018): e1426519, which is incorporated herein by reference in its entirety. In some embodiments, the subject is treated by cells that express TCR or antigen-binding molecules as described herein and one or more bifunctional trap fusion proteins.

In some embodiments, the additional therapeutic agent can comprise one or more inhibitors selected from the group consisting of an inhibitor of B-Raf, an EGFR inhibitor, an

inhibitor of a MEK, an inhibitor of ERK, an inhibitor of K-Ras, an inhibitor of c-Met, an inhibitor of anaplastic lymphoma kinase (ALK), an inhibitor of a phosphatidylinositol 3-kinase (PI3K), an inhibitor of an Akt, an inhibitor of mTOR, a dual PI3K/mTOR inhibitor, an inhibitor of Bruton's tyrosine kinase (BTK), and an inhibitor of Isocitrate dehydrogenase 1 (IDH1) and/or Isocitrate dehydrogenase 2 (IDH2). In some embodiments, the additional therapeutic agent is an inhibitor of indoleamine 2,3-dioxygenase-1 (IDO1) (e.g., epacadostat). In some embodiments, the additional therapeutic agent can comprise one or more inhibitors selected from the group consisting of an inhibitor of HER3, an inhibitor of LSD1, an inhibitor of MDM2, an inhibitor of BCL2, an inhibitor of CHK1, an inhibitor of activated hedgehog signaling pathway, and an agent that selectively degrades the estrogen receptor.

In some embodiments, the additional therapeutic agent can comprise one or more therapeutic agents selected from the group consisting of Trabectedin, nab-paclitaxel, Trebananib, Pazopanib, Cediranib, Palbociclib, everolimus, fluoropyrimidine, IFL, regorafenib, Reolysin, Alimta, Zykadia, Sutent, temsirolimus, axitinib, everolimus, sorafenib, Votrient, Pazopanib, IMA-901, AGS-003, cabozantinib, Vinflunine, an Hsp90 inhibitor, Ad-GM-CSF, Temazolomide, IL-2, IFN α , vinblastine, Thalomid, dacarbazine, cyclophosphamide, lenalidomide, azacytidine, lenalidomide, bortezomid, amrubicine, carfilzomib, pralatrexate, and enzastaurin.

In some embodiments, the additional therapeutic agent can comprise one or more therapeutic agents selected from the group consisting of an adjuvant, a TLR agonist, tumor necrosis factor (TNF) α , IL-1, HMGB1, an IL-10 antagonist, an IL-4 antagonist, an IL-13 antagonist, an IL-17 antagonist, an HVEM antagonist, an ICOS agonist, a treatment targeting CX3CL1, a treatment targeting CXCL9, a treatment targeting CXCL10, a treatment targeting CCL5, an LFA-1 agonist, an ICAM1 agonist, and a Selectin agonist.

In some embodiments, carboplatin, nab-paclitaxel, paclitaxel, cisplatin, pemetrexed, gemcitabine, FOLFOX, or FOLFIRI are administered to the subject. In some embodiments, the additional therapeutic agent is selected from asparaginase, busulfan, carboplatin, cisplatin, daunorubicin, doxorubicin, fluorouracil, gemcitabine, hydroxyurea, methotrexate, paclitaxel, rituximab, vinblastine, vincristine and/or combinations thereof.

COMPOSITIONS AND FORMULATIONS

The present disclosure provides compositions (including pharmaceutical and therapeutic

compositions) containing the engineered cells and populations thereof, produced by the methods disclosed herein. Also provided are methods, e.g., therapeutic methods for administering the engineered T cells and compositions thereof to subjects, e.g., patients.

Compositions including the engineered T cells for administration, including
5 pharmaceutical compositions and formulations, such as unit dose form compositions including the number of cells for administration in a given dose or fraction thereof are provided. The pharmaceutical compositions and formulations can include one or more optional pharmaceutically acceptable carrier or excipient. In some embodiments, the composition includes at least one additional therapeutic agent.

10 A pharmaceutically acceptable carrier refers to an ingredient in a pharmaceutical composition, other than an active ingredient. The pharmaceutically acceptable carrier does not interfere with the active ingredient and is nontoxic to a subject. A pharmaceutically acceptable carrier can include, but is not limited to, a buffer, excipient, stabilizer, or preservative. The pharmaceutical formulation refers to process in which different substances and/or agents are
15 combined to produce a final medicinal product. The formulation studies involve developing a preparation of drug acceptable for patient. Additionally, a preparation which is in such form as to permit the biological activity of an active ingredient contained therein to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered.

20 In some embodiments, the choice of carrier is determined in part by the particular cell (e.g., T cell or NK cell) and/or by the method of administration. A variety of suitable formulations are available. For example, the pharmaceutical composition can contain preservatives. Suitable preservatives can include, for example, methylparaben, propylparaben, sodium benzoate, and benzalkonium chloride. In some embodiments, a mixture of two or more
25 preservatives is used. The preservative or mixtures thereof are typically present in an amount of about 0.0001% to about 2% by weight of the total composition. Carriers are described, e.g., by Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980). Pharmaceutically acceptable carriers are generally nontoxic to recipients at the dosages and concentrations employed, and include, but are not limited to: buffers such as phosphate, citrate, and other
30 organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride;

benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as
5 glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants such as polyethylene glycol (PEG).

10 Suitable buffering agents include, for example, citric acid, sodium citrate, phosphoric acid, potassium phosphate, and various other acids and salts. In some embodiments, a mixture of two or more buffering agents is used. The buffering agent or mixtures thereof are typically present in an amount of about 0.001% to about 4% by weight of the total composition. Methods for preparing administrable pharmaceutical compositions are known. Exemplary methods are
15 described in more detail in, for example, Remington: The Science and Practice of Pharmacy, Lippincott Williams & Wilkins; 21st ed. (May 1, 2005).

The formulations can include aqueous solutions. The formulation or composition can also contain more than one active ingredient useful for a particular indication, disease, or condition being treated with the engineered cells, preferably those with activities complementary
20 to the cells, where the respective activities do not adversely affect one another. Such active ingredients are suitably present in combination in amounts that are effective for the purpose intended. Thus, in some embodiments, the pharmaceutical composition can further include other pharmaceutically active agents or drugs, such as checkpoint inhibitors, fusion proteins, chemotherapeutic agents, e.g., asparaginase, busulfan, carboplatin, cisplatin, daunorubicin,
25 doxorubicin, fluorouracil, gemcitabine, hydroxyurea, methotrexate, paclitaxel, rituximab, vinblastine, and/or vincristine.

The pharmaceutical composition in some embodiments contains the cells in amounts effective to treat or prevent the disease or condition, such as a therapeutically effective or prophylactically effective amount. Therapeutic or prophylactic efficacy in some embodiments is
30 monitored by periodic assessment of treated subjects. The desired dosage can be delivered by a single bolus administration of the cells, by multiple bolus administrations of the cells, or by

continuous infusion administration of the cells.

The cells and compositions can be administered using standard administration techniques, formulations, and/or devices. Administration of the cells can be autologous or heterologous. For example, immunoresponsive T cells or progenitors can be obtained from one
5 subject, and administered to the same subject or a different, compatible subject after genetically modifying them in accordance with various embodiments described herein. Peripheral blood derived immunoresponsive T cells or their progeny (e.g., *in vivo*, *ex vivo* or *in vitro* derived) can be administered via localized injection, including catheter administration, systemic injection, localized injection, intravenous injection, or parenteral administration. Usually, when
10 administering a therapeutic composition (e.g., a pharmaceutical composition containing a genetically modified immunoresponsive cell), it is generally formulated in a unit dosage injectable form (solution, suspension, emulsion).

Formulations disclosed herein include those for oral, intravenous, intraperitoneal, subcutaneous, pulmonary, transdermal, intramuscular, intranasal, buccal, sublingual, or
15 suppository administration. In some embodiments, the cell populations are administered parenterally. The term “parenteral,” as used herein, includes intravenous, intramuscular, subcutaneous, rectal, vaginal, and intraperitoneal administration. In some embodiments, the cells are administered to the subject using peripheral systemic delivery by intravenous, intraperitoneal, or subcutaneous injection.

20 The compositions in some embodiments are provided as sterile liquid preparations, e.g., isotonic aqueous solutions, suspensions, emulsions, dispersions, or viscous compositions, which can in some aspects be buffered to a selected pH. Liquid preparations are normally easier to prepare than gels, other viscous compositions, and solid compositions. Additionally, liquid compositions are somewhat more convenient to administer, especially by injection. Viscous
25 compositions, on the other hand, can be formulated within the appropriate viscosity range to provide longer contact periods with specific tissues. Liquid or viscous compositions can comprise carriers, which can be a solvent or dispersing medium containing, for example, water, saline, phosphate buffered saline, polyol (for example, glycerol, propylene glycol, liquid polyethylene glycol) and suitable mixtures thereof.

30 Sterile injectable solutions can be prepared by incorporating the cells in a solvent, such as in admixture with a suitable carrier, diluent, or excipient such as sterile water, physiological

saline, glucose, dextrose, or the like. The compositions can contain auxiliary substances such as wetting, dispersing, or emulsifying agents (e.g., methylcellulose), pH buffering agents, gelling or viscosity enhancing additives, preservatives, flavoring agents, and/or colors, depending upon the route of administration and the preparation desired. Standard texts can in some aspects be consulted to prepare suitable preparations.

Various additives which enhance the stability and sterility of the compositions, including antimicrobial preservatives, antioxidants, chelating agents, and buffers, can be added. Prevention of the action of microorganisms can be ensured by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, and sorbic acid. Prolonged absorption of the injectable pharmaceutical form can be brought about by the use of agents delaying absorption, for example, aluminum monostearate and gelatin.

The formulations to be used for in vivo administration are generally sterile. Sterility can be readily accomplished, e.g., by filtration through sterile filtration membranes.

The compositions or pharmaceutical compositions as described herein can be included in a container, pack, or dispenser together with instructions for administration.

METHODS OF ADMINISTRATION

Provided are also methods of administering the cells, populations, and compositions, and uses of such cells, populations, and compositions to treat or prevent diseases, conditions, and disorders, including cancers. In some embodiments, the methods described herein can reduce the risk of the developing diseases, conditions, and disorders as described herein.

In some embodiments, the cells, populations, and compositions, described herein are administered to a subject or patient having a particular disease or condition to be treated, e.g., via adoptive cell therapy, such as adoptive T cell therapy. In some embodiments, cells and compositions prepared by the provided methods, such as engineered compositions and end-of-production compositions following incubation and/or other processing steps, are administered to a subject, such as a subject having or at risk for the disease or condition. In some aspects, the methods thereby treat, e.g., ameliorate one or more symptom of, the disease or condition, such as by lessening tumor burden in cancer expressing an antigen recognized by the engineered T cells.

Methods for administration of cells for adoptive cell therapy are known and can be used in connection with the provided methods and compositions. For example, adoptive T cell therapy

methods are described, e.g., in U.S. 2003/0170238; U.S. Pat. No. 4,690,915; Rosenberg, "Cell transfer immunotherapy for metastatic solid cancer—what clinicians need to know." *Nature reviews Clinical oncology* 8.10 (2011): 577; Themeli *et al.* "Generation of tumor-targeted human T lymphocytes from induced pluripotent stem cells for cancer therapy." *Nature biotechnology* 31.10 (2013): 928; Tsukahara *et al.* "CD19 target-engineered T-cells accumulate at tumor lesions in human B-cell lymphoma xenograft mouse models." *Biochemical and biophysical research communications* 438.1 (2013): 84-89; Davila *et al.* "CD19 CAR-targeted T cells induce long-term remission and B Cell Aplasia in an immunocompetent mouse model of B cell acute lymphoblastic leukemia." *PloS one* 8.4 (2013); each of which is incorporated herein by reference in its entirety.

In some embodiments, the cell therapy, e.g., adoptive T cell therapy, is carried out by autologous transfer, in which the T cells are isolated and/or otherwise prepared from the subject who is to receive the cell therapy, or from a sample derived from such a subject. Thus, in some aspects, the cells are derived from a subject, e.g., patient, in need of a treatment and the cells, following isolation and processing are administered to the same subject.

In some embodiments, the cell therapy, e.g., adoptive T cell therapy, is carried out by allogeneic transfer, in which the T cells are isolated and/or otherwise prepared from a subject other than a subject who is to receive or who ultimately receives the cell therapy, e.g., a first subject. In such embodiments, the cells then are administered to a different subject, e.g., a second subject, of the same species. In some embodiments, the first and second subjects are genetically identical. In some embodiments, the first and second subjects are genetically similar. In some embodiments, the second subject expresses the same HLA class or supertype as the first subject.

In some embodiments, the subject has been treated with a therapeutic agent targeting the disease or condition, e.g. the tumor, prior to administration of the cells or composition containing the cells. In some aspects, the subject is refractory or non-responsive to the other therapeutic agent. In some embodiments, the subject has persistent or relapsed disease, e.g., following treatment with another therapeutic intervention, including chemotherapy, radiation, and/or hematopoietic stem cell transplantation (HSCT), e.g., allogenic HSCT. In some embodiments, the administration effectively treats the subject despite the subject having become resistant to another therapy.

In some embodiments, the subject is responsive to the other therapeutic agent, and

treatment with the therapeutic agent reduces disease burden. In some aspects, the subject is initially responsive to the therapeutic agent, but exhibits a relapse of the disease or condition over time. In some embodiments, the subject has not relapsed. In some such embodiments, the subject is determined to be at risk for relapse, such as at high risk of relapse, and thus the cells are administered prophylactically, e.g., to reduce the likelihood of or prevent relapse. In some
5 embodiments, the subject has not received prior treatment with another therapeutic agent.

In some embodiments, the cells are administered at a desired dosage, which in some aspects includes a desired dose or number of cells or cell type(s) and/or a desired ratio of cell types. Thus, the dosage of cells in some embodiments is based on a total number of cells (or
10 number per kg body weight) and a desired ratio of the individual populations or sub-types, such as the CD4⁺ to CD8⁺ ratio. In some embodiments, the dosage of cells is based on a desired total number (or number per kg of body weight) of cells in the individual populations or of individual cell types. In some embodiments, the dosage is based on a combination of such features, such as a desired number of total cells, desired ratio, and desired total number of cells in the individual
15 populations.

In some embodiments, the populations or sub-types of cells, such as CD8⁺ and CD4⁺ T cells, are administered at or within a tolerated difference of a desired dose of total cells, such as a desired dose of T cells. In some embodiments, the desired dose is a desired number of cells or a desired number of cells per unit of body weight of the subject to whom the cells are
20 administered, e.g., cells/kg. In some embodiments, the desired dose is at or above a minimum number of cells or minimum number of cells per unit of body weight. In some embodiments, among the total cells, administered at the desired dose, the individual populations or sub- types are present at or near a desired output ratio (such as CD4⁺ to CD8⁺ ratio), e.g., within a certain tolerated difference or error of such a ratio.

In some embodiments, the cells are administered at or within a tolerated difference of a desired dose of one or more of the individual populations or sub-types of cells, such as a desired dose of CD4⁺ cells and/or a desired dose of CD8⁺ cells. In some embodiments, the desired dose is a desired number of cells of the sub-type or population, or a desired number of such cells per unit of body weight of the subject to whom the cells are administered, e.g., cells/kg. In some
25 embodiments, the desired dose is at or above a minimum number of cells of the population or sub-type, or minimum number of cells of the population or sub-type per unit of body weight.
30

Thus, in some embodiments, the dosage is based on a desired fixed dose of total cells and a desired ratio, and/or based on a desired fixed dose of one or more, e.g., each, of the individual sub-types or sub-populations. Thus, in some embodiments, the dosage is based on a desired fixed or minimum dose of T cells and a desired ratio of CD4+ to CD8+ cells, and/or is
 5 based on a desired fixed or minimum dose of CD4+ and/or CD8+ cells.

In certain embodiments, the cells or individual populations of sub-types of cells, are administered to the subject at a range of about one million to about 100 billion cells, such as, e.g., 1 million to about 50 billion cells (e.g., about 5 million cells, about 25 million cells, about 500 million cells, about 1 billion cells, about 5 billion cells, about 20 billion cells, about 30 billion
 10 cells, about 40 billion cells, or a range defined by any two of the foregoing values), such as about 10 million to about 100 billion cells (e.g., about 20 million cells, about 30 million cells, about 40 million cells, about 60 million cells, about 70 million cells, about 80 million cells, about 90 million cells, about 10 billion cells, about 25 billion cells, about 50 billion cells, about 75 billion cells, about 90 billion cells, or a range defined by any two of the foregoing values), and in
 15 some cases about 100 million cells to about 50 billion cells (e.g., about 120 million cells, about 250 million cells, about 350 million cells, about 450 million cells, about 650 million cells, about 800 million cells, about 900 million cells, about 3 billion cells, about 30 billion cells, about 45 billion cells) or any value in between these ranges.

In some embodiments, the dose of total cells and/or dose of individual sub-populations of cells is within a range of between at or about 10^4 and at or about 10^9 cells/kilograms (kg) body weight, such as between 10^5 and 10^6 cells/kg body weight, for example, at least or at least about or at or about 1×10^5 cells/kg, 1.5×10^5 cells/kg, 2×10^5 cells/kg, or 1×10^6 cells/kg body weight. For example, in some embodiments, the cells are administered at, or within a certain range of error of, between at or about 10^4 and at or about 10^9 T cells/kilograms (kg) body weight, such as
 20 between 10^5 and 10^6 T cells/kg body weight, for example, at least or at least about or at or about 1×10^5 T cells/kg, 1.5×10^5 T cells/kg, 2×10^5 T cells/kg, or 1×10^6 T cells/kg body weight.

In some embodiments, the cells are administered at or within a certain range of error of between at or about 10^4 and at or about 10^9 CD4+ and/or CD8+ cells/kilograms (kg) body weight, such as between 10^5 and 10^6 CD4+ and/or CD8+ cells/kg body weight, for example, at
 30 least or at least about or at or about 1×10^5 CD4+ and/or CD8+ cells/kg, 1.5×10^5 CD4+ and/or CD8+ cells/kg, 2×10^5 CD4+ and/or CD8+ cells/kg, or 1×10^6 CD4+ and/or CD8+ cells/kg body

weight.

In some embodiments, the cells are administered at or within a certain range of error of, greater than, and/or at least about 1×10^6 , about 2.5×10^6 , about 5×10^6 , about 7.5×10^6 , or about 9×10^6 CD4+ cells, and/or at least about 1×10^6 , about 2.5×10^6 , about 5×10^6 , about 7.5×10^6 , or about 9×10^6 CD8+ cells, and/or at least about 1×10^6 , about 2.5×10^6 , about 5×10^6 , about 7.5×10^6 , or about 9×10^6 T cells. In some embodiments, the cells are administered at or within a certain range of error of between about 10^8 and 10^{12} or between about 10^{10} and 10^{11} T cells, between about 10^8 and 10^{12} or between about 10^{10} and 10^{11} CD4+ cells, and/or between about 10^8 and 10^{12} or between about 10^{10} and 10^{11} CD8+ cells.

In some embodiments, the cells are administered at or within a tolerated range of a desired output ratio of multiple cell populations or sub-types, such as CD4+ and CD8+ cells or sub- types. In some aspects, the desired ratio can be a specific ratio or can be a range of ratios. for example, in some embodiments, the desired ratio (e.g., ratio of CD4+ to CD8+ cells) is between at or about 1:5 and at or about 5:1 (or greater than about 1:5 and less than about 5:1), or between at or about 1:3 and at or about 3:1 (or greater than about 1:3 and less than about 3:1), such as between at or about 2:1 and at or about 1:5 (or greater than about 1:5 and less than about 2:1, such as at or about 5:1, 4.5:1, 4:1, 3.5:1, 3:1, 2.5:1, 2:1, 1.9:1, 1.8:1, 1.7:1, 1.6:1, 1.5:1, 1.4:1, 1.3:1, 1.2:1, 1.1:1, 1:1, 1:1.1, 1:1.2, 1:1.3, 1:1.4, 1:1.5, 1:1.6, 1:1.7, 1:1.8, 1:1.9, 1:2, 1:2.5, 1:3, 1:3.5, 1:4, 1:4.5, or 1:5. In some aspects, the tolerated difference is within about 1%, about 2%, about 3%, about 4%, about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50% of the desired ratio, including any value in between these ranges. In some aspects, the TCR described here provides improved expression and activity, thereby providing therapeutic effects even at a low effector to target (E:T) ratio.

Optimal response to therapy can depend on the ability of the engineered recombinant receptors such as TCRs, to be consistently and reliably expressed on the surface of the cells and/or bind the target antigen. For example, in some cases, properties of certain recombinant receptors, e.g., TCRs, can affect the expression and/or activity of the recombinant receptor, in some cases when expressed in a cell, such as a human T cell, used in cell therapy. In some contexts, the level of expression of particular recombinant receptors, e.g., TCRs, can be low, and activity of the engineered cells, such as human T cells, expressing such recombinant receptors, may be limited due to poor expression or poor signaling activity. In some cases, consistency

and/or efficiency of expression of the recombinant receptor, and activity of the receptor is limited in certain cells or certain cell populations of available therapeutic approaches. In some cases, a large number of engineered T cells (a high effector to target (E:T) ratio) is required to exhibit functional activity. In some embodiments, the desired ratio (E:T ratio) is between at or about 1:10 and at or about 10:1 (or greater than about 1:10 and less than about 10:1), or between at or about 1:1 and at or about 10:1 (or greater than about 1:1 and less than about 5:1), such as between at or about 2:1 and at or about 10:1. In some embodiments, the E:T ratio is greater than or about 1:1, 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1, or 10:1.

For the prevention or treatment of disease, the appropriate dosage may depend on the type of disease to be treated, the type of cells or recombinant receptors, the severity and course of the disease, whether the cells are administered for preventive or therapeutic purposes, previous therapy, the subject's clinical history and response to the cells, and the discretion of the attending physician. The compositions and cells are in some embodiments suitably administered to the subject at one time or over a series of treatments.

The cells described herein can be administered by any suitable means, for example, by bolus infusion, by injection, e.g., intravenous or subcutaneous injections, intraocular injection, periocular injection, subretinal injection, intravitreal injection, trans-septal injection, subcleral injection, intrachoroidal injection, intracameral injection, subconjunctival injection, subconjunctival injection, sub-Tenon's injection, retrobulbar injection, peribulbar injection, or posterior juxtasceral delivery. In some embodiments, they are administered by parenteral, intrapulmonary, and intranasal, and, if desired for local treatment, intralesional administration. Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous administration. In some embodiments, a given dose is administered by a single bolus administration of the cells. In some embodiments, it is administered by multiple bolus administrations of the cells, for example, over a period of no more than 3 days, or by continuous infusion administration of the cells.

In some embodiments, the cells are administered as part of a combination treatment, such as simultaneously with or sequentially with, in any order, another therapeutic intervention, such as an antibody or engineered cell or receptor or agent, such as a cytotoxic or therapeutic agent. The cells in some embodiments are co-administered with one or more additional therapeutic agents or in connection with another therapeutic intervention, either simultaneously

or sequentially in any order. In some contexts, the cells are co-administered with another therapy sufficiently close in time such that the cell populations enhance the effect of one or more additional therapeutic agents, or vice versa. In some embodiments, the cells are administered prior to the one or more additional therapeutic agents. In some embodiments, the cells are administered after the one or more additional therapeutic agents. In some embodiments, the one or more additional agents includes a cytokine, such as IL-2, for example, to enhance persistence. In some embodiments, the methods comprise administration of a chemotherapeutic agent.

Following administration of the cells, the biological activity of the engineered cell populations in some embodiments is measured, e.g., by any of a number of known methods.

Parameters to assess include specific binding of engineered T cells to the antigen, in vivo, e.g., by imaging, or ex vivo, e.g., by ELISA or flow cytometry. In certain embodiments, the ability of the engineered cells to destroy target cells can be measured using any suitable method known in the art, such as cytotoxicity assays described in, for example, Kochenderfer *et al.* "Construction and pre-clinical evaluation of an anti-CD19 chimeric antigen receptor." *Journal of immunotherapy* (Hagerstown, Md.: 1997) 32.7 (2009): 689 and Hermans *et al.* "The VITAL assay: a versatile fluorometric technique for assessing CTL-and NKT-mediated cytotoxicity against multiple targets in vitro and in vivo." *Journal of immunological methods* 285.1 (2004): 25-40. In certain embodiments, the biological activity of the cells is measured by assaying expression and/or secretion of one or more cytokines, such as CD107a, IFN γ , IL-2, and TNF. In some aspects the biological activity is measured by assessing clinical outcome, such as reduction in tumor burden or load.

DOSING SCHEDULE AND TREATMENT REGIMENS

Repeated dosing methods are provided in which a first dose of cells is given followed by one or more second consecutive doses. The timing and size of the multiple doses of cells generally are designed to increase the efficacy and/or activity and/or function of engineered cells as described herein, when administered to a subject in adoptive therapy methods. In some embodiments, the repeated dosing reduce the downregulation or inhibiting activity that can occur when inhibitory immune molecules, such as PD-1 and/or PD-L1 are upregulated on engineered T cells. The methods involve administering a first dose, generally followed by one or more consecutive doses, with particular time frames between the different doses.

In the context of adoptive cell therapy, administration of a given “dose” encompasses administration of the given amount or number of cells as a single composition and/or single uninterrupted administration, e.g., as a single injection or continuous infusion, and also encompasses administration of the given amount or number of cells as a split dose, provided in multiple individual compositions or infusions, over a specified period of time (e.g., no more than 3 days). Thus, in some contexts, the first or consecutive dose is a single or continuous administration of the specified number of cells, given or initiated at a single point in time. In some contexts, however, the first or consecutive dose is administered in multiple injections or infusions over a limited time period (e.g., no more than three days), such as once a day for three days or for two days or by multiple infusions over a single day period.

The cells of the first dose are administered in a single pharmaceutical composition. In some embodiments, the cells of the consecutive dose are administered in a single pharmaceutical composition.

In some embodiments, the cells of the first dose are administered in a plurality of compositions, collectively containing the cells of the first dose. In some embodiments, the cells of the consecutive dose are administered in a plurality of compositions, collectively containing the cells of the consecutive dose. In some aspects, additional consecutive doses can be administered in a plurality of compositions over a period of no more than 3 days.

The term “split dose” refers to a dose that is split so that it is administered over more than one day. This type of dosing is encompassed by the present methods and is considered to be a single dose. Thus, in some embodiments, the first dose and/or consecutive dose(s) can be administered as a split dose. For example, in some embodiments, the dose can be administered to the subject over 2 days or over 3 days. Exemplary methods for split dosing include administering 25% of the dose on the first day and administering the remaining 75% of the dose on the second day. In other embodiments, 33% of the first dose can be administered on the first day and the remaining 67% administered on the second day. In some aspects, 10% of the dose is administered on the first day, 30% of the dose is administered on the second day, and 60% of the dose is administered on the third day. In some embodiments, the split dose is not spread over more than 3 days.

With reference to a prior dose, such as a first dose, the term “consecutive dose” refers to a dose that is administered to the same subject after the prior, e.g., first, dose without any

intervening doses having been administered to the subject in the interim. Nonetheless, the term does not encompass the second, third, and/or so forth, injection or infusion in a series of infusions or injections comprised within a single split dose. Thus, unless otherwise specified, a second infusion within a one, two or three-day period is not considered to be a “consecutive” dose as used herein. Likewise, a second, third, and so-forth in the series of multiple doses within a split dose also is not considered to be an “intervening” dose in the context of the meaning of “consecutive” dose. Thus, unless otherwise specified, a dose administered a certain period of time, greater than three days, after the initiation of a first or prior dose, is considered to be a “consecutive” dose even if the subject receives a second or subsequent injection or infusion of the cells following the initiation of the first dose, so long as the second or subsequent injection or infusion occurred within the three-day period following the initiation of the first or prior dose.

Thus, unless otherwise specified, multiple administrations of the same cells over a period of up to 3 days is considered to be a single dose, and administration of cells within 3 days of an initial administration is not considered a consecutive dose and is not considered to be an intervening dose for purposes of determining whether a second dose is “consecutive” to the first.

In some embodiments, multiple consecutive doses are given, in some aspects using the same timing guidelines as those with respect to the timing between the first dose and first consecutive dose, e.g., by administering a first and multiple consecutive doses, with each consecutive dose given within a period of time in which an inhibitory immune molecule, such as PD-1 and/or PD-L1, has been upregulated in cells in the subject from an administered first dose. It is within the level of a skilled artisan to empirically determine when to provide a consecutive dose, such as by assessing levels of PD-1 and/or PD-L1 in antigen-expressing, such as TCR-expressing cells, from peripheral blood or other bodily fluid.

In some embodiments, the timing between the first dose and first consecutive dose, or a first and multiple consecutive doses, is such that each consecutive dose is given within a period of time is greater than about 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, 16 days, 17 days, 18 days, 19 days, 20 days, 21 days, 22 days, 23 days, 24 days, 25 days, 26 days, 27 days, 28 days or more. In some embodiments, the consecutive dose is given within a time period that is less than about 28 days after the administration of the first or immediately prior dose. The additional multiple additional consecutive dose or doses also are

referred to as subsequent dose or subsequent consecutive dose.

The size of the first and/or one or more consecutive doses of cells are generally designed to provide improved efficacy and/or reduced risk of toxicity. In some aspects, a dosage amount or size of a first dose or any consecutive dose is any dosage or amount as described
5 above. In some embodiments, the number of cells in the first dose or in any consecutive dose is between about 0.5×10^6 cells/kg body weight of the subject and 5×10^6 cells/kg, between about 0.75×10^6 cells/kg and 3×10^6 cells/kg or between about 1×10^6 cells/kg and 2×10^6 cells/kg.

As used herein, "first dose" is used to describe the timing of a given dose being prior to the administration of a consecutive or subsequent dose. The term does not necessarily imply
10 that the subject has never before received a dose of cell therapy or even that the subject has not before received a dose of the same cells or cells expressing the same recombinant receptor or targeting the same antigen.

In some embodiments, multiple doses can be administered to a subject over an extended period of time (e.g., over a period of at least 1 week, 2 weeks, 3 weeks, 1 month, 2 months, 3
15 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 12 months, 1 year, 2 years, 3 years, 4 years, or 5 years). A skilled medical professional may determine the length of the treatment period using any of the methods described herein for diagnosing or following the effectiveness of treatment (e.g., the observation of at least one symptom of cancer).

In some embodiments, the engineered receptor, e.g., the TCR, expressed by the cells in the consecutive dose contains at least one immunoreactive epitope as the receptor, e.g., the TCR, expressed by the cells of the first dose. In some embodiments, the receptor, e.g., the TCR, expressed by the cells administered in the consecutive dose is identical to the receptor, e.g., the TCR, expressed by the first dose or is substantially identical to the receptor, e.g., the TCR,
20 expressed by the cells of administered in the first dose.

The receptors, such as TCRs, expressed by the cells administered to the subject in the various doses generally recognize or specifically bind to a molecule that is expressed in, associated with, and/or specific for the disease or condition or cells thereof being treated. Upon specific binding to the molecule, e.g., antigen, the receptor generally delivers an
25 immunostimulatory signal, such as an ITAM-transduced signal, into the cell, thereby promoting an immune response targeted to the disease or condition. For example, in some embodiments, the

cells in the first dose express a TCRs that specifically binds to an antigen expressed.

EXAMPLES

- 5 The invention is further described in the following examples, which do not limit the scope of the invention described in the claims.

EXAMPLE 1: Vector Construct, Cell Line and Media

10 For E202 TCR-T cells, an MP71 retroviral vector construct containing 2 coding regions was generated: (1) the variable region of the alpha chain of a human anti-E6 TCR fused to the constant region of the mouse TCR alpha chain; (2) the variable region of the beta chain of same human anti-E6 TCR fused to the constant region of the mouse TCR beta chain (FIG. 1). The sequence is set forth in SEQ ID NO: 20. The full vector sequence is set forth in SEQ ID NO: 26.

15 HEK-293T, Ca Ski, and T2 cells were purchased from American Type Culture Collection (ATCC). Peripheral blood mononuclear cells (PBMCs) from anonymous donors were purchased from Hemacare. Ca Ski E6/E7 cells were produced by retroviral transduction of Ca Ski cells with a vector overexpressing human E6 and E7. Cells were cultured in DMEM (Dulbecco's Modified Eagle Medium) + 10% FBS (Fetal Bovine Serum), or RPMI (Roswell Park Memorial Institute medium) + 10% FBS.

20

EXAMPLE 2: Retroviral vector production, T cell transduction and expansion, and TCR Staining

25 Retroviral vectors were prepared by transient transfection of HEK-293T cells using a standard calcium phosphate precipitation protocol. Viral supernatants were harvested after 48 hours and used to transduce T cells. Before retroviral transduction, PBMCs were activated for 2 days by culturing with T cell activator beads and human IL-2. For transduction, freshly harvested retroviral supernatant was spin-loaded onto non-tissue culture-treated 24-well plates coated with 15 µg RetroNectin per/well (Clontech Laboratories) by centrifuging for 2 hours at 2,000 g at 32°C. Activated PBMCs were loaded onto the plates and spun at 600 g at 32°C for 30 minutes.

30 T cells were incubated at 37°C and 5% CO₂. Culture medium was replenished every 2 days. All antibodies were purchased from Biolegend. Expression of the recombinant TCR was

detected 48 hours after transduction. Mouse TCR beta chain was stained by an antibody, followed by flow cytometry analysis. CD3, CD4, and CD8 staining was performed simultaneously. The results showed that the anti-E6 TCR was abundantly expressed in human T cells (FIGS. 2A-2B).

5

EXAMPLE 3: *In vitro* TCR-T intracellular IFN- γ production

TCR-T cells were co-cultured with HPV peptide pulsed T2 cells at various effector-to-target ratios. Intracellular IFN- γ expression was measured by flow cytometry according to the manufacturer's instructions. It was found that TCR-T cells containing the anti-E6 TCR can be specifically activated by target cells, which can be measured by intracellular IFN- γ expression (FIGS. 3A-3B).

10

EXAMPLE 4: EC₅₀ of E202 TCR via peptide titration

Further TCR-T cells were co-cultured overnight with different concentrations of HPV peptides pulsed into T2 antigen presenting cells. TCR-T cells and APCs cells were co-cultured at a 1:1 effector-to-target ratio. The T cells were then collected and the intracellular IFN- γ expression was measured to determine the EC₅₀.

15

As shown in FIG. 4, E202 TCR-T cells recognized APC pulsed with E6 peptide at an EC₅₀ of 0.045 μ g/mL.

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EXAMPLE 5: *in vitro* specific killing of E202 TCR-T cells

For E202 TCR-T cell killing assays, Ca Ski E6/E7 cells were pre-stained with CFSE (Carboxyfluorescein succinimidyl ester) and then co-cultured overnight with untransduced or TCR transduced T cells at 1:2, 1:1, 3:1, or 10:1 effector-to-target ratio. The cytotoxicity of T cells against target cells was measured by Annexin V/7-AAD staining. As shown in FIG. 5, E202 TCR-T cells killed the E6+ target cells (Ca Ski E6/E7) in a specific manner. With higher E:T ratio, the TCR-T cells have higher killing capacity.

25

EXAMPLE 6: Construct design

For E202P03 TCR-T cells, an MP71 retroviral vector construct containing 3 coding regions was generated using standard molecular biology techniques: (1) the variable region of

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the alpha chain of a human anti-HPV16 E6 TCR fused to the constant region of the mouse TCR alpha chain; (2) the variable region of the beta chain of same human anti-HPV16 E6 TCR fused to the constant region of the mouse TCR beta chain; (3) the variable regions of the heavy and light chain of an immune checkpoint inhibitor (ICI) linked with a GS linker (FIGS. 6A-6B). The sequence for E202P03 is set forth in SEQ ID NO: 25.

EXAMPLE 7: TCR Staining

Primary T cells were transduced with the constructs of E202 or E202P03 TCR. Expression of the recombinant TCR was detected 13 days after transduction. Mouse TCR beta chain was stained by an antibody, followed by flow cytometry analysis. CD3, CD4, and CD8 staining was performed simultaneously. The results showed that in non-transduced primary T cells, no recombinant TCR was detected (FIG. 7A). The anti-E6 TCR was abundantly expressed in human T cells transduced with the constructs of E202 or E202P03 (FIGS. 7B-7C). All antibodies were purchased from BioLegend. A viable CD3⁺ lymphocyte gating strategy was used.

EXAMPLE 8: *In vitro* intracellular IFN- γ production

TCR-T cells were co-cultured with HPV peptide pulsed T2 cells at various effector-to-target ratios. Intracellular IFN- γ expression was measured by flow cytometry according to the manufacturer's instructions. It was found that TCR-T cells transduced by the constructs of E202 or E202P03 to express anti-E6 TCR can be specifically activated by target cells, which can be measured by intracellular IFN- γ expression (FIGS. 8B-8C). Non-transduced T cells were not activated by target cells (FIG. 8A).

EXAMPLE 9: *In vitro* TCR-T IFN- γ secretion

TCR-T cells were co-cultured overnight with target cells expressing the HPV E6 antigen at 1:0, 1:1, 3:1, or 10:1 effector-to-target ratios. The cell culture supernatant was then collected and the IFN- γ expression in the supernatant was measured using a human IFN- γ ELISA kit according to the manufacturer's instructions (FIG. 9).

TCR-T cells containing the E6 TCR could be activated by target cells, as measured by IFN- γ expression. Stimulated either by peptide-pulsed APCs or E6⁺ target cells (Ca Ski E6/E7),

the E202 and E202P03 TCR-T cells had much higher IFN- γ production than non-transduced T cells.

EXAMPLE 10: *In vitro* TCR-T CD107a expression

TCR-T cells were co-cultured overnight with target cells expressing the HPV E6 antigen at a 1:1 effector-to-target ratio. The T cells were then collected and the CD107a expression was measured in either CD8 (FIGS. 10A-10C) or CD4 (FIGS. 10D-10F) subpopulations by flow cytometry. The results showed that CD107a was expressed in the CD8 subpopulation but not in the CD4 subpopulation.

EXAMPLE 11: *In vitro* specific killing of E202 or E202P03 TCR-T cells

Target cells expressing HPV E6 antigen were pre-stained with CFSE and then co-cultured overnight with TCR-T cells at 1:1, 3:1, or 10:1 effector-to-target ratios. The cytotoxicity of T cells against target cells was measured by 7-AAD staining.

Both E202 and E202P03 TCR-T cells killed LMP2⁺ target cells (Ca Ski) in a specific manner. Non-transduced T cells killed target cells more weakly than the E202 and E202P03 TCR-T cells (FIG. 11). Thus, E202 and E202P03 TCR-T cells have higher killing capacity than the non-transduced TCR-T cells.

EXAMPLE 12: *In vitro* anti-PD-1 scFv expression in E202P03 TCR-T cell culture

Either E202 or E202P03 TCR-T cells were seeded in a 24-well plate at 3×10^6 /ml for 48 hours. Supernatant was then collected from the cell culture and the anti-PD-1 expression in the supernatant was measured. The results showed that the E202P03 TCR-T cells expressed more anti-PD-1 scFv than the E202 TCR-T cells (FIG. 12).

EXAMPLE 13: TCR Staining of E203, E204, and E205 TCR-T cells

Retroviral vector constructs were also generated using the methods as described herein to express E203, E204, or E205 TCR. Human PBMCs were transduced with the constructs of E203, E204, or E205 TCR. Expression of the recombinant TCR was detected 5 days after transduction.

Mouse TCR beta chain was stained by an antibody, followed by flow cytometry analysis. CD3 staining was performed simultaneously. The results showed that in untransduced human PBMCs,

no recombinant TCR was detected (FIG. 13A). The anti-E6 TCR was abundantly expressed in human PBMCs transduced with the constructs of E203, E204, or E205 TCR (FIGS. 13B-13D). A viable CD3⁺ lymphocyte gating strategy was used.

5 EXAMPLE 14: *In vitro* intracellular IFN- γ production of E203, E204, and E205 TCR-T cells

0.2 $\times$ 10⁶ untransduced (UT), E203, E204, or E205 TCR-T cells were co-cultured overnight with 0.4 $\times$ 10⁶ Ca Ski E6/E7 cells (HPV E6 and E7 overexpressed Ca Ski cells), Ca Ski cells or 293T cells. Cells were then treated with Brefeldin A and Monensin for 4 hours, after
 10 which levels of intracellular IFN- γ were measured by flow cytometry. Both CD4⁺ (FIGS. 14A-14P) and CD8⁺ (FIGS. 15A-15P) T cell populations were analyzed. Specifically for CD8⁺ T cells, the results showed that TCR-T cells transduced by the constructs of E203 or E205 to express anti-E6 TCR can be specifically activated by Ca Ski E6/E7 cells, as determined by intracellular IFN- γ expression (FIG. 15F and FIG. 15N). By contrast, untransduced T cells were
 15 not activated by Ca Ski E6/E7 cells (FIG. 15B).

EXAMPLE 15: *in vitro* specific killing of E203, E204, or E205 TCR-T cells

0.03 $\times$ 10⁶ Ca Ski E6/E7 cells were labeled with CellTraceTM CFSE and 0.03 $\times$ 10⁶ 293T cells were labeled with CellTraceTM Violet. The labeled Ca Ski E6/E7 cells and labeled 293T
 20 cells were mixed and co-cultured overnight with untransduced (UT), E203, E204, or E205 TCR-T cells at 0:1, 0.4: 1, 2:1, or 10:1 effector-to-target cell ratio (4 replicates in 96-well plates). Afterwards, the live Ca Ski E6/E7 cells and live 293T cells were quantified by flow cytometry. Beads were added as a reference for flow cytometry analysis. Absolute killing efficacy was calculated based on the ratio of the live Ca Ski E6/E7 cells over beads (FIG. 16A), and
 25 competitive killing efficacy was calculated based on the ratio of the live Ca Ski E6/E7 cells over the live 293T cells (FIG. 16B). The results showed that the target cell (Ca Ski E6/E7) killing specificity was ranked as: E203 TCR-T cells > E205 TCR-T cells > E204 TCR-T cells.

EXAMPLE 16: EC50 of E203 TCR via peptide titration

30 E203 TCR-T cells were co-cultured overnight with antigen presenting cells (APCs) at a 1:1 effector-to-target cell ratio. The APCs were pulsed with different concentrations of HPV

peptides. The T cells were then collected and the intracellular IFN- γ expression was measured to determine the EC50.

As shown in FIG. 17A, when CD8+ E203 TCR-T cells were used to recognize HPV peptide-pulsed APCs, the EC50 was 1.045 ng/ml. As shown in FIG. 17B, when CD4+ E203
5 TCR-T cells were used to recognize HPV peptide-pulsed APCs, the EC50 was 17.62 ng/ml.

EXAMPLE 17. EC50 of E204 TCR via peptide titration

E204 TCR-T cells were co-cultured overnight with antigen presenting cells (APCs) at a 1:1 effector-to-target cell ratio. The APCs were pulsed with different concentrations of HPV
10 peptides. The T cells were then collected and the intracellular IFN- γ expression was measured to determine the EC50.

As shown in FIGS. 18A-18B, E204 TCR-T cells (including CD8+ and CD4+ T cells) did not exhibit HPV peptide-dependent activation. EC50 was not determined for E204 TCR-T cells.

EXAMPLE 18: EC50 of E205 TCR via peptide titration

E205 TCR-T cells were co-cultured overnight with antigen presenting cells (APCs) at a 1:1 effector-to-target cell ratio. The APCs were pulsed with different concentrations of HPV
15 peptides. The T cells were then collected and the intracellular IFN- γ expression was measured to determine the EC50.

As shown in FIG. 19A, when CD8+ E205 TCR-T cells were used to recognize HPV peptide-pulsed APCs, the EC50 was 0.9167 ng/ml. As shown in FIG. 19B, when CD4+ E205
20 TCR-T cells were used to recognize HPV peptide-pulsed APCs, the EC50 was 10.42 ng/ml.

OTHER EMBODIMENTS

25 It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

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