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(54) Title: ENGINEERED T CELL RECEPTORS TARGETING EGFR ANTIGENS AND METHODS OF USE

(57) Abstract: This disclosure provides for engineered T cell Receptors (TCRs), cells comprising the TCRs, and methods of making and using the TCRs. The current disclosure relates to TCRs that specifically recognize EGFR neoantigens comprising L858R mutations and restricted to HLA class I A31 and A33 allotypes. Accordingly, aspects of the disclosure relate to a polypeptide comprising an antigen binding variable region comprising the amino acid sequence of a CDR3 of the disclosure or an amino acid sequence with at least 80% sequence identity to a CDR3 of the disclosure. Further aspects relate to an engineered T-cell Receptor (TCR) comprising a TCR-b polypeptide and a TCR-a polypeptide, wherein the TCR-b polypeptide comprises an amino acid sequence of a CDR3 of the disclosure or an amino acid sequence with at least 80% sequence identity to a CDR3 of the disclosure and the TCR-a polypeptide comprises the amino acid sequence of a CDR3 of the disclosure or an amino acid sequence with at least 80% sequence identity to a CDR3 of the disclosure.



**ENGINEERED T CELL RECEPTORS TARGETING EGFR ANTIGENS AND
METHODS OF USE**

BACKGROUND OF THE INVENTION

5 **[0001]** This application is claims benefit of priority of U.S. Provisional Application No. 63/110,116, filed November 5, 2020, which is hereby incorporated by reference in its entirety.

II. Sequence Listing

10 **[0002]** The instant application contains a Sequence Listing which has been submitted in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on October 25, 2021, is named MDACP1244WO_ST25.txt and is 100,085 bytes in size.

III. Field of the Invention

[0003] This invention relates to the field of cancer therapy.

IV. Background

15 **[0004]** Adoptive T-cell therapy is one potentially powerful treatment for cancer that genetically modifies natural T cells to make them tumor-specific and to improve their ability to destroy tumor cells. The genetically modified T cells are able to express chimeric antigen receptors (CARs) or T-cell receptors (TCRs), showing impressive results in multiple clinical trials. TCR-engineered T (TCR-T) cells have shown great promise against tumors. The potency of TCRs relies on their interaction with peptide-major histocompatibility complex (pMHC), complexes formed by peptide bound to MHC. Intracellular antigens are cut up into peptide chains and displayed by MHC molecules to form pMHCs. Cytoplasmic proteins to be expressed by class I MHC proteins, most of which are defective ribosomal translation products, are cleaved into peptide chains by proteolysis. These peptides are then bound to class I MHC proteins, which are expressed on all nucleated cells' cell surface. Some cells, called antigen-presenting cells (APCs), express class II MHC proteins. They internalize foreign material proteins by endocytosis and cleave them into peptide chains to bind with class II MHC proteins. T-cell receptors from T cells, which must be matched to human leukocyte antigen (HLA) alleles of patients, recognize these pMHCs and cause the killing of cancer cells. (Human class I MHC protein is expressed from 3 gene regions: HLA-A, HLA-B, and HLA-C, and human class II MHC protein is also expressed from 3 gene regions: HLA-DR, HLA-DP, and HLA-DQ.) There is a need in the art for the engineering of TCRs that are directed to cancer-specific antigens and useful for the treatment of cancer.

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SUMMARY OF THE INVENTION

[0005] This disclosure provides for engineered T cell Receptors (TCRs), cells comprising the TCRs, and methods of making and using the TCRs. The current disclosure relates to TCRs that specifically recognize EGFR neoantigens comprising L858R mutations and restricted to HLA class I A31 and A33 allotypes.

[0006] Accordingly, aspects of the disclosure relate to a polypeptide comprising an antigen binding variable region comprising the amino acid sequence of a CDR3 of the disclosure or an amino acid sequence with at least 80% sequence identity to a CDR3 of the disclosure. Further aspects relate to an engineered T-cell Receptor (TCR) comprising a TCR-b polypeptide and a TCR-a polypeptide, wherein the TCR-b polypeptide comprises an amino acid sequence of a CDR3 of the disclosure or an amino acid sequence with at least 80% sequence identity to a CDR3 of the disclosure and the TCR-a polypeptide comprises the amino acid sequence of a CDR3 of the disclosure or an amino acid sequence with at least 80% sequence identity to a CDR3 of the disclosure. Further aspects relate to a nucleic acid encoding a TCR-b polypeptide comprising an amino acid sequence of a CDR3 of the disclosure or comprising an amino acid sequence with at least 80% sequence identity to a CDR3 of the disclosure and/or a TCR-a polypeptide comprising an amino acid sequence of a CDR3 of the disclosure or having the amino acid sequence with at least 80% sequence identity to a CDR3 of the disclosure. The CDR3 may be a CDR3 of SEQ ID NO:8, 14, 28, 22, 42, 36, 56, 50, 70, 64, 84, or 78. TCR-a CDR3 aspects include CDRs with an amino acid sequence of SEQ ID NO:14, 22, 36, 50, 64, and 78. TCR-b CDR3 aspects include CDRs with an amino acid sequence of SEQ ID NO:8, 28, 42, 56, 70, and 84.

[0007] Yet further aspects relate to nucleic acids encoding the polypeptides and engineered TCRs, nucleic acid vectors comprising one or more nucleic acids of the disclosure, and cells comprising the polypeptides, engineered TCRs and/or nucleic acids of the disclosure. Also provided are compositions comprising the polypeptides, cells, nucleic acids, or engineered TCRs of the disclosure. Further aspects relate to a method of making an engineered cell comprising transferring a nucleic acid or vector of the disclosure into a cell. Further aspects relate to a method for treating cancer in a subject comprising administering a polypeptide, composition, cell, nucleic acid, or engineered TCR to a subject in need thereof. Methods also include methods of reducing tumor burden; methods of lysing a cancer cell; methods of killing tumor/cancerous cells; methods of increasing overall survival; methods of reducing the risk of getting cancer or of getting a tumor; methods of increasing recurrent free survival; methods of

preventing cancer; and/or methods of reducing, eliminating, or decreasing the spread or metastasis of cancer, the method comprising administering a polypeptide, composition, cell, nucleic acid, or engineered TCR to a subject in need thereof.

[0008] Further aspects relate to a fusion protein comprising a TCR of the disclosure and a CD3 binding region. In some aspects, the CD3 binding region comprises a CD3-specific fragment antigen binding (Fab), single chain variable fragment (scFv), single domain antibody, or single chain antibody. Exemplary CD3-specific fragment antigen binding (Fab) are known in the art. For example, US20180222981, which is herein incorporated by reference, discloses variable regions that bind specifically to CD3, which may be used in aspects of this disclosure. Anti-CD3 antibodies and variable regions are disclosed in US20180117152, which is also incorporated by reference.

[0009] Nucleic acids of the disclosure include those that encode for CDR regions, variable regions, engineered TCRs, polypeptides, TCR-a polypeptides, TCR-b polypeptides, and fusion proteins described herein. In some aspects, the nucleic acid comprises one of SEQ ID NOS:1, 15, 29, 43, 57, or 71, or a fragment thereof. In some aspects, the nucleic acid comprises a nucleotide having or having at least 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% (or any derivable range therein) sequence identity to one of SEQ ID NOS:1, 15, 29, 43, 57, or 71, or a fragment thereof.

[0010] In some aspects, the polypeptide of the disclosure or the TCR-a polypeptide comprises a CDR3 comprising an amino acid sequence having or having 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% (or any derivable range therein) sequence identity to one of SEQ ID NOS:14, 22, 36, 50, 64, and 78. In some aspects, the polypeptide comprises a CDR3 comprising the amino acid sequence of one of SEQ ID NOS:14, 22, 36, 50, 64, and 78. In some aspects, the polypeptide of the disclosure or the TCR-b polypeptide comprises a CDR3 comprising an amino acid sequence having or having at least 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% (or any derivable range therein) sequence identity to one of SEQ ID NO:8, 28, 42, 56, 70, and 84. In some aspects, the polypeptide of the disclosure or the TCR-b polypeptide comprises a CDR3 comprising the amino acid sequence of one of SEQ ID NO:8, 28, 42, 56, 70, and 84. In some aspects, the engineered TCR comprises a TCR-a comprising a CDR3 having an amino acid having or having at least 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% (or any derivable range therein) sequence identity to one of SEQ ID NOS:14, 22, 36, 50, 64, and 78 and a TCR-b polypeptide comprising a CDR3 having an amino acid having or having at least 75, 76, 77,

78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% (or any derivable range therein) sequence identity to one of SEQ ID NO:8, 28, 42, 56, 70, and 84.

[0011] In some aspects, the nucleic acid encodes a TCR-a polypeptide comprising a CDR3 comprising an amino acid sequence having or having at least 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% (or any derivable range therein) sequence identity to one of SEQ ID NOS:14, 22, 36, 50, 64, and 78 and/or the TCR-b polypeptide comprises a CDR3 comprising an amino acid sequence having or having at least 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% (or any derivable range therein) sequence identity to one of SEQ ID NOS:8, 28, 42, 56, 70, and 84.

[0012] In some aspects, the polypeptide or TCR comprises a variable region comprising a CDR1, CDR2, and/or CDR3 from a TCR-a polypeptide and/or a TCR-b polypeptide. In some aspects, the variable region comprises a CDR1 with an amino acid sequence of one of SEQ ID NOS:12, 6, 20, 26, 34, 40, 48, 54, 62, 68, 76, and 82 or with at least 80% sequence identity to one of SEQ ID NOS:12, 6, 20, 26, 34, 40, 48, 54, 62, 68, 76, and 82. In some aspects, the variable region comprises a CDR1 having or having at least 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% (or any derivable range therein) sequence identity to sequence identity to a one of SEQ ID NOS:12, 6, 20, 26, 34, 40, 48, 54, 62, 68, 76, and 82.

[0013] In some aspects, the polypeptide, TCR, or the TCR-a polypeptide comprises a CDR1 comprising an amino acid sequence having or having at least 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% (or any derivable range therein) sequence identity to one of SEQ ID NOS:12, 20, 34, 48, 62, and 76. In some aspects, the polypeptide, TCR, or the TCR-a polypeptide comprises a CDR1 comprising the amino acid sequence of one of SEQ ID NOS:12, 20, 34, 48, 62, and 76. In some aspects, the polypeptide, TCR, or the TCR-b polypeptide comprises a CDR1 comprising an amino acid sequence having or having at least 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% (or any derivable range therein) sequence identity to one of SEQ ID NOS:6, 26, 40, 54, 68, and 82. In some aspects, the polypeptide, TCR, or the TCR-b polypeptide comprises a CDR1 comprising the amino acid sequence of one of SEQ ID NOS:6, 26, 40, 54, 68, and 82.

[0014] In some aspects, the engineered TCR comprises a TCR-a comprising a CDR1 having an amino acid having or having at least 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% (or any derivable range therein) sequence

identity to one of SEQ ID NOS:12, 20, 34, 48, 62, and 76 and a TCR-b polypeptide comprising a CDR1 having an amino acid having or having at least 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% (or any derivable range therein) sequence identity to one of SEQ ID NOS:6, 26, 40, 54, 68, and 82. In some aspects, the engineered TCR comprises a TCR-a comprising a CDR1 having the amino acid sequence of one of SEQ ID NOS:12, 20, 34, 48, 62, and 76 and a TCR-b polypeptide comprising a CDR1 having the amino acid sequence of one of SEQ ID NOS:6, 26, 40, 54, 68, and 82.

[0015] In some aspects, the nucleic acid encodes a TCR-a polypeptide comprising a CDR1 comprising an amino acid sequence having or having at least 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% (or any derivable range therein) sequence identity to one of SEQ ID NOS:12, 20, 34, 48, 62, and 76 and/or the TCR-b polypeptide comprises a CDR1 comprising an amino acid sequence having or having at least 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% (or any derivable range therein) sequence identity to one of SEQ ID NOS:6, 26, 40, 54, 68, and 82. In some aspects, the nucleic acid encodes a TCR-a polypeptide comprising a CDR1 comprising the amino acid sequence of one of SEQ ID NOS:12, 20, 34, 48, 62, and 76 and/or the TCR-b polypeptide comprises a CDR1 comprising the amino acid sequence of one of SEQ ID NOS:6, 26, 40, 54, 68, and 82.

[0016] In some aspects, the polypeptides, TCR-a or TCR-b may comprise a variable region. The variable region may comprise a CDR2 with an amino acid sequence of one of SEQ ID NOS:13, 7, 21, 27, 35, 41, 49, 55, 63, 69, 77, and 83 or with at least 80% sequence identity to one of SEQ ID NOS:13, 7, 21, 27, 35, 41, 49, 55, 63, 69, 77, and 83. In some aspects, the variable region comprises a CDR2 having or having at least 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% (or any derivable range therein) sequence identity to sequence identity to a one of SEQ ID NOS:13, 7, 21, 27, 35, 41, 49, 55, 63, 69, 77, and 83.

[0017] In some aspects, the polypeptide of the disclosure or the TCR-a polypeptide comprises a CDR2 comprising an amino acid sequence having or having at least 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% (or any derivable range therein) sequence identity to one of SEQ ID NOS:13, 21, 35, 49, 63, and 77. In some aspects, the polypeptide of the disclosure or the TCR-a polypeptide comprises a CDR2 comprising the amino acid sequence of one of SEQ ID NOS:13, 21, 35, 49, 63, and 77. In some aspects, the polypeptide of the disclosure or the TCR-b polypeptide comprises a CDR2 comprising an amino acid sequence having or having at least 75, 76, 77, 78, 79, 80, 81, 82, 83,

84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% (or any derivable range therein) sequence identity to one of SEQ ID NOS:7, 27, 41, 55, 69, and 83. In some aspects, the polypeptide of the disclosure or the TCR-b polypeptide comprises a CDR2 comprising the amino acid sequence of one of SEQ ID NOS:7, 27, 41, 55, 69, and 83.

5 **[0018]** In some aspects, the engineered TCR comprises a TCR-a comprising a CDR2 having an amino acid having or having at least 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% (or any derivable range therein) sequence identity to one of SEQ ID NOS:13, 21, 35, 49, 63, and 77 and a TCR-b polypeptide comprising a CDR2 having an amino acid having or having at least 75, 76, 77, 78, 79, 80, 81, 82, 83, 84,
10 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% (or any derivable range therein) sequence identity to one of SEQ ID NOS:7, 27, 41, 55, 69, and 83. In some aspects, the engineered TCR comprises a TCR-a comprising a CDR2 having the amino acid sequence of one of SEQ ID NOS:13, 21, 35, 49, 63, and 77 and a TCR-b polypeptide comprising a CDR2 having the amino acid sequence of one of SEQ ID NOS:7, 27, 41, 55, 69, and 83.

15 **[0019]** In some aspects, the nucleic acid encodes a TCR-a polypeptide comprising a CDR2 comprising an amino acid sequence having or having at least 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% (or any derivable range therein) sequence identity to one of SEQ ID NOS:13, 21, 35, 49, 63, and 77 and/or the TCR-b polypeptide comprises a CDR2 comprising an amino acid sequence having or having at least
20 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% (or any derivable range therein) sequence identity to one of SEQ ID NOS:7, 27, 41, 55, 69, and 83. In some aspects, the nucleic acid encodes a TCR-a polypeptide comprising a CDR2 comprising the amino acid sequence of one of SEQ ID NOS:13, 21, 35, 49, 63, and 77 and/or the TCR-b polypeptide comprises a CDR2 comprising the amino acid sequence of one
25 of SEQ ID NOS:7, 27, 41, 55, 69, and 83.

[0020] In some aspects, the variable region comprises an amino acid sequence with at least 70% sequence identity to one of SEQ ID NOS:10, 4, 18, 24, 32, 38, 46, 52, 60, 66, 74, and 80. In some aspects, the variable region comprises an amino acid sequence having or having at least 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98,
30 99, or 100% (or any derivable range therein) sequence identity to one of SEQ ID NOS:10, 4, 18, 24, 32, 38, 46, 52, 60, 66, 74, and 80. In some aspects, the variable region comprises the amino acid sequence of one of SEQ ID NOS:10, 4, 18, 24, 32, 38, 46, 52, 60, 66, 74, and 80. In some aspects, the TCR-a variable region comprises an amino acid sequence with at least 70% sequence identity to one of SEQ ID NOS:10, 18, 32, 46, 60, and 74. In some aspects, the

TCR-a variable region comprises an amino acid sequence having or having at least 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% (or any derivable range therein) sequence identity to one of SEQ ID NOS:10, 18, 32, 46, 60, and 74. In some aspects, the TCR-a variable region comprises the amino acid sequence of one of
5 SEQ ID NOS:10, 18, 32, 46, 60, and 74. In some aspects, the TCR-b variable region comprises an amino acid sequence with at least 70% sequence identity to one of SEQ ID NOS:4, 24, 38, 52, 66, and 80. In some aspects, the TCR-b variable region comprises an amino acid sequence having or having at least 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% (or any derivable range therein) sequence identity to one of
10 SEQ ID NOS:4, 24, 38, 52, 66, and 80. In some aspects, the TCR-b variable region comprises the amino acid sequence of one of SEQ ID NOS:4, 24, 38, 52, 66, and 80.

[0021] In some aspects, the polypeptide comprises a T cell receptor alpha (TCR-a) variable region. In some aspects, the variable region comprises a CDR1, CDR2, and/or CDR3. In some aspects, the polypeptide comprises a TCR-a variable and constant region. In some aspects, the
15 polypeptide further comprises a signal peptide. In some aspects, the signal peptide comprises an amino acid sequence with at least 80% identity to one of SEQ ID NOS:11, 5, 19, 25, 33, 39, 47, 53, 61, 67, 75, and 81. In some aspects, the signal peptide comprises an amino acid sequence having or having at least 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% (or any derivable range therein) sequence identity
20 to one of SEQ ID NOS:11, 5, 19, 25, 33, 39, 47, 53, 61, 67, 75, and 81. In some aspects, the signal peptide comprises an amino acid sequence of one of SEQ ID NOS:11, 5, 19, 25, 33, 39, 47, 53, 61, 67, 75, and 81.

[0022] TCR aspects of the disclosure relate to a TCR comprising a TCR-a polypeptide and a TCR-b polypeptide, wherein the CDR1, CDR2, and CDR3 of the TCR-a polypeptide
25 comprise the amino acid sequence of SEQ ID NO:12, 13, and 14, respectively and wherein the CDR1, CDR3, and CDR3 of the TCR-b polypeptide comprise the amino acid sequence of SEQ ID NO:6, 7, and 8, respectively. In some aspects, the TCR comprises a TCR-a polypeptide and a TCR-b polypeptide, wherein the CDR1, CDR2, and CDR3 of the TCR-a polypeptide
30 comprise the amino acid sequence of SEQ ID NO:20, 21, and 22, respectively and wherein the CDR1, CDR3, and CDR3 of the TCR-b polypeptide comprise the amino acid sequence of SEQ ID NO:26, 27, and 28, respectively. In some aspects, the TCR comprises a TCR-a polypeptide and a TCR-b polypeptide, wherein the CDR1, CDR2, and CDR3 of the TCR-a polypeptide
comprise the amino acid sequence of SEQ ID NO:34, 35, and 36, respectively and wherein the CDR1, CDR3, and CDR3 of the TCR-b polypeptide comprise the amino acid sequence of SEQ

ID NO:40, 41, and 42, respectively. In some aspects, the TCR comprises a TCR-a polypeptide and a TCR-b polypeptide, wherein the CDR1, CDR2, and CDR3 of the TCR-a polypeptide comprise the amino acid sequence of SEQ ID NO:48, 49, and 50, respectively and wherein the CDR1, CDR3, and CDR3 of the TCR-b polypeptide comprise the amino acid sequence of SEQ ID NO:54, 55, and 56, respectively. In some aspects, the TCR comprises a TCR-a polypeptide and a TCR-b polypeptide, wherein the CDR1, CDR2, and CDR3 of the TCR-a polypeptide comprise the amino acid sequence of SEQ ID NO:62, 63, and 64, respectively and wherein the CDR1, CDR3, and CDR3 of the TCR-b polypeptide comprise the amino acid sequence of SEQ ID NO:68, 69, and 70, respectively. In some aspects, the TCR comprises a TCR-a polypeptide and a TCR-b polypeptide, wherein the CDR1, CDR2, and CDR3 of the TCR-a polypeptide comprise the amino acid sequence of SEQ ID NO:76, 77, and 78, respectively and wherein the CDR1, CDR3, and CDR3 of the TCR-b polypeptide comprise the amino acid sequence of SEQ ID NO:82, 83, and 84, respectively.

[0023] In some aspects, the TCR-a polypeptide comprises the amino acid sequence of SEQ ID NO:9 or an amino acid sequence having or having at least 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% (or any derivable range therein) sequence identity to SEQ ID NO:9 and the TCR-b polypeptide comprises the amino acid sequence of SEQ ID NO:3 or an amino acid sequence having or having at least 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% (or any derivable range therein) sequence identity to SEQ ID NO:3. In some aspects, the TCR-a polypeptide comprises the amino acid sequence of SEQ ID NO:17 or an amino acid sequence having or having at least 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% (or any derivable range therein) sequence identity to SEQ ID NO:17 and the TCR-b polypeptide comprises the amino acid sequence of SEQ ID NO:23 or an amino acid sequence having or having at least 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% (or any derivable range therein) sequence identity to SEQ ID NO:23. In some aspects, the TCR-a polypeptide comprises the amino acid sequence of SEQ ID NO:31 or an amino acid sequence having or having at least 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% (or any derivable range therein) sequence identity to SEQ ID NO:31 and the TCR-b polypeptide comprises the amino acid sequence of SEQ ID NO:37 or an amino acid sequence having or having at least 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% (or any derivable range therein) sequence identity to SEQ ID NO:37. In some aspects, the TCR-a polypeptide comprises the amino acid sequence of SEQ

ID NO:45 or an amino acid sequence having or having at least 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% (or any derivable range therein) sequence identity to SEQ ID NO:45 and the TCR-b polypeptide comprises the amino acid sequence of SEQ ID NO:51 or an amino acid sequence having or having at least 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% (or any derivable range therein) sequence identity to SEQ ID NO:51. In some aspects, the TCR-a polypeptide comprises the amino acid sequence of SEQ ID NO:59 or an amino acid sequence having or having at least 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% (or any derivable range therein) sequence identity to SEQ ID NO:59 and the TCR-b polypeptide comprises the amino acid sequence of SEQ ID NO:65 or an amino acid sequence having or having at least 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% (or any derivable range therein) sequence identity to SEQ ID NO:65. In some aspects, the TCR-a polypeptide comprises the amino acid sequence of SEQ ID NO:73 or an amino acid sequence having or having at least 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% (or any derivable range therein) sequence identity to SEQ ID NO:73 and the TCR-b polypeptide comprises the amino acid sequence of SEQ ID NO:79 or an amino acid sequence having or having at least 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% (or any derivable range therein) sequence identity to SEQ ID NO:79.

[0024] In some aspects, the TCR comprises a modification or is chimeric. In some aspects, the variable region of the TCR is fused to a TCR constant region that is different from the constant region of the cloned TCR that specifically binds to a peptide of the disclosure.

[0025] In some aspects, the TCR-a polypeptide and TCR-b polypeptide are operably linked.

The term “operably linked” can refer to a covalent linkage, such as a peptide bond (eg. the two elements are polypeptides and are on the same polypeptide), or a non-covalent linkage, such as Van der Waals force (e.g. two polypeptides that have a certain degree of specific binding affinity for each other). In some aspects, the TCR-a polypeptide and TCR-b polypeptide are operably linked through a peptide bond. In some aspects, the TCR-a polypeptide and TCR-b polypeptide are on the same polypeptide and wherein the TCR-b is amino-proximal to the TCR-a. In some aspects, the TCR-a polypeptide and TCR-b polypeptide are on the same polypeptide and wherein the TCR-a is amino-proximal to the TCR-b. The polypeptide or TCR of the disclosure may be further defined as a single-chain TCR. The TCR may comprise a linker between the TCR-a and TCR-b polypeptide. The linker may comprise glycine and serine

residues. In some aspects, the linker is composed of only glycine and serine residues (a glycine-serine linker). The linker may be a flexible linker. Exemplary flexible linkers include glycine polymers (G)_n, glycine-serine polymers (including, for example, (GS)_n, (GSGGS-SEQ ID NO:86)_n, (G₄S)_n and (GGGS-SEQ ID NO:87)_n, where n is an integer of at least one. In some aspects, n is at least, at most, or exactly 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 (or any derivable range therein). Glycine-alanine polymers, alanine-serine polymers, and other flexible linkers known in the art and may be used as a linker in the polypeptides of the disclosure. Exemplary linkers can comprise or consist of GGSG (SEQ ID NO:88), GGS GG (SEQ ID NO:89), GSGSG (SEQ ID NO:90), GSGGG (SEQ ID NO:91), GGGSG (SEQ ID NO:92), GSSSG (SEQ ID NO:93), and the like. Further linkers useful in the polypeptides and TCRs of the disclosure are described herein. A first region is carboxy-proximal to a second region when the first region is attached to the carboxy terminus of the second region. There may be further intervening amino acid residues between the first and second regions. Thus, the regions need not be immediately adjacent, unless specifically specified as not having intervening amino acid residues. The term “amino-proximal” is similarly defined in that a first region is amino-proximal to a second region when the first region is attached to the amino terminus of the second region. Similarly, there may be further intervening amino acid residues between the first and second regions unless stated otherwise.

[0026] A CDR may also comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 16, 18, 19, 20, 21, 22, 23, or more contiguous amino acid residues (or any range derivable therein) flanking one or both sides of a particular CDR sequence in the context of the variable region of the TCR-a or TCR-b polypeptide; therefore, there may be one or more additional amino acids at the N-terminal or C-terminal end of a particular CDR sequence, such as those shown in the variable regions of SEQ ID NOS:10, 4, 18, 24, 32, 38, 46, 52, 60, 66, 74, and 80. Alternatively, or in combination, a CDR may also be a fragment of a CDR described herein and may lack at least 1, 2, 3, 4, or 5 amino acids from the C-terminal or N-terminal end of a particular CDR sequence.

[0027] In some aspects, the TCR or fusion protein is conjugated to a detection or therapeutic agent. In some aspects, the agent comprises a fluorescent molecule, radiative molecule, or toxin. In some aspects, the TCR or fusion protein is conjugated to an agent described herein.

[0028] In some aspects, the nucleic acid comprises a TCR-a (TRA) and TCR-b (TRB) gene. In some aspects, the nucleic acid is polycistronic. In some aspects, the nucleic acid comprises an internal ribosome entry site (IRES) or a 2A cleavable linker, such as a P2A linker. In some aspects, the nucleic acid comprises a cDNA encoding the TCR-a and/or TCR-b genes. In some

aspects, the nucleic acid further encodes for a polypeptide comprising a CD3 binding region. In some aspects, the CD3 binding region comprises a CD3-specific fragment antigen binding (Fab), single chain variable fragment (scFv), single domain antibody, or single chain antibody.

[0029] In some aspects, the vector comprises both of the TCR-a and TCR-b genes. In some

5 aspects, the vector comprises a promoter that directs the expression of the nucleic acid. In some aspects, the promoter comprises a murine stem cell virus (MSCV) promoter. In some aspects, the cell comprises a stem cell, a progenitor cell, an immune cell, or a natural killer (NK) cell. In some aspects, the cell comprises a hematopoietic stem or progenitor cell, a T cell, a cell differentiated from mesenchymal stem cells (MSCs) or an induced pluripotent stem
10 cell (iPSC). In some aspects, the cell is isolated or derived from peripheral blood mononuclear cell (PBMCs). In some aspects, the T cell comprises a cytotoxic T lymphocyte (CTL), a CD8+ T cell, a CD4+ T cell, an invariant NK T (iNKT) cell, a gamma-delta T cell, a NKT cell, or a regulatory T cell. In some aspects, the cell is isolated from a cancer patient. In some aspects, the cell is isolated from a non-cancerous patient. In some aspects, the cell is isolated from a
15 healthy patient. In some aspects, the cell is frozen or has never been frozen. In some aspects, the cell is in cell culture. In some aspects, the cell lacks endogenous expression of TCR genes. In some aspects, the cell further comprises a chimeric antigen receptor (CAR).

[0030] In some aspects, the composition has been determined to be serum-free, mycoplasma-free, endotoxin-free, and/or sterile. In some aspects, the method further
20 comprises culturing the cell in media, incubating the cell at conditions that allow for the division of the cell, screening the cell, and/or freezing the cell.

[0031] In some aspects, the cancer comprises lung cancer. In some aspects, the subject has been diagnosed with cancer, such as lung cancer. In some aspects, the cancer comprises a solid tumor. In some aspects, the subject has previously been treated for the cancer. In some aspects,
25 the subject has been determined to be resistant to the previous treatment. In some aspects, the method further comprises the administration of an additional therapy. In some aspects, the subject is a mammal. In some aspects, the subject comprises a laboratory test animal, such as a mouse, rat, rabbit, dog, cat, horse, or pig. In some aspects, the subject is a human. In some aspects, the subject has been determined to be HLA-A31 or HLA-A33 positive. In some
30 aspects, the subject harbors an EGFR L858R mutation. In some aspects, the subject is one that has been determined to have an EGFR protein with a L858R mutation or one to have a genetic mutation in the EGFR gene that results in an EGFR L858R mutant protein. In some aspects, the cancer comprises an EGFR L858R positive lung cancer.

[0032] In some aspects, the lung cancer comprises small cell lung cancer, non-small cell lung cancer, metastatic lung cancer, lung nodules, adenosquamous carcinoma, large cell neuroendocrine carcinoma, salivary gland-type lung carcinoma, lung carcinoids, or mesothelioma. In some aspects, a lung cancer such as small cell lung cancer, non-small cell lung cancer, metastatic lung cancer, lung nodules, adenosquamous carcinoma, large cell neuroendocrine carcinoma, salivary gland-type lung carcinoma, lung carcinoids, or mesothelioma is excluded. In some aspects, the cancer comprises lung adenocarcinoma, non-small cell lung carcinoma, squamous cell lung carcinoma, or small cell lung carcinoma.

[0033] In some aspects, the compositions of the disclosure are formulated as a vaccine. In some aspects, the compositions and methods of the disclosure provide for prophylactic therapies to prevent cancer. In some aspects, the compositions and methods of the disclosure provide for therapeutic therapies to treat existing cancers, such as for the treatment of patients with cancer. In some aspects, the composition further comprises an adjuvant. Adjuvants are known in the art and include, for example, TLR agonists and aluminum salts.

[0034] In some aspects the methods of the disclosure further comprise screening the cell for one or more cellular properties, such as for TCR expression, incorporation of nucleic acids encoding TCR genes, or for immunogenic properties, such as binding of the TCR to a cancer antigen. In some aspects, the subject is one that has or has been determined to have an EGFR mutation at L858R.

[0035] In some aspects, the method comprises administering a cell or a composition comprising a cell and wherein the cell comprises an autologous cell. In some aspects, the cell comprises a non-autologous cell.

[0036] “Treatment” or treating may refer to any treatment of a disease in a mammal, including: (i) preventing the disease, that is, causing the clinical symptoms of the disease not to develop by administration of a protective composition prior to the induction of the disease; (ii) suppressing the disease, that is, causing the clinical symptoms of the disease not to develop by administration of a protective composition after the inductive event but prior to the clinical appearance or reappearance of the disease; (iii) inhibiting the disease, that is, arresting the development of clinical symptoms by administration of a protective composition after their initial appearance; and/or (iv) relieving the disease, that is, causing the regression of clinical symptoms by administration of a protective composition after their initial appearance. In some aspects, the treatment may exclude prevention of the disease.

[0037] Throughout this application, the term “about” is used according to its plain and ordinary meaning in the area of cell and molecular biology to indicate that a value includes the standard deviation of error for the device or method being employed to determine the value.

[0038] The use of the word “a” or “an” when used in conjunction with the term “comprising” may mean “one,” but it is also consistent with the meaning of “one or more,” “at least one,” and “one or more than one.”

[0039] As used herein, the terms “or” and “and/or” are utilized to describe multiple components in combination or exclusive of one another. For example, “x, y, and/or z” can refer to “x” alone, “y” alone, “z” alone, “x, y, and z,” “(x and y) or z,” “x or (y and z),” or “x or y or z.” It is specifically contemplated that x, y, or z may be specifically excluded from an embodiment or aspect.

[0040] The words “comprising” (and any form of comprising, such as “comprise” and “comprises”), “having” (and any form of having, such as “have” and “has”), “including” (and any form of including, such as “includes” and “include”), “characterized by” (and any form of including, such as “characterized as”), or “containing” (and any form of containing, such as “contains” and “contain”) are inclusive or open-ended and do not exclude additional, unrecited elements or method steps.

[0041] The compositions and methods for their use can “comprise,” “consist essentially of,” or “consist of” any of the ingredients or steps disclosed throughout the specification. The phrase “consisting of” excludes any element, step, or ingredient not specified. The phrase “consisting essentially of” limits the scope of described subject matter to the specified materials or steps and those that do not materially affect its basic and novel characteristics. It is contemplated that embodiments and aspects described in the context of the term “comprising” may also be implemented in the context of the term “consisting of” or “consisting essentially of.”

[0042] Any method in the context of a therapeutic, diagnostic, or physiologic purpose or effect may also be described in “use” claim language such as “Use of” any compound, composition, or agent discussed herein for achieving or implementing a described therapeutic, diagnostic, or physiologic purpose or effect.

[0043] Use of the one or more sequences or compositions may be employed based on any of the methods described herein. Other embodiments are discussed throughout this application. Any embodiment or aspect discussed with respect to one aspect of the disclosure applies to other aspects of the disclosure as well and vice versa.

[0044] It is specifically contemplated that any limitation discussed with respect to one embodiment or aspect of the invention may apply to any other embodiment or aspect of the invention. Furthermore, any composition of the invention may be used in any method of the invention, and any method of the invention may be used to produce or to utilize any composition of the invention. Aspects of an embodiment set forth in the Examples are also embodiments that may be implemented in the context of embodiments discussed elsewhere in a different Example or elsewhere in the application, such as in the Summary of Invention, Detailed Description of the Embodiments, Claims, and description of Figure Legends.

[0045] Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating specific embodiments and aspects of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0046] The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

[0047] FIG. 1. TCRA31-1 and TCRA31-2 mediated cytotoxicity

[0048] FIG. 2. Interferon-gamma release in response to titrated amounts of HVKITDFGR (SEQ ID NO:85) peptide.

[0049] FIG. 3. IFN-gamma ELISPOT to determine the avidity of two EGFR-A*3303 TCRs.

[0050] FIG. 4. Peptide Titration to determine the killing efficacy of TCR33-32H-transduced T cells

[0051] FIG. 5. TCR33-32H and TCRA33-32L mediated cytotoxicity assays. The FACS plots on the left show PBMC transduced with TCR construct 33-32H or 33-32L.

[0052] FIG. 6. TCRA33-32H mediated cytotoxicity against EGFR(L858R) gene-transduced target cells.

[0053] FIG. 7. Killing activity of EGFR-A33 TCR-T cells expressing T33-32H, T33-37-3 or T33-37-4.

DETAILED DESCRIPTION OF THE INVENTION

[0054] The current disclosure provides a T-cell receptor (TCR) which recognizes a epitopes of EGFR that span the L858R mutation. The present disclosure also provides a nucleotide sequence encoding this TCR, a expression vector comprising this nucleotide sequence which
5 can be used to modify cells, such as peripheral blood mononuclear cells, to generate the EGFR-L858R-specific T cells. The present disclosure also provides the use of EGFR-L858R-specific T cells for adoptive immunotherapy for cancer patients harboring the EGFR-L858R mutation.

I. Engineered T Cell Receptors

[0055] T-cell receptors comprise two different polypeptide chains, termed the T-cell
10 receptor α (TCR α) and β (TCR β) chains, linked by a disulfide bond. These α : β heterodimers are very similar in structure to the Fab fragment of an immunoglobulin molecule, and they account for antigen recognition by most T cells. A minority of T cells bear an alternative, but structurally similar, receptor made up of a different pair of polypeptide chains designated γ and δ . Both types of T-cell receptor differ from the membrane-bound immunoglobulin that serves
15 as the B-cell receptor: a T-cell receptor has only one antigen-binding site, whereas a B-cell receptor has two, and T-cell receptors are never secreted, whereas immunoglobulin can be secreted as antibody.

[0056] Both chains of the T-cell receptor have an amino-terminal variable (V) region with homology to an immunoglobulin V domain, a constant (C) region with homology to an
20 immunoglobulin C domain, and a short hinge region containing a cysteine residue that forms the interchain disulfide bond. Each chain spans the lipid bilayer by a hydrophobic transmembrane domain, and ends in a short cytoplasmic tail.

[0057] The three-dimensional structure of the T-cell receptor has been determined. The structure is indeed similar to that of an antibody Fab fragment, as was suspected from earlier
25 studies on the genes that encoded it. The T-cell receptor chains fold in much the same way as those of a Fab fragment, although the final structure appears a little shorter and wider. There are, however, some distinct differences between T-cell receptors and Fab fragments. The most striking difference is in the C α domain, where the fold is unlike that of any other immunoglobulin-like domain. The half of the domain that is juxtaposed with the C β domain
30 forms a β sheet similar to that found in other immunoglobulin-like domains, but the other half of the domain is formed of loosely packed strands and a short segment of α helix. The intramolecular disulfide bond, which in immunoglobulin-like domains normally joins two β strands, in a C α domain joins a β strand to this segment of α helix.

[0058] There are also differences in the way in which the domains interact. The interface between the V and C domains of both T-cell receptor chains is more extensive than in antibodies, which may make the hinge joint between the domains less flexible. And the interaction between the C α and C β domains is distinctive in being assisted by carbohydrate, with a sugar group from the C α domain making a number of hydrogen bonds to the C β domain. Finally, a comparison of the variable binding sites shows that, although the complementarity-determining region (CDR) loops align fairly closely with those of antibody molecules, there is some displacement relative to those of the antibody molecule. This displacement is particularly marked in the V α CDR2 loop, which is oriented at roughly right angles to the equivalent loop in antibody V domains, as a result of a shift in the β strand that anchors one end of the loop from one face of the domain to the other. A strand displacement also causes a change in the orientation of the V β CDR2 loop in two of the seven V β domains whose structures are known. As yet, the crystallographic structures of seven T-cell receptors have been solved to this level of resolution.

[0059] Aspects of the disclosure relate to engineered T cell receptors. The term “engineered” refers to T cell receptors that have TCR variable regions grafted onto TCR constant regions to make a chimeric polypeptide that binds to peptides and antigens of the disclosure. In certain aspects, the TCR comprises intervening sequences that are used for cloning, enhanced expression, detection, or for therapeutic control of the construct, but are not present in endogenous TCRs, such as multiple cloning sites, linker, hinge sequences, modified hinge sequences, modified transmembrane sequences, a detection polypeptide or molecule, or therapeutic controls that may allow for selection or screening of cells comprising the TCR.

[0060] In some aspects, the TCR comprises non-TCR sequences. Accordingly, certain aspects relate to TCRs with sequences that are not from a TCR gene. In some aspects, the TCR is chimeric, in that it contains sequences normally found in a TCR gene, but contains sequences from at least two TCR genes that are not necessarily found together in nature.

[0061] In some aspects the engineered TCRs of the disclosure comprise an aspect as shown below:

Table 1: TCR Aspects

Description	Sequence	SEQ ID NO:
#1. EGFR31 TCRA31-1 TRBV2*01 J2- 7*01 / TRAV14/DV4*01 C2 J48*01	ATGGATACCTGGCTCGTATGCTGGGCAATTTTTAGTCTCTT GAAAGCAGGACTCACAGAACCTGAAGTCACCCAGACTCCC AGCCATCAGGTCACACAGATGGGACAGGAAGTGATCTTGC GCTGTGTCCCATCTCTAATCACTTATACTTCTATTGGTAC AGACAAATCTTGGGGCAGAAAGTCGAGTTTCTGGTTTCCTT TTATAATAATGAAATCTCAGAGAAGTCTGAAATATTCGAT GATCAATTCTCAGTTGAAAGGCCTGATGGATCAAATTTCA CTCTGAAGATCCGGTCCACAAAGCTGGAGGACTCAGCCAT GTACTTCTGTGCCAGCAGTGGTGCCGGGGGTAGGTACGAG CAGTACTTCGGGCCGGGCACCAGGCTCACGGTCACAGAGG ACCTGAAAAACGTGTTCCACCCGAGGTCGCTGTGTTTGA GCCATCAGAAGCAGAGATCTCCACACCCAAAAGGCCACA CTGGTATGCCTGGCCACAGGCTTCTACCCCGACCACGTGG AGCTGAGCTGGTGGGTGAATGGGAAGGAGGTGCACAGTG GGGTCAGCACAGACCCGCAGCCCCTCAAGGAGCAGCCCGC CCTCAATGACTCCAGATACTGCCTGAGCAGCCGCCTGAGG GTCTCGGCCACCTTCTGGCAGAACCCCCGCAACCACTTCCG CTGTCAAGTCCAGTTCTACGGGCTCTCGGAGAATGACGAG TGGACCCAGGATAGGGCCAAACCCGTCACCCAGATCGTCA GCGCCGAGGCCTGGGGTAGAGCAGACTGTGGCTTCACCTC CGAGTCTTACCAGCAAGGGGTCTGTCTGCCACCATCCTCT ATGAGATCTTGCTAGGGAAGGCCACCTTGTATGCCGTGCT GGTCAGTGCCCTCGTGCTGATGGCCATGGTCAAGAGAAAG GATTTTCAGAGGCCGTGCCAAGCGAAGCGGATCTGGCGCCA CGAACTTCTCTCTGTAAAGCAAGCAGGAGATGTTGAAGA AAACCCCGGGCCTATGTCACTTTCTAGCCTGCTGAAGGTG GTCACAGCTTCACTGTGGCTAGGACCTGGCATTGCCCAGA AGATAACTCAAACCCAACCAGGAATGTTTCGTGCAGGAAAA GGAGGCTGTGACTCTGGACTGCACATATGACACCAGTGAT CCAAGTTATGGTCTATTCTGGTACAAGCAGCCCAGCAGTG GGGAAATGATTTTTCTTATTTATCAGGGGTCTTATGACCAG CAAAATGCAACAGAAGGTCGCTACTCATTGAATTTCCAGA AGGCAAGAAAATCCGCCAACCTTGTATCTCCGCTTCACA ACTGGGGGACTCAGCAATGTACTTCTGTGCAATGAGAGGT ATATCTAACTTTGGAAATGAGAAATTAACCTTTGGGACTG GAACAAGACTCACCATCATACCCAATATCCAGAAGCCTGA CCCTGCCGTGTACCAGCTGAGAGACTCTAAATCCAGTGAC AAGTCTGTCTGCCTATTCACCGATTTTGATTCTCAAACAAA TGTGTCACAAAGTAAGGATTCTGATGTGTATATCACAGAC AAAAGTGTGCTAGACATGAGGTCTATGGACTTCAAGAGCA ACAGTGCTGTGGCCTGGAGCAACAAATCTGACTTTGCATG TGCAAACGCCTTCAACAACAGCATTATTCCAGCAGACACC TTCTTCCCCAGCCCAGAAAGTTCCTGTGATGTCAAGCTGGT CGAGAAAAGCTTTGAAACAGATACGAACCTAACTTTCAA AACCTGTCAGTGATTGTGTTCCGAATCCTCCTCCTGAAAGT GGCCGGGTTTAATCTGCTCATGACGCTGCGGCTGTGGTCCA GCTGA	1

#1. EGFA31 TCRA31-1 TRBV2*01 J2- 7*01 / TRAV14/DV4*01 C2 J48*01	MDTWLVCWAIFSLLKAGLTEPEVTQTPSHQVTQMGQEVILRCVPISNHLIFYWYRQILGQKVEFLVSFYNNNEISEKSEIFDDQFSVERPDGSNFTLKIRSTKLEDSAMYFCASSGAGGRYEQYFGPGTRLTVTEDLKNVFPPEVAVFEPSEAEISHTQKATLVCLATGFYPDHVELSWWVNGKEVHSGVSTDPQPLKEQPALNDSRYCLSSRLRVSATFWQNPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFTSESYQQGVLSATILYEILLGKATLYAVLVLSALVLMAMVKKRDFRGRAKRSGSGATNFSLLKQAGDVEENPGPMSLSLLKVVTASLWLGPGIAQKITQTQPGMFVQEKEAVTLTDCYDTSFPSYGLFWYKQPSSGEMIFLIYQGSYDQQNATEGRYSLNFQKARKSANLVISASQLGDSAMYFCAMRGISNFGNEKLTFTGTGTRLTIIPNIQKPDPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKSDSVYITDKTVLDMRSMDFKSNSAVAWSNKSDFACANAFNNSIIPADTFFPSPSSCDVKLVEKSFETDTNLFQNLVIVFRILLKLVAGFNLLMTLRLWSS	2
#1. EGFA31 TCRA31-1-TCR- beta chain	EPEVTQTPSHQVTQMGQEVILRCVPISNHLIFYWYRQILGQKVEFLVSFYNNNEISEKSEIFDDQFSVERPDGSNFTLKIRSTKLEDSAMYFCASSGAGGRYEQYFGPGTRLTVTEDLKNVFPPEVAVFEPSEAEISHTQKATLVCLATGFYPDHVELSWWVNGKEVHSGVSTDPQPLKEQPALNDSRYCLSSRLRVSATFWQNPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFTSESYQQGVLSATILYEILLGKATLYAVLVLSALVLMAMVKKRDFRGRAKRSGSGATNFSLLKQAGDVEENPGP	3
#1. EGFA31 TCRA31-1-TCR- beta - variable region	EPEVTQTPSHQVTQMGQEVILRCVPISNHLIFYWYRQILGQKVEFLVSFYNNNEISEKSEIFDDQFSVERPDGSNFTLKIRSTKLEDSAMYFCASSGAGGRYEQYFGPGT	4
#1. EGFA31 TCRA31-1-TCR- beta signal sequence	MDTWLVCWAIFSLLKAGLT	5
#1. EGFA31 TCRA31-1-TCR- beta CDR1	SNHLYF	6
#1. EGFA31 TCRA31-1-TCR- beta CDR2	FYNNE	7
#1. EGFA31 TCRA31-1-TCR- beta CDR3	ASSGAGGRYEQY	8
#1. EGFA31 TCRA31-1-TCR- alpha chain	AQKITQTQPGMFVQEKEAVTLTDCYDTSFPSYGLFWYKQPSSGEMIFLIYQGSYDQQNATEGRYSLNFQKARKSANLVISASQLGDSAMYFCAMRGISNFGNEKLTFTGTGTRLTIIPNIQKPDPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKSDSVYITDKTVLDMRSMDFKSNSAVAWSNKSDFACANAFNNSIIPADTFFPSPSSCDVKLVEKSFETDTNLFQNLVIVFRILLKLVAGFNLLMTLRLWSS	9

#1. EGFRA31 TCRA31-1-TCR- alpha - variable region	AQKITQTQPGMFVQEKEAVTLDCTYDTSFPSYGLFWYKQPSS GEMIFLIYQGSYDQQNATEGRYSLNFQKARKSANLVISASQL GDSAMYFCAMRGISNFGNEKLTFGTGTRLTIIP	10
#1. EGFRA31 TCRA31-1-TCR- alpha signal sequence	MSLSSLLKVVTASLWLGPGI	11
#1. EGFRA31 TCRA31-1-TCR- alpha CDR1	TSDPSYG	12
#1. EGFRA31 TCRA31-1-TCR- alpha CDR2	QGSYDQQN	13
#1. EGFRA31 TCRA31-1-TCR- alpha CDR3	AMRGISNFGNEKL T	14
#2. EGFRA31 RTCA31-2 TRAV14/DV4*01 J48*01/TRBV2*0 1C2J2-7*01	ATGTCACCTTTCTAGCCTGCTGAAGGTGGTCACAGCTTCACT GTGGCTAGGACCTGGCATTGCCCAGAAGATAACTCAAACC CAACCAGGAATGTTTCGTGCAGGAAAAGGAGGCTGTGACTC TGGACTGCACATATGACACCAGTGATCCAAGTTATGGTCT ATTCTGGTACAAGCAGCCCAGCAGTGGGGAAATGATTTTT CTTATTTATCAGGGGTCTTATGACCAGCAAAATGCAACAG AAGGTCGCTACTCATTGAATTTCCAGAAGGCAAGAAAATC CGCCAACCTTGTCTATCTCCGCTTCACAACTGGGGGACTCAG CAATGTACTTCTGTGCAATGAGAGTTATATCTAACTTTGGA AATGAGAAATTAACCTTTGGGACTGGAACAAGACTCACCA TCATACCCAATATCCAGAAGCCTGACCCTGCCGTGTACCA GCTGAGAGACTCTAAATCCAGTGACAAGTCTGTCTGCCTA TTCACCGATTTTGATTCTCAAACAAATGTGTACAAAGTAA GGATTCTGATGTGTATATCACAGACAAAAGTGTGCTAGAC ATGAGGTCTATGGACTTCAAGAGCAACAGTGCTGTGGCCT GGAGCAACAAATCTGACTTTGCATGTGCAAACGCCTTCAA CAACAGCATTATTCCAGCAGACACCTTCTTCCCCAGCCCAG AAAGTTCCTGTGATGTCAAGCTGGTCGAGAAAAGCTTTGA AACAGATACGAACCTAACTTTCAAACCTGTCTAGTGATT GGGTTCCGAATCCTCCTCCTGAAAGTGGCCGGGTTTTATCT GCTCATGACGCTGCGGCTGTGGTCCAGCGTCGACGTGCCA AGCGAAGCGGATCTGGCGCCACGAACCTTCTCTGTGTTAA GCAAGCAGGAGATGTTGAAGAAAACCCCGGGCCTCTCGAG ATGGATACCTGGCTCGTATGCTGGGCAATTTTTAGTCTCTT GAAAGCAGGACTCACAGAACCTGAAGTCACCCAGACTCCC AGCCATCAGGTCACACAGATGGGACAGGAAGTGATCTTGC GCTGTGTCCCCTCTCTAATCACTTATACTTCTATTGGTAC AGACAAATCTTGGGGCAGAAAGTCGAGTTTCTGGTTTCCTT TTATAATAATGAAATCTCAGAGAAGTCTGAAATATTCGAT GATCAATTCTCAGTTGAAAGGCCTGATGGATCAAATTTCA CTCTGAAGATCCGGTCCACAAAGCTGGAGGACTCAGCCAT GTACTTCTGTGCCAGCAGTCTCGGCGGGGGCACCTACGAG CAGTACTTCGGGCGGGGCACCAGGCTCACGGTCACAGAGG ACCTGAAAAACGTGTTCCACCCGAGGTCGCTGTGTTTGA	15

	GCCATCAGAAGCAGAGATCTCCCACACCCAAAAGGCCACA CTGGTGTGCCTGGCCACAGGCTTCTTCCCTGACCACGTGGA GCTGAGCTGGTGGGTGAATGGGAAGGAGGTGCACAGTGG GGTCAGCACAGACCCGCAGCCCCCTCAAGGAGCAGCCCGCC CTCAATGACTCCAGATACTGCCTGAGCAGCCGCCTGAGGG TCTCGGCCACCTTCTGGCAGAACCCCCGCAACCACTTCCGC TGTCAAGTCCAGTTCTACGGGCTCTCGGAGAATGACGAGT GGACCCAGGATAGGGCCAAACCTGTCACCCAGATCGTCAG CGCCGAGGCCTGGGGTAGAGCAGACTGTGGCTTCACCTCC GAGTCTTACCAGCAAGGGGTCCTGTCTGCCACCATCCTCTA TGAGATCTTGCTAGGGAAGGCCACCTTGTATGCCGTGCTG GTCAGTGCCCTCGTGCTGATGGCCATGGTCAAGAGAAAGG ATTCCAGAGGCTAG	
#2. EGFR31 RTCRA31-2 TRAV14/DV4*01 J48*01/TRBV2*0 1C2J2-7*01	MSLSSLLKVVVTASLWLGPGIAQKITQTQPGMFVQEKEAVTLD CTYDTS DPSYGLFWYKQPSSGEMIFLIYQGSYDQQNATEGRY SLNFQKARKSANLVISASQLGDSAMYFCAMRVISNFGNEKLT FGTGTRLTIIPNIQKPDPAVYQLRDSKSSDKSVCLFTDFDSQTN VSQSKDSDVYITDKTVLDMRSMDFKSNSAVAWSNKSDFACA NAFNNSIIPADTFFPSPESCDVKLVEKSFETDTNLFQNL SVI GFRILLKLVAGFYLLMTLRLWSSVDRAKRSGSGATNFSLLKQ AGDVEENPGPLEMDTWLVCWAIFSLKAGLTEPEVTQTPSHQ VTQMGEVILRCVPISNHL YFYWYRQILGQKVEFLVSFYNN ISEKSEIFDDQFSVERPDGSNFTLKIRSTKLEDSAMYFCASSLG GGTYEQYFGPGTRLTVTEDLKNVPPEVAVFEPSEAEISHTQK ATLVCLATGFFPDHVELS WVVNGKEVHSGVSTDPQPLKEQP ALNDSRYCLSSRLRVSAFTWQNP RNHFRCQVQFYGLSENDE WTQDRAKPVTQIVSAEAWGRADCGFTSESYQQGVLSATILY EILLGKATLYAVLV SALVLMAMVKRKDSRG	16
#2. EGFR31 RTCRA31-2- TCR-alpha chain	AQKITQTQPGMFVQEKEAVTLDCTYDTS DPSYGLFWYKQPSS GEMIFLIYQGSYDQQNATEGRYSLNFQKARKSANLVISASQL GDSAMYFCAMRVISNFGNEKLTFGTGTRLTIIPNIQKPDPAVY QLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKTVLDM RSMDFKSNSAVAWSNKSDFACANAFNNSIIPADTFFPSPESCD VKLVEKSFETDTNLFQNL SVIGFRILLKLVAGFYLLMTLRL WSSVDRAKRSGSGATNFSLLKQAGDVEENPGPLE	17
#2. EGFR31 RTCRA31-2- TCR-alpha variable region	AQKITQTQPGMFVQEKEAVTLDCTYDTS DPSYGLFWYKQPSS GEMIFLIYQGSYDQQNATEGRYSLNFQKARKSANLVISASQL GDSAMYFCAMRVISNFGNEKLTFGTGTRLTIIP	18
#2. EGFR31 RTCRA31-2- TCR-alpha signal sequence	MSLSSLLKVVVTASLWLGPGI	19
#2. EGFR31 RTCRA31-2- TCR-alpha CDR1	TSDPSYG	20
#2. EGFR31 RTCRA31-2- TCR-alpha CDR2	QGSYDQQN	21

#2. EGFA31 RTCRA31-2- TCR-alpha CDR3	AMRVISNFGNEKLT	22
#2. EGFA31 RTCRA31-2- TCR-beta chain	EPEVTQTPSHQVTQMGQEVILRCVPISNHL YFYWYRQILGQK VEFLVSFYNN EISEKSEIFDDQFSVERPDGSNFTLKIRSTKLED SAMYFCASSLGGGT YEQYFGPGTRLTVTEDLKNVFPPEVAVF EPSEAEISHTQKATLVCLATGFFPDHVELSWVNGKEVHSGV STDPQPLKEQPALNDSRYCLSSRLRV SATFWQNPRNHFRCQV QFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFTSESYQ QGVLSATILYEILLGKATLYAVLV SALVLMAMVKKR KDSRG	23
#2. EGFA31 RTCRA31-2- TCR-beta variable region	EPEVTQTPSHQVTQMGQEVILRCVPISNHL YFYWYRQILGQK VEFLVSFYNN EISEKSEIFDDQFSVERPDGSNFTLKIRSTKLED SAMYFCASSLGGGT YEQYFGPGTRLTVT	24
#2. EGFA31 RTCRA31-2- TCR-beta signal sequence	MDTWLVCWAIFSLLKAGLT	25
#2. EGFA31 RTCRA31-2- TCR-beta CDR1	SNHLY	26
#2. EGFA31 RTCRA31-2- TCR-beta CDR2	FYNNEI	27
#2. EGFA31 RTCRA31-2- TCR-beta CDR3	ASSLGGGT YEQYF	28

#1 EGFR33 TCR33-32L TRAV19*01 J33*01 / TRBV12- 3*01C2 J2-1*01	ATGCTGTTCTCCAGCCTGCTGTGTGTATTTGTGGCCTTCAG CTACTCTGGATCAAGTGTGGCCCAGAAGGTTACTCAAGCC CAGTCATCAGTATCCATGCCAGTGAGGAAAGCAGTCACCC TGAAGTGCCTGTATGAAACAAGTTGGTGGTCATATTATATT TTTTGGTACAAGCAACTTCCCAGCAAAGAGATGATTTTCCT TATTCGCCAGGGTTCTGATGAACAGAATGCAAAAAGTGGT CGCTATTCTGTCAACTTCAAGAAAGCAGCGAAATCCGTCG CCTTAACCATTTTCAGCCTTACAGCTAGAAGATTCAGCAAA GTACTTTTGTGCTCTTGGGGATAGCAACTATCAGTTAATCT GGGGCGCTGGGACCAAGCTAATTATAAAGCCAGATATCCA GAACCTTGACCCTGCCGTGTACCAGCTGAGAGACTCTAAA TCCAGTGACAAGTCTGTCTGCCTATTCACCGATTTTGATT TCAAACAAATGTGTACAAAGTAAGGATTCTGATGTGTAT ATCACAGACAAATGCGTGCTAGACATGAGGTCTATGGACT TCAAGAGCAACAGTGCTGTGGCCTGGAGCAACAAATCTGA CTTTGCATGTGCAAACGCCTTCAACAACAGCATTATTCCAG AAGACACCTTCTTCCCCAGCCCAGAAAGTTCCTGTGATGTC AAGCTGGTCGAGAAAAGCTTTGAAACAGATACGAACCTAA ACTTTCAAACCTGTCAAGTGATTGGGTTCGAATCCTCCTC CTGAAAGTGGCCGGGTTTAATCTGCTCATGACGCTGCGGC TGTGGTCCAGCCGTGCCAAGCGAAGCGGATCTGGCGCCAC GAAGTTCTCTCTGTAAAGCAAGCAGGAGATGTTGAAGAA AACCCCGGGCCTATGGACTCCTGGACCTTCTGCTGTGTGTC CCTTTGCATCCTGGTAGCGAAGCATAACAGATGCTGGAGTT ATCCAGTCACCCCGCCATGAGGTGACAGAGATGGGACAAG AAGTGAAGTCTGAGATGTAAACCAATTTTCAGGCCACAATC CCTTTTCTGGTACAGACAGACCATGATGCGGGGACTGGAG TTGCTCATTTACTTTAACAACAACGTTCCGATAGATGATTC AGGGATGCCCCGAGGATCGATTCTCAGCTAAGATGCCTAAT GCATCATTCTCCACTCTGAAGATCCAGCCCTCAGAACCCA GGGACTCAGCTGTGTACTTCTGTGCCAGCAGAGCCGGGAC AGGGGAGGTCAATGAGCAGTTCTTCGGGCCAGGGACACGG CTCACCGTGCTAGAGGACCTGAAAAACGTGTTCCACCCG AGGTCGCTGTGTTTGAGCCATCAGAAGCAGAGATCTCCCA CACCCAAAAGGCCACACTGGTGTGCCTGGCCACAGGCTTC TACCCCGACCACGTGGAGCTGAGCTGGTGGGTGAATGGGA AGGAGGTGCACAGTGGGGTCTGCACAGACCCGCAGCCCCT CAAGGAGCAGCCCGCCCTCAATGACTCCAGATACTGCCTG AGCAGCCGCCTGAGGGTCTCGGCCACCTTCTGGCAGAACC CCCGCAACCACTTCCGCTGTCAAGTCCAGTTCTACGGGCTC TCGGAGAATGACGAGTGGACCCAGGATAGGGCCAAACCT GTCACCCAGATCGTCAGCGCCGAGGCCTGGGGTAGAGCAG ACTGTGGCTTCACCTCCGAGTCTTACCAGCAAGGGGTCCTG TCTGCCACCATCCTCTATGAGATCTTGCTAGGGAAGGCCAC CTTGTATGCCGTGCTGGTCAGTGCCCTCGTGCTGATGGCCA TGGTCAAGAGAAAGGATTCCAGAGGCTAA	29
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#1 EGFR33 TCR33-32L TRAV19*01 J33*01 / TRBV12- 3*01C2 J2-1*01	MLFSSLLCVFVAFSYSGSSVAQKVTQAQSSVSMPVRKAVTLN CLYETSWWSYYIFWYKQLPSKEMIFLIRQGSDEQNAKSGRYS VNFKKA AKSVALTISALQLEDS AKYFCALGDSNYQLIWGAG TKLIIKPD IQNPDP AVYQLRDSKSSDKSVCLFTDFDSQTNVSQ SKDSDVYITDKCVLDMRSMDFKSNSAVAWSNKSDFACANAF NNSIIPEDTFFPSPESSCDVKLVEKSFETDTNLFQNL SVIGFRI LLLKVAGFNLLMTLRLWSSRAKRSGSGATNFSLLKQAGDVE ENPGPMDSWTFCCVSLCILVAKHTDAGVIQSPRHEVTEMGQE VTLRCKPISGHNSLFWYRQTM MRGLELLIYFNNNPIDDSGM PEDRFS AKMPNASFSTLKIQPSEPRDS AVYFCASRAGTGEVNE QFFGPGTRLTVLEDLKNVFPPEVAVFEPSEAEISHTQKATLVC LATGFYPDHVELSWWVNGKEVHSGVCTDPQPLKEQPALNDS RYCLSSRLRVSATFWQNPRNHFRCQVQFYGLSENDEWTQDR AKPVTQIVSAEAWGRADCGFTSESYQQGVLSATILYEILLGK ATLYAVLVSALVLMAMVKRKDSRG	30
#1 EGFR33 TCR33-32L-TCR- alpha chain	QKVTQAQSSVSMPVRKAVTLNCLYETSWWSYYIFWYKQLPS KEMIFLIRQGSDEQNAKSGRYSVNFKKA AKSVALTISALQLE DS AKYFCALGDSNYQLIWGAGTKLIIKPD IQNPDP AVYQLRD SKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKCVLDMRSM DKSNSAVAWSNKSDFACANAFNNSIIPEDTFFPSPESSCDVKL VEKSFETDTNLFQNL SVIGFRILLKVAGFNLLMTLRLWSSR AKRSGSGATNFSLLKQAGDVEENPGP	31
#1 EGFR33 TCR33-32L-TCR- alpha - variable region	QKVTQAQSSVSMPVRKAVTLNCLYETSWWSYYIFWYKQLPS KEMIFLIRQGSDEQNAKSGRYSVNFKKA AKSVALTISALQLE DS AKYFCALGDSNYQLIWGAGTKLIIKPD	32
#1 EGFR33 TCR33-32L-TCR- alpha signal sequence	MLFSSLLCVFVAFSYSGSSVA	33
#1 EGFR33 TCR33-32L-TCR- alpha CDR1	TSWWSYY	34
#1 EGFR33 TCR33-32L-TCR- alpha CDR2	QGSDEQNA	35
#1 EGFR33 TCR33-32L-TCR- alpha CDR3	LGDSNYQLI	36
#1 EGFR33 TCR33-32L-TCR- beta chain	GVIQSPRHEVTEMGQEVTLRCKPISGHNSLFWYRQTM MRGL ELLIYFNNNPIDDSGM PEDRFS AKMPNASFSTLKIQPSEPRDS AVYFCASRAGTGEVNEQFFGPGTRLTVLEDLKNVFPPEVAVF EPSEAEISHTQKATLVCLATGFYPDHVELSWWVNGKEVHSG VCTDPQPLKEQPALNDSRYCLSSRLRVSATFWQNPRNHFRCQ VQFYGLSENDEWTQDR AKPVTQIVSAEAWGRADCGFTSESY QQGVLSATILYEILLGKATLYAVLVSALVLMAMVKRKDSRG	37

#1 EGFR33 TCR33-32L-TCR- beta - variable region	GVIQSPRHEVTEMGQEVTLRCKPISGHNSLFWYRQTMMRGL ELLIYFNNNVPIDDSGMPEDRFS AKMPNASFSTLKIQPSEPRDS AVYFCASRAGTGEVNEQFFGPGTRLTVL	38
#1 EGFR33 TCR33-32L-TCR- beta signal sequence	MDSWTFCCVSLCILVAKHTDA	39
#1 EGFR33 TCR33-32L-TCR- beta CDR1	SGHNS	40
#1 EGFR33 TCR33-32L-TCR- beta CDR2	FNNNVP	41
#1 EGFR33 TCR33-32L-TCR- beta CDR3	ASRAGTGEVNEQF	42
#2. EGFR33 TCRA33-32H TRAV14/DV4*02 TRAJ48*01 /TRBV16*01 C2 J2-1*01	ATGTCACCTTTCTAGCCTGCTGAAGGTGGTCACAGCTTCACT GTGGCTAGGACCTGGCATTGCCCAGAAGATAACTCAAACC CAACCAGGAATGTTTCGTGCAGGAAAAGGAGGCTGTGACTC TGGACTGCACATATGACACCAGTGATCAAAGTTATGGTCT ATTCTGGTACAAGCAGCCCAGCAGTGGGGAAATGATTTTT CTTATTTATCAGGGGTCTTATGACGAGCAAAATGCAACAG AAGGTCGCTACTCATTGAATTTCCAGAAGGCAAGAAAATC CGCCAACCTTGTCTATCTCCGCTTCACAACTGGGGGACTCAG CAATGTATTTCTGTGCAATGAGAGAGGGTCTTTGGAAATGA GAAATTAACCTTTGGGACTGGAACAAGACTCACCATCATA CCCAATATCCAGAACCCTGACCCTGCCGTGTACCAGCTGA GAGACTCTAAATCCAGTGACAAGTCTGTCTGCCTATTACC GATTTTGATTCTCAAACAAATGTGTACAAAGTAAGGATT CTGATGTGTATATCACAGACAAATGCGTGCTAGACATGAG GTCTATGGACTTCAAGAGCAACAGTGCTGTGGCCTGGAGC AACAAATCTGACTTTGCATGTGCAAACGCCTTCAACAACA GCATTATTCCAGAAGACACCTTCTTCCCCAGCCCAGAAAG TTCCTGTGATGTCAAGCTGGTCGAGAAAAGCTTTGAAACA GATACGAACCTAACTTTCAAAACCTGTCAGTGATTGGGT TCCGAATCCTCCTCCTGAAAGTGGCCGGGTTTAAATCTGCTC ATGACGCTGCGGCTGTGGTCCAGCCGTGCCAAGCGAAGCG GATCTGGCGCCACGAACCTTCTCTGTAAAGCAAGCAGG AGATGTTGAAGAAAACCCCGGGCCTATGAGCCCAATATTC ACCTGCATCACAATCCTTTGTCTGCTGGCTGCAGGTTCTCC TGGTGAAGAAGTCGCCCAGACTCCAAAACATCTTGTCAGA GGGGAAGGACAGAAAGCAAAATTATATTGTGCCCCAATAA AAGGACACAGTTATGTTTTTTGGTACCAACAGGTCCTGAA AAACGAGTTCAAGTTCTTGATTTCCTTCCAGAATGAAAATG TCTTTGATGAAACAGGTATGCCCAAGGAAAGATTTTCAGC TAAGTGCCTCCCAAATTCACCCTGTAGCCTTGAGATCCAGG CTACGAAGCTTGAGGATTCAGCAGTGTATTTTTGTGCCAGC AGCCCTCCTCCCTCAAACACCTACAATGAGCAGTTCTTCGG GCCAGGGACACGGCTCACCGTGCTAGAGGACCTGAAAAAC GTGTTCCCAACCCGAGGTCGCTGTGTTTGAGCCATCAGAAG	43

	CAGAGATCTCCACACCCAAAAGGCCACACTGGTGTGCCT GGCCACAGGCTTCTACCCCGACCACGTGGAGCTGAGCTGG TGGGTGAATGGGAAGGAGGTGCACAGTGGGGTCTGCACA GACCCGCAGCCCCTCAAGGAGCAGCCCGCCCTCAATGACT CCAGATACTGCCTGAGCAGCCGCCTGAGGGTCTCGGCCAC CTTCTGGCAGAACCCCCGCAACCACTTCCGCTGTCAAGTCC AGTTCTACGGGCTCTCGGAGAATGACGAGTGGACCCAGGA TAGGGCCAAACCTGTACCCAGATCGTCAGCGCCGAGGCC TGGGGTAGAGCAGACTGTGGCTTCACCTCCGAGTCTTACC AGCAAGGGGTCTGTCTGCCACCATCCTCTATGAGATCTTG CTAGGGAAGGCCACCTTGTATGCCGTGCTGGTCAGTGCCC TCGTGCTGATGGCCATGGTCAAGAGAAAGGATTCCAGAGG CTAA	
#2. EGFR33 TCRA33-32H TRAV14/DV4*02 TRAJ48*01 /TRBV16*01 C2 J2-1*01	MSLSSLLKVVVTASLWLGPGIAQKITQTQPGMFVQEKEAVTLD CTYDTSQSYGLFWYKQPSSGEMIFLIYQGSYDEQNATEGRY SLNFQKARKSANLVISASQLGDSAMYFCAMREVFVNEKLT GTGTRLTIIPNIQNPDPAVYQLRDSKSSDKSVCLFTDFDSQTN VSQSKDSVDVYITDKCVLDMRSMDFKSNSAVAWSNKSDFA NAFNNSIIPEDTFFPSPESSCDVKLVEKSFETDTNLFQNL SVIGFRILLKLVAGFNLLMTLRLWSSRAKRSGSGATNFSLLKQAG DVEENPGPMSPIFTCILCLLAAGSPGEEVAQTPKHLVRGEG QKAKLYCAPIKGHSYVFWYQQVLKNEFKFLISFQENNVFDET GMPKERFSAKCLPNSPCSLEIQATKLEDSAVYFCASSPPPSNT YNEQFFGPGTRLTVLEDLKNVFPPEVAVFEPSEAEISHTQKAT LVCLATGFYDPDHVELSWVNGKEVHSGVCTDPQPLKEQPAL NDSRYCLSSRLRVSAATFWQNPARNHFRQVQFYGLSENDEWT QDRAKPVTQIVSAEAWGRADCGFTSESYQQGVLSATILYEIL LGKATLYAVLVSAVLMLMAMVKRKDSRG	44
#2. EGFR33 TCRA33-32H- TCR-alpha chain	QKITQTQPGMFVQEKEAVTLDCTYDTSQSYGLFWYKQPSS GEMIFLIYQGSYDEQNATEGRYSLNFQKARKSANLVISASQL GDSAMYFCAMREVFVNEKLTFTGTGTRLTIIPNIQNPDPAVYQ LRDSKSSDKSVCLFTDFDSQTNVSQSKDSVDVYITDKCVLDMR SMDFKSNSAVAWSNKSDFAANAFNNSIIPEDTFFPSPESSCD VKLVEKSFETDTNLFQNL SVIGFRILLKLVAGFNLLMTLRL WSSRAKRSGSGATNFSLLKQAGDVEENPGP	45
#2. EGFR33 TCRA33-32H- TCR-alpha variable region	QKITQTQPGMFVQEKEAVTLDCTYDTSQSYGLFWYKQPSS GEMIFLIYQGSYDEQNATEGRYSLNFQKARKSANLVISASQL GDSAMYFCAMREVFVNEKLTFTGTGTRLTIIP	46
#2. EGFR33 TCRA33-32H- TCR-alpha signal sequence	MSLSSLLKVVVTASLWLGPGIA	47
#2. EGFR33 TCRA33-32H- TCR-alpha CDR1	TSDQSYG	48
#2. EGFR33 TCRA33-32H- TCR-alpha CDR2	QGSYDEQN	49

#2. EGFR33 TCRA33-32H- TCR-alpha CDR3	AMREVFVGNEKLT	50
#2. EGFR33 TCRA33-32H- TCR-beta chain	EEVAQTPKHLVRGEGQKAKLYCAPIKGHSYVFWYQQVLKNE FKFLISFQNNENVFDETGMPEKRFSAKCLPNSPCSLEIQATKLED SAVYFCASSPPPSNTYNEQFFGPGTRLTVLLEDLKNVFPPEVAV FEPSEAEISHTQKATLVCLATGFYDPDHVELSWWVNGKEVHSG VCTDPQPLKEQPALNDSRYCLSSRLRVSAFWQNPNNHFRQC VQFYGLSENDEWTQDRAKPVITQIVSAEAWGRADCGFTSESY QQGVLSATILYEILLGKATLYAVLVSALVLMAMVKKRDSRG	51
#2. EGFR33 TCRA33-32H- TCR-beta variable region	EEVAQTPKHLVRGEGQKAKLYCAPIKGHSYVFWYQQVLKNE FKFLISFQNNENVFDETGMPEKRFSAKCLPNSPCSLEIQATKLED SAVYFCASSPPPSNTYNEQFFGPGTRLTVL	52
#2. EGFR33 TCRA33-32H- TCR-beta signal sequence	MSPIFTCITILCLLAAGSPG	53
#2. EGFR33 TCRA33-32H- TCR-beta CDR1	KGHSY	54
#2. EGFR33 TCRA33-32H- TCR-beta CDR2	FQNNENV	55
#2. EGFR33 TCRA33-32H- TCR-beta CDR3	ASSPPPSNTYNEQF	56

#3 EGFR33 TCRA33-37-3 TRAV14/DV4*02 /TRBV16*01 C1 J1-5*01**	ATGTCACTTTCTAGCCTGCTGAAGGTGGTCACAGCTTCACT GTGGCTAGGACCTGGCATTGCCCAGAAGATAACTCAAACC CAACCAGGAATGTTTCGTGCAGGAAAAGGAGGCTGTGACTC TGGACTGCACATATGACACCAGTGATCAAAGTTATGGTCT ATTCTGGTACAAGCAGCCCAGCAGTGGGGAAATGATTTTT CTTATTTATCAGGGGTCTTATGACGAGCAAAATGCAACAG AAGGTCGCTACTCATTGAATTTCCAGAAGGCAAGAAAATC CGCCAACCTTGTCTATCTCCGCTTCACAACTGGGGGACTCAG CAATGTATTTCTGTGCAATGAGAGAGGTCTTTGGAAATGA GAAATTAACCTTTGGGACTGGAACAAGACTCACCATCATA CCCAATATCCAGAACCCTGACCCTGCCGTGTACCAGCTGA GAGACTCTAAATCCAGTGACAAGTCTGTCTGCCTATTACC GATTTTGATTCTCAAACAAATGTGTACAAAAGTAAGGATT CTGATGTGTATATCACAGACAAATGCGTGTAGACATGAG GTCTATGGACTTCAAGAGCAACAGTGCTGTGGCCTGGAGC AACAAATCTGACTTTGCATGTGCAAACGCCTTCAACAACA GCATTATTCCAGAAGACACCTTCTTCCCCAGCCCAGAAAG TTCCTGTGATGTCAAGCTGGTCGAGAAAAGCTTTGAAACA GATACGAACCTAACTTTCAAAACCTGTCAGTGATTGGGT TCCGAATCCTCCTCCTGAAAGTGGCCGGGTTTAATCTGCTC ATGACGCTGCGGCTGTGGTCCAGCCGTGCCAAGCGAAGCG GATCTGGCGCCACGAACCTTCTCTCTGTTAAAGCAAGCAGG AGATGTTGAAGAAAACCCCGGGCCTATGAGCCCAATATTC ACCTGCATCACAATCCTTTGTCTGCTGGCTGCAGGTTCTCC TGGTGAAGAAGTCGCCCAGACTCCAAAACATCTTGTGAGA GGGGAAGGACAGAAAAGCAAAATTATATTGTGCCCCAATAA AAGGACACAGTTATGTTTTTTGGTACCAACAGGTCCTGAA AAACGAGTTCAAGTTCTTGATTTCCTTCCAGAATGAAAATG TCTTTGATGAAACAGGTATGCCCAAGGGAAGATTTTCAGC TAAGTGCCTCCCAAATTCACCCTGTAGCCTTGAGATCCAGG CTACGAAGCTTGAGGATTCAGCAGTGTATTTTGTGCCAGC AGCCATAGAAGTTGGAAGGGGGGTGATACGCAGCCCCAG CATTTTGGTGTGAGGACTCGACTCTCCATCCTAGAGGACCT GAACAAGGTGTTCCCACCCGAGGTCGCTGTGTTTGAGCCA TCAGAAGCAGAGATCTCCACACCCAAAAGGCCACACTGG TGTGCCTGGCCACAGGCTTCTTCCCTGACCACGTGGAGCTG AGCTGGTGGGTGAATGGGAAGGAGGTGCACAGTGGGGTCT GCACGGACCCGCAGCCCCTCAAGGAGCAGCCCGCCCTCAA TGAATCCAGATACTGCCTGAGCAGCCGCCTGAGGGTCTCG GCCACCTTCTGGCAGAACCCCGCAACCACTTCCGCTGTCA AGTCCAGTTCTACGGGCTCTCGGAGAATGACGAGTGGACC CAGGATAGGGCCAAACCCGTCACCCAGATCGTCAGCGCCG AGGCCTGGGGTAGAGCAGACTGTGGCTTTACCTCGGTGTC CTACCAGCAAGGGGTCCTGTCTGCCACCATCCTCTATGAG ATCCTGCTAGGGAAGGCCACCCTGTATGCTGTGCTGGTCA GCGCCCTTGTGTTGATGGCCATGGTCAAGAGAAAGGATTT CTGA	57
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#3 EGFR33 TCRA33-37-3 TRAV14/DV4*02 /TRBV16*01 C1 J1-5*01**	MSLSSLLKVVVTASLWLGPGIAQKITQTQPGMFVQEKEAVTLD CTYDTSQSYGLFWYKQPSSGEMIFLIYQGSYDEQNATEGRY SLNFQKARKSANLVISASQLGDSAMYFCAMREVFGEKLT GTGTRLTIIPNIQNPDPAVYQLRDSKSSDKSVCLFTDFDSQTN VSQSKSDSDVYITDKCVLDMRSMDFKSNSAVAWSNKSDFACA NAFNNSIIPEDTFFPSPESSCDVKLVEKSFETDTNLFQNL SVIGFRILLKLVAGFNLLMTLRLWSSRAKRSRSGGATNFSLLKQAG DVEENPGPMSPIFTCITILCLLAAGSPGEEVAQTPKHLVRGEG QKAKLYCAPIKGHSYVFWYQQVLKNEFKFLISFQENNVFDET GMPKGRFSAKCLPNSPCSLEIQATKLEDSAVYFCASSHRSWK GGDTQPQHFGDGTRLSILEDLNKVFPPVAVFEPSEAEISHTQ KATLVCLATGFFPDHVELSWVWNGKEVHSGVCTDPQPLKEQ PALNDSRYCLSSRLRVSATFWQNPRNHFRQVQFYGLSENDE WTQDRAKPVTQIVSAEAWGRADCGFTSVSYQQGVLSATILY EILLGKATLYAVLVSALVLMAMVKKRDF	58
#3 EGFR33 TCRA33-37-3- TCR-alpha chain	AQKITQTQPGMFVQEKEAVTLDCTYDTSQSYGLFWYKQPS SGEMIFLIYQGSYDEQNATEGRYSLNFQKARKSANLVISASQL GDSAMYFCAMREVFGEKLTFTGTGTRLTIIPNIQNPDPAVYQ LRDSKSSDKSVCLFTDFDSQTNVSQSKSDSDVYITDKCVLDMR SMDFKSNSAVAWSNKSDFACANAFNNSIIPEDTFFPSPESSCD VKLVEKSFETDTNLFQNL SVIGFRILLKLVAGFNLLMTLRL WSSRAKRSRSGGATNFSLLKQAGDVEENPGP	59
#3 EGFR33 TCRA33-37-3- TCR-alpha variable region	AQKITQTQPGMFVQEKEAVTLDCTYDTSQSYGLFWYKQPS SGEMIFLIYQGSYDEQNATEGRYSLNFQKARKSANLVISASQL GDSAMYFCAMREVFGEKLTFTGTGTRLTIIP	60
#3 EGFR33 TCRA33-37-3- TCR-alpha signal sequence	MSLSSLLKVVVTASLWLGPGI	61
#3 EGFR33 TCRA33-37-3- TCR-alpha CDR1	TSDQSYG	62
#3 EGFR33 TCRA33-37-3- TCR-alpha CDR2	QGSYDEQN	63
#3 EGFR33 TCRA33-37-3- TCR-alpha CDR3	AMREVFGEKLT	64
#3 EGFR33 TCRA33-37-3- TCR-beta chain	EEVAQTPKHLVRGEGQKAKLYCAPIKGHSYVFWYQQVLKNE FKFLISFQENNVFDETGMPKGRFSAKCLPNSPCSLEIQATKLE DSAVYFCASSHRSWKGGDTQPQHFGDGTRLSILEDLNKVFPP EVAVFEPSEAEISHTQKATLVCLATGFFPDHVELSWVWNGKE VHSGVCTDPQPLKEQPALNDSRYCLSSRLRVSATFWQNPRNH FRQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFT SVSYQQGVLSATILYEILLGKATLYAVLVSALVLMAMVKKR DF	65

#3 EGFRA33 TCRA33-37-3- TCR-beta variable region	EEVAQTPKHLVRGEGQKAKLYCAPIKGHSYVFWYQQVLKNE FKFLISFQNNENVFDETGMPPKGRFSAKCLPNSPCSLEIQATKLE DSAVYFCASSHRSWKGGDTQPQHFGDGTRLSIL	66
#3 EGFRA33 TCRA33-37-3- TCR-beta signal sequence	MSPIFTCITILCLLAAGSPG	67
#3 EGFRA33 TCRA33-37-3- TCR-beta CDR1	KGHSY	68
#3 EGFRA33 TCRA33-37-3- TCR-beta CDR2	FQNNENV	69
#3 EGFRA33 TCRA33-37-3- TCR-beta CDR3	ASSHRSWKGGDTQPQH	70
#4. EGFRA33 TCRA33-37-4 TRAV14/DV4*02 /TRBV 16*01 C1 J1-5*01**	ATGTCACCTTTCTAGCCTGCTGAAGGTGGTCACAGCTTCACT GTGGCTAGGACCTGGCATTGCCCAGAAGATAACTCAAACC CAACCAGGAATGTTTCGTGCAGGAAAAGGAGGCTGTGACTC TGGACTGCACATATGACACCAGTGATCCAAGTTATGGTCT ATTCTGGTACAAGCAGCCCAGCAGTGGGGAAATGATTTTT CTTATTTATCAGGGGTCTTATGACCAGCAAAATGCAACAG AAGGTCGCTACTCATTGAATTTCCAGAAGGCAAGAAAATC CGCCAACCTTGTCTATCTCCGCTTCACAACTGGGGGACTCAG CAATGTACTTCTGTGCAATGAGGAGTGGGCATCTGTCTAA CTTTGGAAATGAGAAATTAACCTTTGGGACTGGAACAAGA CTCACCATCATACCCAATATCCAGAACCCTGACCCTGCCGT GTACCAGCTGAGAGACTCTAAATCCAGTGACAAGTCTGTC TGCCTATTCACCGATTTTGTATTCTCAAACAAATGTGTACAA AAGTAAGGATTCTGATGTGTATATCACAGACAAATGCGTG CTAGACATGAGGTCTATGGACTTCAAGAGCAACAGTGCTG TGGCCTGGAGCAACAAATCTGACTTTGCATGTGCAAACGC CTTCAACAACAGCATTATTCCAGAAGACACCTTCTTCCCCA GCCCAGAAAAGTTCCTGTGATGTCAAGCTGGTCGAGAAAAG CTTTGAAACAGATACGAACCTAACTTTCAAAACCTGTCA GTGATTGGGTTCCGAATCCTCCTCCTGAAAGTGGCCGGGTT TAATCTGCTCATGACGCTGCGGCTGTGGTCCAGCCGTGCCA AGCGAAGCGGATCTGGCGCCACGAACTTCTCTCTGTAAA GCAAGCAGGAGATGTTGAAGAAAACCCCGGGCCTATGAG CCCAATATTCACCTGCATCACAATCCTTTGTCTGCTGGCTG CAGGTTCTCCTGGTGAAGAAGTCGCCCAGACTCCAAAACA TCTTGTGAGAGGGGAAGGACAGAAAAGCAAAATTATATTGT GCCCCAATAAAAAGGACACAGTTATGTTTTTTGGTACCAAC AGGTCCTGAAAAACGAGTTCAAGTTCTTGATTTCTTCCAG AATGAAAATGTCTTTGATGAAACAGGTATGCCAAGGGAA GATTTTCAGCTAAGTGCCTCCCAATTACCCCTGTAGCCTT GAGATCCAGGCTACGAAGCTTGAGGATTCAGCAGTGTATT TTTGTGCCAGCAGCCATAGAAGTTGGAAGGGGGGTGATAC GCAGCCCCAGCATTTTGGTGTGAGGACTCGACTCTCCATCC TAGAGGACCTGAACAAGGTGTTCCCAACCCGAGGTCGCTGT	71

	GTTTGAGCCATCAGAAGCAGAGATCTCCACACCCAAAAG GCCACACTGGTGTGCCTGGCCACAGGCTTCTTCCCTGACCA CGTGGAGCTGAGCTGGTGGGTGAATGGGAAGGAGGTGCA CAGTGGGGTCTGCACGGACCCGCAGCCCCCTCAAGGAGCAG CCCGCCCTCAATGACTCCAGATACTGCCTGAGCAGCCGCC TGAGGGTCTCGGCCACCTTCTGGCAGAACCCCCGCAACCA CTTCCGCTGTCAAGTCCAGTTCTACGGGCTCTCGGAGAATG ACGAGTGGACCCAGGATAGGGCCAAACCCGTCACCCAGAT CGTCAGCGCCGAGGCCTGGGGTAGAGCAGACTGTGGCTTT ACCTCGGTGTCCTACCAGCAAGGGGTCTGTCTGCCACCAT CCTCTATGAGATCCTGCTAGGGAAGGCCACCCTGTATGCT GTGCTGGTCAGCGCCCTTGTGTTGATGGCCATGGTCAAGA GAAAGGATTTCTGA	
#4. EGFRA33 TCRA33-37-4 TRAV14/DV4*02 /TRBV 16*01 C1 J1-5*01**	MSLSSLLKVVVTASLWLGPGIAQKITQTQPGMFVQEKEAVTLD CTYDTS DPSYGLFWYKQPSSGEMIFLIYQGSYDQQNATEGRY SLNFQKARKSANLVISASQLGDSAMYFCAMRSGHLSNFGNE KLTFGTGTRLTIIPNIQNPDPVYQLRDSKSSDKSVCLFTDFDS QTNVSQSKSDVYITDKCVLDMRSMDFKSNSAVAWSNKSDFA CANAFNNSIIPEDTFFPSPESCDVKLVEKSFETDTNLFQNL SVIGFRILLKLVAGFNLLMTLRLWSSRAKRSGSGATNFSLLKQ AGDVEENPGPMSPIFTCTILCLLAAGSPGEEVAQTPKHLVRG EGQKAKLYCAPIKGHSYVFWYQQVLKNEFKFLISFQENNVFD ETGMPKGRFSAKCLPNSPCSLEIQATKLEDSAVYFCASSHRS WKG GDTQPQHFGDGTLSILEDLNKVFPEVAVFEPSEAEISH TQKATLVCLATGFFPDHVELSWVNGKEVHSGVCTDPQPLK EQPALNDSRYCLSSRLRVSATFWQNP RNHFRCQVQFYGLSEN DEWTQDRAKPVQTIVSAEAWGRADCGFTSVSYQQGVLSATI LYEILLGKATLYAVLVSAVLMLAMVVKRKDF	72
#4. EGFRA33 TCRA33-37-4- TCR-alpha chain	AQKITQTQPGMFVQEKEAVTLDCTYDTS DPSYGLFWYKQPSS GEMIFLIYQGSYDQQNATEGRYSLNFQKARKSANLVISASQL GDSAMYFCAMRSGHLSNFGNEKLTFGTGTRLTIIPNIQNPDP VYQLRDSKSSDKSVCLFTDFDSQTNVSQSKSDVYITDKCVL DMRSMDFKSNSAVAWSNKSDFA CANAFNNSIIPEDTFFPSPES CDVKLVEKSFETDTNLFQNL SVIGFRILLKLVAGFNLLMTL RLWSSRAKRSGSGATNFSLLKQAGDVEENPGP	73
#4. EGFRA33 TCRA33-37-4- TCR-alpha variable region	AQKITQTQPGMFVQEKEAVTLDCTYDTS DPSYGLFWYKQPSS GEMIFLIYQGSYDQQNATEGRYSLNFQKARKSANLVISASQL GDSAMYFCAMRSGHLSNFGNEKLTFGTGTRLTIIP	74
#4. EGFRA33 TCRA33-37-4- TCR-alpha signal sequence	MSLSSLLKVVVTASLWLGPGI	75
#4. EGFRA33 TCRA33-37-4- TCR-alpha CDR1	TSDPSYG	76
#4. EGFRA33 TCRA33-37-4- TCR-alpha CDR2	QGSYDQQN	77

#4. EGFR33 TCRA33-37-4- TCR-alpha CDR3	AMRSGHLSNFGNEKLT	78
#4. EGFR33 TCRA33-37-4- TCR-beta chain	EEVAQTPKHLVRGEGQKAKLYCAPIKGHSYVFWYQQVLKNE FKFLISFQNNENVFDETGMPPKGRFSKCLPNSPCSLEIQATKLE DSAVYFCASSHRSWKGGDTQPQHFGDGTRLSILEDLNKVFPP EVAVFEPSEAEISHTQKATLVCLATGFFPDHVELSWVNGKE VHSGVCTDPQPLKEQPALNDSRYCLSSRLRVSATFWQNPRNH FRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFT SVSYQQGVLSATILYEILLGKATLYAVLVSALVLMAMVKRK DF	79
#4. EGFR33 TCRA33-37-4- TCR-beta variable region	EEVAQTPKHLVRGEGQKAKLYCAPIKGHSYVFWYQQVLKNE FKFLISFQNNENVFDETGMPPKGRFSKCLPNSPCSLEIQATKLE DSAVYFCASSHRSWKGGDTQPQHFGDGTRLSIL	80
#4. EGFR33 TCRA33-37-4- TCR-beta signal sequence	MSPIFTCITILCLLAAGSPG	81
#4. EGFR33 TCRA33-37-4- TCR-beta CDR1	KGHSY	82
#4. EGFR33 TCRA33-37-4- TCR-beta CDR2	FQNNENV	83
#4. EGFR33 TCRA33-37-4- TCR-beta CDR3	ASSHRSWKGGDTQPQH	84

II. Proteinaceous Compositions

[0062] As used herein, a “protein” “peptide” or “polypeptide” refers to a molecule comprising at least five amino acid residues. As used herein, the term “wild-type” refers to the endogenous version of a molecule that occurs naturally in an organism. In some aspects, wild-type versions of a protein or polypeptide are employed, however, in many aspects of the disclosure, a modified protein or polypeptide is employed to generate an immune response. The terms described above may be used interchangeably. A “modified protein” or “modified polypeptide” or a “variant” refers to a protein or polypeptide whose chemical structure, particularly its amino acid sequence, is altered with respect to the wild-type protein or polypeptide. In some aspects, a modified/variant protein or polypeptide has at least one modified activity or function (recognizing that proteins or polypeptides may have multiple activities or functions). It is specifically contemplated that a modified/variant protein or

polypeptide may be altered with respect to one activity or function yet retain a wild-type activity or function in other respects, such as immunogenicity.

[0063] Where a protein is specifically mentioned herein, it is in general a reference to a native (wild-type) or recombinant (modified) protein or, optionally, a protein in which any signal sequence has been removed. The protein may be isolated directly from the organism of which it is native, produced by recombinant DNA/exogenous expression methods, or produced by solid-phase peptide synthesis (SPPS) or other in vitro methods. In particular aspects, there are isolated nucleic acid segments and recombinant vectors incorporating nucleic acid sequences that encode a polypeptide (e.g., an antibody or fragment thereof). The term “recombinant” may be used in conjunction with a polypeptide or the name of a specific polypeptide, and this generally refers to a polypeptide produced from a nucleic acid molecule that has been manipulated in vitro or that is a replication product of such a molecule.

[0064] In certain aspects the size of a protein or polypeptide (wild-type or modified) may comprise, but is not limited to, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 525, 550, 575, 600, 625, 650, 675, 700, 725, 750, 775, 800, 825, 850, 875, 900, 925, 950, 1000, 1200, 1400, 1600, 1800, or 2000 amino acid residues or nucleic acid residues or greater, and any range derivable therein, or derivative of a corresponding amino sequence described or referenced herein. It is contemplated that polypeptides may be mutated by truncation, rendering them shorter than their corresponding wild-type form, also, they might be altered by fusing or conjugating a heterologous protein or polypeptide sequence with a particular function (e.g., for targeting or localization, for enhanced immunogenicity, for purification purposes, etc.).

[0065] The polypeptides, proteins, or polynucleotides encoding such polypeptides or proteins of the disclosure may include 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 (or any derivable range therein) or more variant amino acids or nucleic acid substitutions or be at least 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% (or any derivable range therein) similar, identical, or homologous to at least, or at most 3, 4, 5,

6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 300, 400, 500, 550, 1000 or more contiguous amino acids or nucleic acids, or any range derivable therein, of SEQ ID NOS:1-93. In specific aspects, the peptide or polypeptide is or is based on a human sequence. In certain aspects, the peptide or polypeptide is not naturally occurring and/or is in a combination of peptides or polypeptides.

[0066] In some aspects, the protein, polypeptide, or nucleic acid may comprise amino acids or nucleotides 1 to 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, or 320 (or any derivable range therein) of SEQ ID NOS:1-93.

[0067] In some aspects, the protein, polypeptide, or nucleic acid may comprise, may comprise at least, or may comprise at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, or 320 (or any derivable range therein) contiguous amino acids or nucleic acids of SEQ ID NOS:1-93.

[0068] In some aspects, the polypeptide, protein, or nucleic acid may comprise, may comprise at least, or may comprise at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303,

304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, or 320 (or any derivable range therein) contiguous amino acids of SEQ ID NOS:1-93 that are at least, at most, or exactly 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% (or any derivable range therein) similar, identical, or homologous to one of SEQ ID NOS:1-93.

[0069] In some aspects there is a nucleic acid molecule or polypeptide starting at position 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 409, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, 500, 501, 502, 503, 504, 505, 506, 507, 508, 509, 510, 511, 512, 513, 514, 515, 516, 517, 518, 519, 520, 521, 522, 523, 524, 525, 526, 527, 528, 529, 530, 531, 532, 533, 534, 535, 536, 537, 538, 539,

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[0070] The polypeptides of the disclosure may include at least, at most, or exactly 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 409, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, 500, 501, 502, 503, 504, 505, 506, 507, 508, 509, 510, 511, 512, 513, 514, 515, 516, 517, 518, 519, 520, 521, 522, 523, 524, 525, 526, 527, 528, 529, 530, 531, 532, 533, 534, 535, 536, 537, 538, 539, 540, 541, 542, 543, 544, 545, 546, 547, 548, 549, 550, 551, 552, 553, 554, 555, 556, 557, 558, 559, 560,

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5 **[0071]** The substitution may be at amino acid position 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 10 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 15 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 20 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 25 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 409, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 30 491, 492, 493, 494, 495, 496, 497, 498, 499, 500, 501, 502, 503, 504, 505, 506, 507, 508, 509, 510, 511, 512, 513, 514, 515, 516, 517, 518, 519, 520, 521, 522, 523, 524, 525, 526, 527, 528, 529, 530, 531, 532, 533, 534, 535, 536, 537, 538, 539, 540, 541, 542, 543, 544, 545, 546, 547, 548, 549, 550, 551, 552, 553, 554, 555, 556, 557, 558, 559, 560, 561, 562, 563, 564, 565, 566, 567, 568, 569, 570, 571, 572, 573, 574, 575, 576, 577, 578, 579, 580, 581, 582, 583, 584, 585,

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[0072] The nucleotide as well as the protein, polypeptide, and peptide sequences for various genes have been previously disclosed, and may be found in the recognized computerized databases. Two commonly used databases are the National Center for Biotechnology Information's Genbank and GenPept databases (on the World Wide Web at ncbi.nlm.nih.gov/) and The Universal Protein Resource (UniProt; on the World Wide Web at uniprot.org). The coding regions for these genes may be amplified and/or expressed using the techniques disclosed herein or as would be known to those of ordinary skill in the art.

[0073] It is contemplated that in compositions of the disclosure, there is between about 0.001 mg and about 10 mg of total polypeptide, peptide, and/or protein per ml. The concentration of protein in a composition can be about, at least about or at most about 0.001, 0.010, 0.050, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, 10.0 mg/ml or more (or any range derivable therein).

[0074] The following is a discussion of changing the amino acid subunits of a protein to create an equivalent, or even improved, second-generation variant polypeptide or peptide. For example, certain amino acids may be substituted for other amino acids in a protein or polypeptide sequence with or without appreciable loss of interactive binding capacity with structures such as, for example, antigen-binding regions of antibodies or binding sites on substrate molecules. Since it is the interactive capacity and nature of a protein that defines that protein's functional activity, certain amino acid substitutions can be made in a protein sequence and in its corresponding DNA coding sequence, and nevertheless produce a protein with similar or desirable properties. It is thus contemplated by the inventors that various changes may be made in the DNA sequences of genes which encode proteins without appreciable loss of their biological utility or activity.

[0075] The term "functionally equivalent codon" is used herein to refer to codons that encode the same amino acid, such as the six different codons for arginine. Also considered are "neutral substitutions" or "neutral mutations" which refers to a change in the codon or codons that encode biologically equivalent amino acids.

[0076] Amino acid sequence variants of the disclosure can be substitutional, insertional, or deletion variants. A variation in a polypeptide of the disclosure may affect 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, or more non-contiguous or contiguous amino acids of the protein or polypeptide, as compared to wild-type (or any range derivable therein). A variant can comprise an amino acid sequence that is at least 50%, 60%, 70%, 80%, or 90%, including all values and ranges there between, identical to any sequence provided or referenced herein. A variant can include 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more substitute amino acids.

[0077] It also will be understood that amino acid and nucleic acid sequences may include additional residues, such as additional N- or C-terminal amino acids, or 5' or 3' sequences, respectively, and yet still be essentially identical as set forth in one of the sequences disclosed herein, so long as the sequence meets the criteria set forth above, including the maintenance of biological protein activity where protein expression is concerned. The addition of terminal sequences particularly applies to nucleic acid sequences that may, for example, include various non-coding sequences flanking either of the 5' or 3' portions of the coding region.

[0078] Deletion variants typically lack one or more residues of the native or wild type protein. Individual residues can be deleted or a number of contiguous amino acids can be deleted. A stop codon may be introduced (by substitution or insertion) into an encoding nucleic acid sequence to generate a truncated protein.

[0079] Insertional mutants typically involve the addition of amino acid residues at a non-terminal point in the polypeptide. This may include the insertion of one or more amino acid residues. Terminal additions may also be generated and can include fusion proteins which are multimers or concatemers of one or more peptides or polypeptides described or referenced herein.

[0080] Substitutional variants typically contain the exchange of one amino acid for another at one or more sites within the protein or polypeptide, and may be designed to modulate one or more properties of the polypeptide, with or without the loss of other functions or properties. Substitutions may be conservative, that is, one amino acid is replaced with one of similar chemical properties. "Conservative amino acid substitutions" may involve exchange of a member of one amino acid class with another member of the same class. Conservative substitutions are well known in the art and include, for example, the changes of: alanine to serine; arginine to lysine; asparagine to glutamine or histidine; aspartate to glutamate; cysteine to serine; glutamine to asparagine; glutamate to aspartate; glycine to proline; histidine to

asparagine or glutamine; isoleucine to leucine or valine; leucine to valine or isoleucine; lysine to arginine; methionine to leucine or isoleucine; phenylalanine to tyrosine, leucine or methionine; serine to threonine; threonine to serine; tryptophan to tyrosine; tyrosine to tryptophan or phenylalanine; and valine to isoleucine or leucine. Conservative amino acid substitutions may encompass non-naturally occurring amino acid residues, which are typically incorporated by chemical peptide synthesis rather than by synthesis in biological systems. These include peptidomimetics or other reversed or inverted forms of amino acid moieties.

[0081] Alternatively, substitutions may be “non-conservative”, such that a function or activity of the polypeptide is affected. Non-conservative changes typically involve substituting an amino acid residue with one that is chemically dissimilar, such as a polar or charged amino acid for a nonpolar or uncharged amino acid, and vice versa. Non-conservative substitutions may involve the exchange of a member of one of the amino acid classes for a member from another class.

[0082] One skilled in the art can determine suitable variants of polypeptides as set forth herein using well-known techniques. One skilled in the art may identify suitable areas of the molecule that may be changed without destroying activity by targeting regions not believed to be important for activity. The skilled artisan will also be able to identify amino acid residues and portions of the molecules that are conserved among similar proteins or polypeptides. In further aspects, areas that may be important for biological activity or for structure may be subject to conservative amino acid substitutions without significantly altering the biological activity or without adversely affecting the protein or polypeptide structure.

[0083] In making such changes, the hydropathy index of amino acids may be considered. The hydropathy profile of a protein is calculated by assigning each amino acid a numerical value (“hydropathy index”) and then repetitively averaging these values along the peptide chain. Each amino acid has been assigned a value based on its hydrophobicity and charge characteristics. They are: isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cysteine (+2.5); methionine (+1.9); alanine (+1.8); glycine (−0.4); threonine (−0.7); serine (−0.8); tryptophan (−0.9); tyrosine (−1.3); proline (1.6); histidine (−3.2); glutamate (−3.5); glutamine (−3.5); aspartate (−3.5); asparagine (−3.5); lysine (−3.9); and arginine (−4.5).

The importance of the hydropathy amino acid index in conferring interactive biologic function on a protein is generally understood in the art (Kyte et al., J. Mol. Biol. 157:105-131 (1982)). It is accepted that the relative hydropathic character of the amino acid contributes to the secondary structure of the resultant protein or polypeptide, which in turn defines the interaction of the protein or polypeptide with other molecules, for example, enzymes, substrates, receptors,

DNA, antibodies, antigens, and others. It is also known that certain amino acids may be substituted for other amino acids having a similar hydropathy index or score, and still retain a similar biological activity. In making changes based upon the hydropathy index, in certain aspects, the substitution of amino acids whose hydropathy indices are within ± 2 is included. In some aspects of the invention, those that are within ± 1 are included, and in other aspects of the invention, those within ± 0.5 are included.

[0084] It also is understood in the art that the substitution of like amino acids can be effectively made based on hydrophilicity. U.S. Patent 4,554,101, incorporated herein by reference, states that the greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with a biological property of the protein. In certain aspects, the greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with its immunogenicity and antigen binding, that is, as a biological property of the protein. The following hydrophilicity values have been assigned to these amino acid residues: arginine (+3.0); lysine (+3.0); aspartate (+3.0 $\pm$ 1); glutamate (+3.0 $\pm$ 1); serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); threonine (−0.4); proline (−0.5 $\pm$ 1); alanine (−0.5); histidine (−0.5); cysteine (−1.0); methionine (−1.3); valine (−1.5); leucine (−1.8); isoleucine (−1.8); tyrosine (−2.3); phenylalanine (−2.5); and tryptophan (−3.4). In making changes based upon similar hydrophilicity values, in certain aspects, the substitution of amino acids whose hydrophilicity values are within ± 2 are included, in other aspects, those which are within ± 1 are included, and in still other aspects, those within ± 0.5 are included. In some instances, one may also identify epitopes from primary amino acid sequences based on hydrophilicity. These regions are also referred to as “epitopic core regions.” It is understood that an amino acid can be substituted for another having a similar hydrophilicity value and still produce a biologically equivalent and immunologically equivalent protein.

[0085] Additionally, one skilled in the art can review structure-function studies identifying residues in similar polypeptides or proteins that are important for activity or structure. In view of such a comparison, one can predict the importance of amino acid residues in a protein that correspond to amino acid residues important for activity or structure in similar proteins. One skilled in the art may opt for chemically similar amino acid substitutions for such predicted important amino acid residues.

[0086] One skilled in the art can also analyze the three-dimensional structure and amino acid sequence in relation to that structure in similar proteins or polypeptides. In view of such information, one skilled in the art may predict the alignment of amino acid residues of an

antibody with respect to its three-dimensional structure. One skilled in the art may choose not to make changes to amino acid residues predicted to be on the surface of the protein, since such residues may be involved in important interactions with other molecules. Moreover, one skilled in the art may generate test variants containing a single amino acid substitution at each desired amino acid residue. These variants can then be screened using standard assays for binding and/or activity, thus yielding information gathered from such routine experiments, which may allow one skilled in the art to determine the amino acid positions where further substitutions should be avoided either alone or in combination with other mutations. Various tools available to determine secondary structure can be found on the world wide web at [expasy.org/proteomics/protein structure](https://expasy.org/proteomics/protein%20structure).

[0087] In some aspects of the invention, amino acid substitutions are made that: (1) reduce susceptibility to proteolysis, (2) reduce susceptibility to oxidation, (3) alter binding affinity for forming protein complexes, (4) alter ligand or antigen binding affinities, and/or (5) confer or modify other physicochemical or functional properties on such polypeptides. For example, single or multiple amino acid substitutions (in certain aspects, conservative amino acid substitutions) may be made in the naturally occurring sequence. Substitutions can be made in that portion of the antibody that lies outside the domain(s) forming intermolecular contacts. In such aspects, conservative amino acid substitutions can be used that do not substantially change the structural characteristics of the protein or polypeptide (e.g., one or more replacement amino acids that do not disrupt the secondary structure that characterizes the native antibody).

III. Nucleic Acids

[0088] In certain aspects, nucleic acid sequences can exist in a variety of instances such as: isolated segments and recombinant vectors of incorporated sequences or recombinant polynucleotides encoding one or both chains of an antibody, or a fragment, derivative, mutein, or variant thereof, polynucleotides sufficient for use as hybridization probes, PCR primers or sequencing primers for identifying, analyzing, mutating or amplifying a polynucleotide encoding a polypeptide, anti-sense nucleic acids for inhibiting expression of a polynucleotide, and complementary sequences of the foregoing described herein. Nucleic acids that encode the epitope to which certain of the antibodies provided herein are also provided. Nucleic acids encoding fusion proteins that include these peptides are also provided. The nucleic acids can be single-stranded or double-stranded and can comprise RNA and/or DNA nucleotides and artificial variants thereof (e.g., peptide nucleic acids).

[0089] The term “polynucleotide” refers to a nucleic acid molecule that either is recombinant or has been isolated from total genomic nucleic acid. Included within the term “polynucleotide” are oligonucleotides (nucleic acids 100 residues or less in length), recombinant vectors, including, for example, plasmids, cosmids, phage, viruses, and the like.

5 Polynucleotides include, in certain aspects, regulatory sequences, isolated substantially away from their naturally occurring genes or protein encoding sequences. Polynucleotides may be single- stranded (coding or antisense) or double- stranded, and may be RNA, DNA (genomic, cDNA or synthetic), analogs thereof, or a combination thereof. Additional coding or non-coding sequences may, but need not, be present within a polynucleotide.

10 [0090] In this respect, the term “gene,” “polynucleotide,” or “nucleic acid” is used to refer to a nucleic acid that encodes a protein, polypeptide, or peptide (including any sequences required for proper transcription, post-translational modification, or localization). As will be understood by those in the art, this term encompasses genomic sequences, expression cassettes, cDNA sequences, and smaller engineered nucleic acid segments that express, or may be
15 adapted to express, proteins, polypeptides, domains, peptides, fusion proteins, and mutants. A nucleic acid encoding all or part of a polypeptide may contain a contiguous nucleic acid sequence encoding all or a portion of such a polypeptide. It also is contemplated that a particular polypeptide may be encoded by nucleic acids containing variations having slightly different nucleic acid sequences but, nonetheless, encode the same or substantially similar
20 protein.

[0091] In certain aspects, there are polynucleotide variants having substantial identity to the sequences disclosed herein; those comprising at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% or higher sequence identity, including all values and ranges there between, compared to a polynucleotide sequence provided herein using the methods described herein
25 (e.g., BLAST analysis using standard parameters). In certain aspects, the isolated polynucleotide will comprise a nucleotide sequence encoding a polypeptide that has at least 90%, preferably 95% and above, identity to an amino acid sequence described herein, over the entire length of the sequence; or a nucleotide sequence complementary to said isolated polynucleotide.

30 [0092] The nucleic acid segments, regardless of the length of the coding sequence itself, may be combined with other nucleic acid sequences, such as promoters, polyadenylation signals, additional restriction enzyme sites, multiple cloning sites, other coding segments, and the like, such that their overall length may vary considerably. The nucleic acids can be any length. They can be, for example, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 75, 100, 125, 175, 200,

250, 300, 350, 400, 450, 500, 750, 1000, 1500, 3000, 5000 or more nucleotides in length, and/or can comprise one or more additional sequences, for example, regulatory sequences, and/or be a part of a larger nucleic acid, for example, a vector. It is therefore contemplated that a nucleic acid fragment of almost any length may be employed, with the total length preferably being limited by the ease of preparation and use in the intended recombinant nucleic acid protocol. In some cases, a nucleic acid sequence may encode a polypeptide sequence with additional heterologous coding sequences, for example to allow for purification of the polypeptide, transport, secretion, post-translational modification, or for therapeutic benefits such as targeting or efficacy. As discussed above, a tag or other heterologous polypeptide may be added to the modified polypeptide-encoding sequence, wherein "heterologous" refers to a polypeptide that is not the same as the modified polypeptide.

A. Hybridization

[0093] The nucleic acids that hybridize to other nucleic acids under particular hybridization conditions. Methods for hybridizing nucleic acids are well known in the art. See, e.g., Current Protocols in Molecular Biology, John Wiley and Sons, N.Y. (1989), 6.3.1-6.3.6. As defined herein, a moderately stringent hybridization condition uses a prewashing solution containing 5× sodium chloride/sodium citrate (SSC), 0.5% SDS, 1.0 mM EDTA (pH 8.0), hybridization buffer of about 50% formamide, 6×SSC, and a hybridization temperature of 55° C. (or other similar hybridization solutions, such as one containing about 50% formamide, with a hybridization temperature of 42° C), and washing conditions of 60° C. in 0.5×SSC, 0.1% SDS. A stringent hybridization condition hybridizes in 6×SSC at 45° C., followed by one or more washes in 0.1×SSC, 0.2% SDS at 68° C. Furthermore, one of skill in the art can manipulate the hybridization and/or washing conditions to increase or decrease the stringency of hybridization such that nucleic acids comprising nucleotide sequence that are at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to each other typically remain hybridized to each other.

[0094] The parameters affecting the choice of hybridization conditions and guidance for devising suitable conditions are set forth by, for example, Sambrook, Fritsch, and Maniatis (Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., chapters 9 and 11 (1989); Current Protocols in Molecular Biology, Ausubel et al., eds., John Wiley and Sons, Inc., sections 2.10 and 6.3-6.4 (1995), both of which are herein incorporated by reference in their entirety for all purposes) and can be readily determined by

those having ordinary skill in the art based on, for example, the length and/or base composition of the DNA.

B. Mutation

[0095] Changes can be introduced by mutation into a nucleic acid, thereby leading to changes in the amino acid sequence of a polypeptide (e.g., an antibody or antibody derivative) that it encodes. Mutations can be introduced using any technique known in the art. In one aspect, one or more particular amino acid residues are changed using, for example, a site-directed mutagenesis protocol. In another aspect, one or more randomly selected residues are changed using, for example, a random mutagenesis protocol. However it is made, a mutant polypeptide can be expressed and screened for a desired property.

[0096] Mutations can be introduced into a nucleic acid without significantly altering the biological activity of a polypeptide that it encodes. For example, one can make nucleotide substitutions leading to amino acid substitutions at non-essential amino acid residues. Alternatively, one or more mutations can be introduced into a nucleic acid that selectively changes the biological activity of a polypeptide that it encodes. See, eg., Romain Studer et al., *Biochem. J.* 449:581-594 (2013). For example, the mutation can quantitatively or qualitatively change the biological activity. Examples of quantitative changes include increasing, reducing or eliminating the activity. Examples of qualitative changes include altering the antigen specificity of an antibody.

C. Probes

[0097] In another aspect, nucleic acid molecules are suitable for use as primers or hybridization probes for the detection of nucleic acid sequences. A nucleic acid molecule can comprise only a portion of a nucleic acid sequence encoding a full-length polypeptide, for example, a fragment that can be used as a probe or primer or a fragment encoding an active portion of a given polypeptide.

[0098] In another aspect, the nucleic acid molecules may be used as probes or PCR primers for specific antibody sequences. For instance, a nucleic acid molecule probe may be used in diagnostic methods or a nucleic acid molecule PCR primer may be used to amplify regions of DNA that could be used, inter alia, to isolate nucleic acid sequences for use in producing variable domains of antibodies. See, eg., Gaily Kivi et al., *BMC Biotechnol.* 16:2 (2016). In a preferred aspect, the nucleic acid molecules are oligonucleotides. In a more preferred aspect, the oligonucleotides are from highly variable regions of the heavy and light or alpha and beta

chains of the antibody or TCR of interest. In an even more preferred aspect, the oligonucleotides encode all or part of one or more of the CDRs or TCRs.

[0099] Probes based on the desired sequence of a nucleic acid can be used to detect the nucleic acid or similar nucleic acids, for example, transcripts encoding a polypeptide of interest. The probe can comprise a label group, e.g., a radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor. Such probes can be used to identify a cell that expresses the polypeptide.

IV. Polypeptide Expression

[0100] In some aspects, there are nucleic acid molecule encoding polypeptides or peptides of the disclosure (e.g TCR genes). These may be generated by methods known in the art, e.g., isolated from B cells of mice that have been immunized and isolated, phage display, expressed in any suitable recombinant expression system and allowed to assemble to form antibody molecules or by recombinant methods.

A. Expression

[0101] The nucleic acid molecules may be used to express large quantities of polypeptides. If the nucleic acid molecules are derived from a non-human, non-transgenic animal, the nucleic acid molecules may be used for humanization of the TCR genes.

B. Vectors

[0102] In some aspects, contemplated are expression vectors comprising a nucleic acid molecule encoding a polypeptide of the desired sequence or a portion thereof (e.g., a fragment containing one or more CDRs or one or more variable region domains). Expression vectors comprising the nucleic acid molecules may encode the heavy chain, light chain, alpha chain, beta chain, or the antigen-binding portion thereof. In some aspects, expression vectors comprising nucleic acid molecules may encode fusion proteins, modified antibodies, antibody fragments, and probes thereof. In addition to control sequences that govern transcription and translation, vectors and expression vectors may contain nucleic acid sequences that serve other functions as well.

[0103] To express the polypeptides or peptides of the disclosure, DNAs encoding the polypeptides or peptides are inserted into expression vectors such that the gene area is operatively linked to transcriptional and translational control sequences. In some aspects, a vector that encodes a functionally complete human CH or CL immunoglobulin or TCR sequence with appropriate restriction sites engineered so that any variable region sequences can be easily inserted and expressed. In some aspects, a vector that encodes a functionally

complete human TCR alpha or TCR beta sequence with appropriate restriction sites engineered so that any variable sequence or CDR1, CDR2, and/or CDR3 can be easily inserted and expressed. Typically, expression vectors used in any of the host cells contain sequences for plasmid or virus maintenance and for cloning and expression of exogenous nucleotide sequences. Such sequences, collectively referred to as “flanking sequences” typically include one or more of the following operatively linked nucleotide sequences: a promoter, one or more enhancer sequences, an origin of replication, a transcriptional termination sequence, a complete intron sequence containing a donor and acceptor splice site, a sequence encoding a leader sequence for polypeptide secretion, a ribosome binding site, a polyadenylation sequence, a polylinker region for inserting the nucleic acid encoding the polypeptide to be expressed, and a selectable marker element. Such sequences and methods of using the same are well known in the art.

C. Expression Systems

[0104] Numerous expression systems exist that comprise at least a part or all of the expression vectors discussed above. Prokaryote- and/or eukaryote-based systems can be employed for use with an aspect to produce nucleic acid sequences, or their cognate polypeptides, proteins and peptides. Commercially and widely available systems include in but are not limited to bacterial, mammalian, yeast, and insect cell systems. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. Those skilled in the art are able to express a vector to produce a nucleic acid sequence or its cognate polypeptide, protein, or peptide using an appropriate expression system.

V. Methods of Gene Transfer

[0105] Suitable methods for nucleic acid delivery to effect expression of compositions are anticipated to include virtually any method by which a nucleic acid (e.g., DNA, including viral and nonviral vectors) can be introduced into a cell, a tissue or an organism, as described herein or as would be known to one of ordinary skill in the art. Such methods include, but are not limited to, direct delivery of DNA such as by injection (U.S. Patents 5,994,624, 5,981,274, 5,945,100, 5,780,448, 5,736,524, 5,702,932, 5,656,610, 5,589,466 and 5,580,859, each incorporated herein by reference), including microinjection (Harland and Weintraub, 1985; U.S. Patent 5,789,215, incorporated herein by reference); by electroporation (U.S. Patent No. 5,384,253, incorporated herein by reference); by calcium phosphate precipitation (Graham and

Van Der Eb, 1973; Chen and Okayama, 1987; Rippe et al., 1990); by using DEAE dextran followed by polyethylene glycol (Gopal, 1985); by direct sonic loading (Fechheimer et al., 1987); by liposome mediated transfection (Nicolau and Sene, 1982; Fraley et al., 1979; Nicolau et al., 1987; Wong et al., 1980; Kaneda et al., 1989; Kato et al., 1991); by microprojectile bombardment (PCT Application Nos. WO 94/09699 and 95/06128; U.S. Patents 5,610,042; 5,322,783, 5,563,055, 5,550,318, 5,538,877 and 5,538,880, and each incorporated herein by reference); by agitation with silicon carbide fibers (Kaeppeler et al., 1990; U.S. Patents 5,302,523 and 5,464,765, each incorporated herein by reference); by Agrobacterium mediated transformation (U.S. Patents 5,591,616 and 5,563,055, each incorporated herein by reference); or by PEG mediated transformation of protoplasts (Omirulleh et al., 1993; U.S. Patents 4,684,611 and 4,952,500, each incorporated herein by reference); by desiccation/inhibition mediated DNA uptake (Potrykus et al., 1985). Other methods include viral transduction, such as gene transfer by lentiviral or retroviral transduction.

A. Host Cells

[0106] In another aspect, contemplated are the use of host cells into which a recombinant expression vector has been introduced. Antibodies can be expressed in a variety of cell types. An expression construct encoding an antibody can be transfected into cells according to a variety of methods known in the art. Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. Some vectors may employ control sequences that allow it to be replicated and/or expressed in both prokaryotic and eukaryotic cells. In certain aspects, the antibody expression construct can be placed under control of a promoter that is linked to T-cell activation, such as one that is controlled by NFAT-1 or NF- κ B, both of which are transcription factors that can be activated upon T-cell activation. Control of antibody expression allows T cells, such as tumor- targeting T cells, to sense their surroundings and perform real-time modulation of cytokine signaling, both in the T cells themselves and in surrounding endogenous immune cells. One of skill in the art would understand the conditions under which to incubate host cells to maintain them and to permit replication of a vector. Also understood and known are techniques and conditions that would allow large-scale production of vectors, as well as production of the nucleic acids encoded by vectors and their cognate polypeptides, proteins, or peptides.

[0107] For stable transfection of mammalian cells, it is known, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a selectable

marker (e.g., for resistance to antibiotics) is generally introduced into the host cells along with the gene of interest. Cells stably transfected with the introduced nucleic acid can be identified by drug selection (e.g., cells that have incorporated the selectable marker gene will survive, while the other cells die), among other methods known in the arts.

B. Isolation

[0108] The nucleic acid molecule encoding either or both of the entire heavy, light, alpha, and beta chains of an antibody or TCR, or the variable regions thereof may be obtained from any source that produces antibodies. Methods of isolating mRNA encoding an antibody are well known in the art. See e.g., Sambrook et al., supra. The sequences of human heavy and light chain constant region genes are also known in the art. See, e.g., Kabat et al., 1991, supra. Nucleic acid molecules encoding the full-length heavy and/or light chains may then be expressed in a cell into which they have been introduced and the antibody isolated.

VI. Additional Therapies

A. Immunotherapy

[0109] In some aspects, the methods comprise administration of an additional therapy. In some aspects, the additional therapy comprises a cancer immunotherapy. Cancer immunotherapy (sometimes called immuno-oncology, abbreviated IO) is the use of the immune system to treat cancer. Immunotherapies can be categorized as active, passive or hybrid (active and passive). These approaches exploit the fact that cancer cells often have molecules on their surface that can be detected by the immune system, known as tumor-associated antigens (TAAs); they are often proteins or other macromolecules (e.g. carbohydrates). Active immunotherapy directs the immune system to attack tumor cells by targeting TAAs. Passive immunotherapies enhance existing anti-tumor responses and include the use of monoclonal antibodies, lymphocytes and cytokines. Immunotherapies are known in the art, and some are described below.

1. Checkpoint Inhibitors and Combination Treatment

[0110] Aspects of the disclosure may include administration of immune checkpoint inhibitors, which are further described below.

a. PD-1, PDL1, and PDL2 inhibitors

[0111] PD-1 can act in the tumor microenvironment where T cells encounter an infection or tumor. Activated T cells upregulate PD-1 and continue to express it in the peripheral tissues. Cytokines such as IFN-gamma induce the expression of PDL1 on epithelial cells and tumor

cells. PDL2 is expressed on macrophages and dendritic cells. The main role of PD-1 is to limit the activity of effector T cells in the periphery and prevent excessive damage to the tissues during an immune response. Inhibitors of the disclosure may block one or more functions of PD-1 and/or PDL1 activity.

5 **[0112]** Alternative names for “PD-1” include CD279 and SLEB2. Alternative names for “PDL1” include B7-H1, B7-4, CD274, and B7-H. Alternative names for “PDL2” include B7-DC, Btdc, and CD273. In some aspects, PD-1, PDL1, and PDL2 are human PD-1, PDL1 and PDL2.

10 **[0113]** In some aspects, the PD-1 inhibitor is a molecule that inhibits the binding of PD-1 to its ligand binding partners. In a specific aspect, the PD-1 ligand binding partners are PDL1 and/or PDL2. In another aspect, a PDL1 inhibitor is a molecule that inhibits the binding of PDL1 to its binding partners. In a specific aspect, PDL1 binding partners are PD-1 and/or B7-1. In another aspect, the PDL2 inhibitor is a molecule that inhibits the binding of PDL2 to its binding partners. In a specific aspect, a PDL2 binding partner is PD-1. The inhibitor may be an
15 antibody, an antigen binding fragment thereof, an immunoadhesin, a fusion protein, or oligopeptide. Exemplary antibodies are described in U.S. Patent Nos. 8,735,553, 8,354,509, and 8,008,449, all incorporated herein by reference. Other PD-1 inhibitors for use in the methods and compositions provided herein are known in the art such as described in U.S. Patent Application Nos. US2014/0294898, US2014/022021, and US2011/0008369, all incorporated
20 herein by reference.

25 **[0114]** In some aspects, the PD-1 inhibitor is an anti-PD-1 antibody (e.g., a human antibody, a humanized antibody, or a chimeric antibody). In some aspects, the anti-PD-1 antibody is selected from the group consisting of nivolumab, pembrolizumab, and pidilizumab. In some aspects, the PD-1 inhibitor is an immunoadhesin (e.g., an immunoadhesin comprising an
30 extracellular or PD-1 binding portion of PDL1 or PDL2 fused to a constant region (e.g., an Fc region of an immunoglobulin sequence). In some aspects, the PDL1 inhibitor comprises AMP-224. Nivolumab, also known as MDX-1106-04, MDX-1106, ONO-4538, BMS-936558, and OPDIVO®, is an anti-PD-1 antibody described in WO2006/121168. Pembrolizumab, also known as MK-3475, Merck 3475, lambrolizumab, KEYTRUDA®, and SCH-900475, is an
anti-PD-1 antibody described in WO2009/114335. Pidilizumab, also known as CT-011, hBAT, or hBAT-1, is an anti-PD-1 antibody described in WO2009/101611. AMP-224, also known as B7-DCIg, is a PDL2-Fc fusion soluble receptor described in WO2010/027827 and WO2011/066342. Additional PD-1 inhibitors include MEDI0680, also known as AMP-514, and REGN2810.

[0115] In some aspects, the immune checkpoint inhibitor is a PDL1 inhibitor such as Durvalumab, also known as MEDI4736, atezolizumab, also known as MPDL3280A, avelumab, also known as MSB00010118C, MDX-1105, BMS-936559, or combinations thereof. In certain aspects, the immune checkpoint inhibitor is a PDL2 inhibitor such as rHIgM12B7.

[0116] In some aspects, the inhibitor comprises the heavy and light chain CDRs or VRs of nivolumab, pembrolizumab, or pidilizumab. Accordingly, in one aspect, the inhibitor comprises the CDR1, CDR2, and CDR3 domains of the VH region of nivolumab, pembrolizumab, or pidilizumab, and the CDR1, CDR2 and CDR3 domains of the VL region of nivolumab, pembrolizumab, or pidilizumab. In another aspect, the antibody competes for binding with and/or binds to the same epitope on PD-1, PDL1, or PDL2 as the above-mentioned antibodies. In another aspect, the antibody has at least about 70, 75, 80, 85, 90, 95, 97, or 99% (or any derivable range therein) variable region amino acid sequence identity with the above-mentioned antibodies.

b. CTLA-4, B7-1, and B7-2

[0117] Another immune checkpoint that can be targeted in the methods provided herein is the cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), also known as CD152. The complete cDNA sequence of human CTLA-4 has the Genbank accession number L15006. CTLA-4 is found on the surface of T cells and acts as an “off” switch when bound to B7-1 (CD80) or B7-2 (CD86) on the surface of antigen-presenting cells. CTLA4 is a member of the immunoglobulin superfamily that is expressed on the surface of Helper T cells and transmits an inhibitory signal to T cells. CTLA4 is similar to the T-cell co-stimulatory protein, CD28, and both molecules bind to B7-1 and B7-2 on antigen-presenting cells. CTLA-4 transmits an inhibitory signal to T cells, whereas CD28 transmits a stimulatory signal. Intracellular CTLA-4 is also found in regulatory T cells and may be important to their function. T cell activation through the T cell receptor and CD28 leads to increased expression of CTLA-4, an inhibitory receptor for B7 molecules. Inhibitors of the disclosure may block one or more functions of CTLA-4, B7-1, and/or B7-2 activity. In some aspects, the inhibitor blocks the CTLA-4 and B7-1 interaction. In some aspects, the inhibitor blocks the CTLA-4 and B7-2 interaction.

[0118] In some aspects, the immune checkpoint inhibitor is an anti-CTLA-4 antibody (e.g., a human antibody, a humanized antibody, or a chimeric antibody), an antigen binding fragment thereof, an immunoadhesin, a fusion protein, or oligopeptide.

[0119] Anti-human-CTLA-4 antibodies (or VH and/or VL domains derived therefrom) suitable for use in the present methods can be generated using methods well known in the art. Alternatively, art recognized anti-CTLA-4 antibodies can be used. For example, the anti-CTLA-4 antibodies disclosed in: US 8,119,129, WO 01/14424, WO 98/42752; WO 00/37504 (CP675,206, also known as tremelimumab; formerly ticilimumab), U.S. Patent No. 6,207,156; Hurwitz et al., 1998; can be used in the methods disclosed herein. The teachings of each of the aforementioned publications are hereby incorporated by reference. Antibodies that compete with any of these art-recognized antibodies for binding to CTLA-4 also can be used. For example, a humanized CTLA-4 antibody is described in International Patent Application No. WO2001/014424, WO2000/037504, and U.S. Patent No. 8,017,114; all incorporated herein by reference.

[0120] A further anti-CTLA-4 antibody useful as a checkpoint inhibitor in the methods and compositions of the disclosure is ipilimumab (also known as 10D1, MDX- 010, MDX- 101, and Yervoy®) or antigen binding fragments and variants thereof (see, e.g., WO 01/14424).

[0121] In some aspects, the inhibitor comprises the heavy and light chain CDRs or VRs of tremelimumab or ipilimumab. Accordingly, in one aspect, the inhibitor comprises the CDR1, CDR2, and CDR3 domains of the VH region of tremelimumab or ipilimumab, and the CDR1, CDR2 and CDR3 domains of the VL region of tremelimumab or ipilimumab. In another aspect, the antibody competes for binding with and/or binds to the same epitope on PD-1, B7-1, or B7-2 as the above- mentioned antibodies. In another aspect, the antibody has at least about 70, 75, 80, 85, 90, 95, 97, or 99% (or any derivable range therein) variable region amino acid sequence identity with the above-mentioned antibodies.

2. Inhibition of co-stimulatory molecules

[0122] In some aspects, the immunotherapy comprises an inhibitor of a co-stimulatory molecule. In some aspects, the inhibitor comprises an inhibitor of B7-1 (CD80), B7-2 (CD86), CD28, ICOS, OX40 (TNFRSF4), 4-1BB (CD137; TNFRSF9), CD40L (CD40LG), GITR (TNFRSF18), and combinations thereof. Inhibitors include inhibitory antibodies, polypeptides, compounds, and nucleic acids.

3. Dendritic cell therapy

[0123] Dendritic cell therapy provokes anti-tumor responses by causing dendritic cells to present tumor antigens to lymphocytes, which activates them, priming them to kill other cells that present the antigen. Dendritic cells are antigen presenting cells (APCs) in the mammalian

immune system. In cancer treatment they aid cancer antigen targeting. One example of cellular cancer therapy based on dendritic cells is sipuleucel-T.

[0124] One method of inducing dendritic cells to present tumor antigens is by vaccination with autologous tumor lysates or short peptides (small parts of protein that correspond to the protein antigens on cancer cells). These peptides are often given in combination with adjuvants (highly immunogenic substances) to increase the immune and anti-tumor responses. Other adjuvants include proteins or other chemicals that attract and/or activate dendritic cells, such as granulocyte macrophage colony-stimulating factor (GM-CSF).

[0125] Dendritic cells can also be activated in vivo by making tumor cells express GM-CSF. This can be achieved by either genetically engineering tumor cells to produce GM-CSF or by infecting tumor cells with an oncolytic virus that expresses GM-CSF.

[0126] Another strategy is to remove dendritic cells from the blood of a patient and activate them outside the body. The dendritic cells are activated in the presence of tumor antigens, which may be a single tumor-specific peptide/protein or a tumor cell lysate (a solution of broken down tumor cells). These cells (with optional adjuvants) are infused and provoke an immune response.

[0127] Dendritic cell therapies include the use of antibodies that bind to receptors on the surface of dendritic cells. Antigens can be added to the antibody and can induce the dendritic cells to mature and provide immunity to the tumor. Dendritic cell receptors such as TLR3, TLR7, TLR8 or CD40 have been used as antibody targets.

4. CAR-T cell therapy

[0128] Chimeric antigen receptors (CARs, also known as chimeric immunoreceptors, chimeric T cell receptors or artificial T cell receptors) are engineered receptors that combine a new specificity with an immune cell to target cancer cells. Typically, these receptors graft the specificity of a monoclonal antibody onto a T cell. The receptors are called chimeric because they are fused of parts from different sources. CAR-T cell therapy refers to a treatment that uses such transformed cells for cancer therapy.

[0129] The basic principle of CAR-T cell design involves recombinant receptors that combine antigen-binding and T-cell activating functions. The general premise of CAR-T cells is to artificially generate T-cells targeted to markers found on cancer cells. Scientists can remove T-cells from a person, genetically alter them, and put them back into the patient for them to attack the cancer cells. Once the T cell has been engineered to become a CAR-T cell, it acts as a “living drug”. CAR-T cells create a link between an extracellular ligand recognition

domain to an intracellular signaling molecule which in turn activates T cells. The extracellular ligand recognition domain is usually a single-chain variable fragment (scFv). An important aspect of the safety of CAR-T cell therapy is how to ensure that only cancerous tumor cells are targeted, and not normal cells. The specificity of CAR-T cells is determined by the choice of molecule that is targeted.

[0130] Exemplary CAR-T therapies include Tisagenlecleucel (Kymriah) and Axicabtagene ciloleucel (Yescarta). In some aspects, the CAR-T therapy targets CD19.

5. Cytokine therapy

[0131] Cytokines are proteins produced by many types of cells present within a tumor. They can modulate immune responses. The tumor often employs them to allow it to grow and reduce the immune response. These immune-modulating effects allow them to be used as drugs to provoke an immune response. Two commonly used cytokines are interferons and interleukins.

[0132] Interferons are produced by the immune system. They are usually involved in anti-viral response, but also have use for cancer. They fall in three groups: type I (IFN α and IFN β), type II (IFN γ) and type III (IFN λ).

[0133] Interleukins have an array of immune system effects. IL-2 is an exemplary interleukin cytokine therapy.

6. Adoptive T-cell therapy

[0134] Adoptive T cell therapy is a form of passive immunization by the transfusion of T-cells (adoptive cell transfer). They are found in blood and tissue and usually activate when they find foreign pathogens. Specifically they activate when the T-cell's surface receptors encounter cells that display parts of foreign proteins on their surface antigens. These can be either infected cells, or antigen presenting cells (APCs). They are found in normal tissue and in tumor tissue, where they are known as tumor infiltrating lymphocytes (TILs). They are activated by the presence of APCs such as dendritic cells that present tumor antigens. Although these cells can attack the tumor, the environment within the tumor is highly immunosuppressive, preventing immune-mediated tumor death.

[0135] Multiple ways of producing and obtaining tumor targeted T-cells have been developed. T-cells specific to a tumor antigen can be removed from a tumor sample (TILs) or filtered from blood. Subsequent activation and culturing is performed ex vivo, with the results reinfused. Activation can take place through gene therapy, or by exposing the T cells to tumor antigens.

B. Chemotherapies

[0136] In some aspects, the additional therapy comprises a chemotherapy. Suitable classes of chemotherapeutic agents include (a) Alkylating Agents, such as nitrogen mustards (e.g., mechlorethamine, cyclophosphamide, ifosfamide, melphalan, chlorambucil), ethylenimines and methylmelamines (e.g., hexamethylmelamine, thiotepa), alkyl sulfonates (e.g., busulfan), nitrosoureas (e.g., carmustine, lomustine, chlorozotocin, streptozocin) and triazines (e.g., dicarbazine), (b) Antimetabolites, such as folic acid analogs (e.g., methotrexate), pyrimidine analogs (e.g., 5-fluorouracil, floxuridine, cytarabine, azauridine) and purine analogs and related materials (e.g., 6-mercaptopurine, 6-thioguanine, pentostatin), (c) Natural Products, such as vinca alkaloids (e.g., vinblastine, vincristine), epipodophylotoxins (e.g., etoposide, teniposide), antibiotics (e.g., dactinomycin, daunorubicin, doxorubicin, bleomycin, plicamycin and mitoxanthrone), enzymes (e.g., L-asparaginase), and biological response modifiers (e.g., Interferon- α), and (d) Miscellaneous Agents, such as platinum coordination complexes (e.g., cisplatin, carboplatin), substituted ureas (e.g., hydroxyurea), methylhydiazine derivatives (e.g., procarbazine), and adreocortical suppressants (e.g., taxol and mitotane). In some aspects, cisplatin is a particularly suitable chemotherapeutic agent.

[0137] Cisplatin has been widely used to treat cancers such as, for example, metastatic testicular or ovarian carcinoma, advanced bladder cancer, head or neck cancer, cervical cancer, lung cancer or other tumors. Cisplatin is not absorbed orally and must therefore be delivered via other routes such as, for example, intravenous, subcutaneous, intratumoral or intraperitoneal injection. Cisplatin can be used alone or in combination with other agents, with efficacious doses used in clinical applications including about 15 mg/m² to about 20 mg/m² for 5 days every three weeks for a total of three courses being contemplated in certain aspects. In some aspects, the amount of cisplatin delivered to the cell and/or subject in conjunction with the construct comprising an Egr-1 promoter operably linked to a polynucleotide encoding the therapeutic polypeptide is less than the amount that would be delivered when using cisplatin alone.

[0138] Other suitable chemotherapeutic agents include antimicrotubule agents, e.g., Paclitaxel ("Taxol") and doxorubicin hydrochloride ("doxorubicin"). The combination of an Egr-1 promoter/TNF α construct delivered via an adenoviral vector and doxorubicin was determined to be effective in overcoming resistance to chemotherapy and/or TNF- α , which suggests that combination treatment with the construct and doxorubicin overcomes resistance to both doxorubicin and TNF- α .

[0139] Doxorubicin is absorbed poorly and is preferably administered intravenously. In certain aspects, appropriate intravenous doses for an adult include about 60 mg/m² to about 75 mg/m² at about 21-day intervals or about 25 mg/m² to about 30 mg/m² on each of 2 or 3 successive days repeated at about 3 week to about 4 week intervals or about 20 mg/m² once a week. The lowest dose should be used in elderly patients, when there is prior bone-marrow depression caused by prior chemotherapy or neoplastic marrow invasion, or when the drug is combined with other myelopoietic suppressant drugs.

[0140] Nitrogen mustards are another suitable chemotherapeutic agent useful in the methods of the disclosure. A nitrogen mustard may include, but is not limited to, mechlorethamine (HN₂), cyclophosphamide and/or ifosfamide, melphalan (L-sarcolysin), and chlorambucil. Cyclophosphamide (CYTOXAN®) is available from Mead Johnson and NEOSTAR® is available from Adria), is another suitable chemotherapeutic agent. Suitable oral doses for adults include, for example, about 1 mg/kg/day to about 5 mg/kg/day, intravenous doses include, for example, initially about 40 mg/kg to about 50 mg/kg in divided doses over a period of about 2 days to about 5 days or about 10 mg/kg to about 15 mg/kg about every 7 days to about 10 days or about 3 mg/kg to about 5 mg/kg twice a week or about 1.5 mg/kg/day to about 3 mg/kg/day. Because of adverse gastrointestinal effects, the intravenous route is preferred. The drug also sometimes is administered intramuscularly, by infiltration or into body cavities.

[0141] Additional suitable chemotherapeutic agents include pyrimidine analogs, such as cytarabine (cytosine arabinoside), 5-fluorouracil (fluoruracil; 5-FU) and floxuridine (fluorodeoxyuridine; FudR). 5-FU may be administered to a subject in a dosage of anywhere between about 7.5 to about 1000 mg/m². Further, 5-FU dosing schedules may be for a variety of time periods, for example up to six weeks, or as determined by one of ordinary skill in the art to which this disclosure pertains.

[0142] Gemcitabine diphosphate (GEMZAR®, Eli Lilly & Co., “gemcitabine”), another suitable chemotherapeutic agent, is recommended for treatment of advanced and metastatic pancreatic cancer, and will therefore be useful in the present disclosure for these cancers as well.

[0143] The amount of the chemotherapeutic agent delivered to the patient may be variable. In one suitable aspect, the chemotherapeutic agent may be administered in an amount effective to cause arrest or regression of the cancer in a host, when the chemotherapy is administered with the construct. In other aspects, the chemotherapeutic agent may be administered in an amount that is anywhere between 2 to 10,000 fold less than the chemotherapeutic effective

dose of the chemotherapeutic agent. For example, the chemotherapeutic agent may be administered in an amount that is about 20 fold less, about 500 fold less or even about 5000 fold less than the chemotherapeutic effective dose of the chemotherapeutic agent. The chemotherapeutics of the disclosure can be tested in vivo for the desired therapeutic activity in combination with the construct, as well as for determination of effective dosages. For example, such compounds can be tested in suitable animal model systems prior to testing in humans, including, but not limited to, rats, mice, chicken, cows, monkeys, rabbits, etc. In vitro testing may also be used to determine suitable combinations and dosages, as described in the examples.

C. Radiotherapy

[0144] In some aspects, the additional therapy or prior therapy comprises radiation, such as ionizing radiation. As used herein, “ionizing radiation” means radiation comprising particles or photons that have sufficient energy or can produce sufficient energy via nuclear interactions to produce ionization (gain or loss of electrons). An exemplary and preferred ionizing radiation is an x-radiation. Means for delivering x-radiation to a target tissue or cell are well known in the art.

D. Surgery

[0145] In some aspects, the additional therapy comprises surgery. Approximately 60% of persons with cancer will undergo surgery of some type, which includes preventative, diagnostic or staging, curative, and palliative surgery. Curative surgery includes resection in which all or part of cancerous tissue is physically removed, excised, and/or destroyed and may be used in conjunction with other therapies, such as the treatment of the present aspects, chemotherapy, radiotherapy, hormonal therapy, gene therapy, immunotherapy, and/or alternative therapies. Tumor resection refers to physical removal of at least part of a tumor. In addition to tumor resection, treatment by surgery includes laser surgery, cryosurgery, electrosurgery, and microscopically-controlled surgery (Mohs’ surgery).

[0146] Upon excision of part or all of cancerous cells, tissue, or tumor, a cavity may be formed in the body. Treatment may be accomplished by perfusion, direct injection, or local application of the area with an additional anti-cancer therapy. Such treatment may be repeated, for example, every 1, 2, 3, 4, 5, 6, or 7 days, or every 1, 2, 3, 4, and 5 weeks or every 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months (or any range derivable therein). These treatments may be of varying dosages as well.

VII. Detection and Therapeutic Agents

[0147] In some aspects of this disclosure, it will be useful to detectably or therapeutically label the TCRs or fusion proteins of the disclosure. Methods for conjugating polypeptides to these agents are known in the art. For the purpose of illustration only, polypeptides can be labeled with a detectable moiety such as a radioactive atom, a chromophore, a fluorophore, or the like. Such labeled polypeptides can be used for diagnostic techniques, either in vivo, or in an isolated test sample or in methods described herein.

[0148] As used herein, the term "label" intends a directly or indirectly detectable compound or composition that is conjugated directly or indirectly to the composition to be detected, e.g., polynucleotide or protein such as an antibody so as to generate a "labeled" composition. The term also includes sequences conjugated to the polynucleotide that will provide a signal upon expression of the inserted sequences, such as green fluorescent protein (GFP) and the like. The label may be detectable by itself (e.g. radioisotope labels or fluorescent labels) or, in the case of an enzymatic label, may catalyze chemical alteration of a substrate compound or composition that is detectable. The labels can be suitable for small scale detection or more suitable for high-throughput screening. As such, suitable labels include, but are not limited to radioisotopes, fluorochromes, chemiluminescent compounds, dyes, and proteins, including enzymes. The label may be simply detected or it may be quantified. A response that is simply detected generally comprises a response whose existence merely is confirmed, whereas a response that is quantified generally comprises a response having a quantifiable (e.g., numerically reportable) value such as an intensity, polarization, and/or other property. In luminescence or fluorescence assays, the detectable response may be generated directly using a luminophore or fluorophore associated with an assay component actually involved in binding, or indirectly using a luminophore or fluorophore associated with another (e.g., reporter or indicator) component.

[0149] Examples of luminescent labels that produce signals include, but are not limited to bioluminescence and chemiluminescence. Detectable luminescence response generally comprises a change in, or an occurrence of, a luminescence signal. Suitable methods and luminophores for luminescently labeling assay components are known in the art and described for example in Haugland, Richard P. (1996) Handbook of Fluorescent Probes and Research Chemicals (6.sup.th ed.). Examples of luminescent probes include, but are not limited to, aequorin and luciferases.

[0150] Examples of suitable fluorescent labels include, but are not limited to, fluorescein, rhodamine, tetramethylrhodamine, eosin, erythrosin, coumarin, methyl-coumarins, pyrene, Malacite green, stilbene, Lucifer Yellow, Cascade Blue.TM., and Texas Red. Other suitable optical dyes are described in the Haugland, Richard P. (1996) Handbook of Fluorescent Probes and Research Chemicals (6.sup.th ed.).

[0151] In another aspect, the fluorescent label is functionalized to facilitate covalent attachment to a cellular component present in or on the surface of the cell or tissue such as a cell surface marker. Suitable functional groups, including, but not are limited to, isothiocyanate groups, amino groups, haloacetyl groups, maleimides, succinimidyl esters, and sulfonyl halides, all of which may be used to attach the fluorescent label to a second molecule. The choice of the functional group of the fluorescent label will depend on the site of attachment to either a linker, the agent, the marker, or the second labeling agent.

[0152] Attachment of the fluorescent label may be either directly to the cellular component or compound or alternatively, can be via a linker. Suitable binding pairs for use in indirectly linking the fluorescent label to the intermediate include, but are not limited to, antigens/polypeptides, e.g., rhodamine/anti-rhodamine, biotin/avidin and biotin/streptavidin.

[0153] The coupling of polypeptides to low molecular weight haptens can increase the sensitivity of the antibody in an assay. The haptens can then be specifically detected by means of a second reaction. For example, it is common to use haptens such as biotin, which reacts with avidin, or dinitrophenol, pyridoxal, and fluorescein, which can react with specific anti-hapten polypeptides. See, Harlow and Lane (1988) supra.

[0154] The conjugated agents can be linked to the polypeptide directly or indirectly, using any of a large number of available methods. For example, an agent can be attached at the hinge region of the reduced antibody component via disulfide bond formation, using cross-linkers such as N-succinyl 3-(2-pyridyldithio)propionate (SPDP), or via a carbohydrate moiety in the Fc region of the antibody (Yu et al., 1994; Upeslakis et al., 1995; Price, 1995).

[0155] Techniques for conjugating agents to polypeptides are well known (Amon et al., 1985; Hellstrom et al., 1987; Thorpe, 1985; Baldwin et al., 1985; Thorpe et al., 1982),

[0156] The polypeptides of the disclosure or antigen-binding regions thereof can be linked to another functional molecule such as ligands, cytotoxic molecules, chemotherapeutic agents, or other agents described as additional therapeutics.

VIII. Formulations and Culture of the Cells

[0157] In particular aspects, the cells of the disclosure may be specifically formulated and/or they may be cultured in a particular medium. The cells may be formulated in such a manner as to be suitable for delivery to a recipient without deleterious effects.

5 [0158] The medium in certain aspects can be prepared using a medium used for culturing animal cells as their basal medium, such as any of AIM V, X-VIVO-15, NeuroBasal, EGM2, TeSR, BME, BGJb, CMRL 1066, Glasgow MEM, Improved MEM Zinc Option, IMDM, Medium 199, Eagle MEM, α MEM, DMEM, Ham, RPMI-1640, and Fischer's media, as well as any combinations thereof, but the medium may not be particularly limited thereto as far as
10 it can be used for culturing animal cells. Particularly, the medium may be xeno-free or chemically defined.

[0159] The medium can be a serum-containing or serum-free medium, or xeno-free medium. From the aspect of preventing contamination with heterogeneous animal-derived components, serum can be derived from the same animal as that of the stem cell(s). The serum-
15 free medium refers to medium with no unprocessed or unpurified serum and accordingly, can include medium with purified blood-derived components or animal tissue-derived components (such as growth factors).

[0160] The medium may contain or may not contain any alternatives to serum. The alternatives to serum can include materials which appropriately contain albumin (such as lipid-
20 rich albumin, bovine albumin, albumin substitutes such as recombinant albumin or a humanized albumin, plant starch, dextrans and protein hydrolysates), transferrin (or other iron transporters), fatty acids, insulin, collagen precursors, trace elements, 2-mercaptoethanol, 3'-thiolglycerol, or equivalents thereto. The alternatives to serum can be prepared by the method disclosed in International Publication No. 98/30679, for example (incorporated herein in its
25 entirety). Alternatively, any commercially available materials can be used for more convenience. The commercially available materials include knockout Serum Replacement (KSR), Chemically-defined Lipid concentrated (Gibco), and Glutamax (Gibco).

[0161] In certain aspects, the medium may comprise one, two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more of the following: Vitamins such
30 as biotin; DL Alpha Tocopherol Acetate; DL Alpha-Tocopherol; Vitamin A (acetate); proteins such as BSA (bovine serum albumin) or human albumin, fatty acid free Fraction V; Catalase; Human Recombinant Insulin; Human Transferrin; Superoxide Dismutase; Other Components such as Corticosterone; D-Galactose; Ethanolamine HCl; Glutathione (reduced); L-Carnitine

HCl; Linoleic Acid; Linolenic Acid; Progesterone; Putrescine 2HCl; Sodium Selenite; and/or T3 (triiodo-L-thyronine). . In specific aspects, one or more of these may be explicitly excluded.

[0162] In some aspects, the medium further comprises vitamins. In some aspects, the

medium comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13 of the following (and any range

5 derivable therein): biotin, DL alpha tocopherol acetate, DL alpha-tocopherol, vitamin A, choline chloride, calcium pantothenate, pantothenic acid, folic acid nicotinamide, pyridoxine, riboflavin, thiamine, inositol, vitamin B12, or the medium includes combinations thereof or salts thereof. In some aspects, the medium comprises or consists essentially of biotin, DL alpha

tocopherol acetate, DL alpha-tocopherol, vitamin A, choline chloride, calcium pantothenate, 10 pantothenic acid, folic acid nicotinamide, pyridoxine, riboflavin, thiamine, inositol, and vitamin B12. In some aspects, the vitamins include or consist essentially of biotin, DL alpha tocopherol acetate, DL alpha-tocopherol, vitamin A, or combinations or salts thereof. In some

aspects, the medium further comprises proteins. In some aspects, the proteins comprise albumin or bovine serum albumin, a fraction of BSA, catalase, insulin, transferrin, superoxide

15 dismutase, or combinations thereof. In some aspects, the medium further comprises one or more of the following: corticosterone, D-Galactose, ethanolamine, glutathione, L-carnitine, linoleic acid, linolenic acid, progesterone, putrescine, sodium selenite, or triiodo-L-thyronine, or combinations thereof. In some aspects, the medium comprises one or more of the following: a

B-27® supplement, xeno-free B-27® supplement, GS21TM supplement, or combinations 20 thereof. In some aspects, the medium comprises or further comprises amino acids, monosaccharides, inorganic ions. In some aspects, the amino acids comprise arginine, cystine, isoleucine, leucine, lysine, methionine, glutamine, phenylalanine, threonine, tryptophan, histidine, tyrosine, or valine, or combinations thereof. In some aspects, the inorganic ions

comprise sodium, potassium, calcium, magnesium, nitrogen, or phosphorus, or combinations 25 or salts thereof. In some aspects, the medium further comprises one or more of the following: molybdenum, vanadium, iron, zinc, selenium, copper, or manganese, or combinations thereof.

In certain aspects, the medium comprises or consists essentially of one or more vitamins discussed herein and/or one or more proteins discussed herein, and/or one or more of the 30 following: corticosterone, D-Galactose, ethanolamine, glutathione, L-carnitine, linoleic acid,

linolenic acid, progesterone, putrescine, sodium selenite, or triiodo-L-thyronine, a B-27® supplement, xeno-free B-27® supplement, GS21TM supplement, an amino acid (such as arginine, cystine, isoleucine, leucine, lysine, methionine, glutamine, phenylalanine, threonine, tryptophan, histidine, tyrosine, or valine), monosaccharide, inorganic ion (such as sodium, potassium, calcium, magnesium, nitrogen, and/or phosphorus) or salts thereof, and/or

molybdenum, vanadium, iron, zinc, selenium, copper, or manganese. In specific aspects, one or more of these may be explicitly excluded.

[0163] The medium can also contain one or more externally added fatty acids or lipids, amino acids (such as non-essential amino acids), vitamin(s), growth factors, cytokines, antioxidant substances, 2-mercaptoethanol, pyruvic acid, buffering agents, and/or inorganic salts. . In specific aspects, one or more of these may be explicitly excluded.

[0164] One or more of the medium components may be added at a concentration of at least, at most, or about 0.1, 0.5, 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 150, 180, 200, 250 ng/L, ng/ml, µg/ml, mg/ml, or any range derivable therein.

[0165] In specific aspects, the cells of the disclosure are specifically formulated. They may or may not be formulated as a cell suspension. In specific cases they are formulated in a single dose form. They may be formulated for systemic or local administration. In some cases the cells are formulated for storage prior to use, and the cell formulation may comprise one or more cryopreservation agents, such as DMSO (for example, in 5% DMSO). The cell formulation may comprise albumin, including human albumin, with a specific formulation comprising 2.5% human albumin. The cells may be formulated specifically for intravenous administration; for example, they are formulated for intravenous administration over less than one hour. In particular aspects the cells are in a formulated cell suspension that is stable at room temperature for 1, 2, 3, or 4 hours or more from time of thawing.

[0166] In particular aspects, the cells of the disclosure comprise an exogenous TCR, which may be of a defined antigen specificity. In some aspects, the TCR can be selected based on absent or reduced alloreactivity to the intended recipient (examples include certain virus-specific TCRs, xeno-specific TCRs, or cancer-testis antigen-specific TCRs). In the example where the exogenous TCR is non-alloreactive, during T cell differentiation the exogenous TCR suppresses rearrangement and/or expression of endogenous TCR loci through a developmental process called allelic exclusion, resulting in T cells that express only the non-alloreactive exogenous TCR and are thus non-alloreactive. In some aspects, the choice of exogenous TCR may not necessarily be defined based on lack of alloreactivity. In some aspects, the endogenous TCR genes have been modified by genome editing so that they do not express a protein. Methods of gene editing such as methods using the CRISPR/Cas9 system are known in the art and described herein.

[0167] In some aspects, the cells of the disclosure further comprise one or more chimeric antigen receptors (CARs). Examples of tumor cell antigens to which a CAR may be directed include at least 5T4, 8H9, αvβ6 integrin, BCMA, B7-H3, B7-H6, CAIX, CA9, CD19, CD20,

CD22, CD30, CD33, CD38, CD44, CD44v6, CD44v7/8, CD70, CD123, CD138, CD171, CEA, CSPG4, EGFR, EGFR family including ErbB2 (HER2), EGFRvIII, EGP2, EGP40, ERBB3, ERBB4, ErbB3/4, EPCAM, EphA2, EpCAM, folate receptor-a, FAP, FBP, fetal AchR, FR α , GD2, G250/CAIX, GD3, Glypican-3 (GPC3), Her2, IL-13R α 2, Lambda, Lewis-
 5 Y, Kappa, KDR, MAGE, MCSP, Mesothelin, Muc1, Muc16, NCAM, NKG2D Ligands, NY-ESO-1, PRAME, PSC1, PSCA, PSMA, ROR1, SP17, Survivin, TAG72, TEMs, carcinoembryonic antigen, HMW-MAA, AFP, CA-125, ETA, Tyrosinase, MAGE, laminin receptor, HPV E6, E7, BING-4, Calcium-activated chloride channel 2, Cyclin-B1, 9D7, EphA3, Telomerase, SAP-1, BAGE family, CAGE family, GAGE family, MAGE family,
 10 SAGE family, XAGE family, NY-ESO-1/LAGE-1, PAME, SSX-2, Melan-A/MART-1, GP100/pmel17, TRP-1/-2, P. polypeptide, MC1R, Prostate-specific antigen, β -catenin, BRCA1/2, CML66, Fibronectin, MART-2, TGF- β RII, or VEGF receptors (e.g., VEGFR2), for example. The CAR may be a first, second, third, or more generation CAR. The CAR may be bispecific for any two nonidentical antigens, or it may be specific for more than two
 15 nonidentical antigens.

IX. Administration of Therapeutic Compositions

[0168] The therapy provided herein may comprise administration of a combination of therapeutic agents, such as a first cancer therapy and a second cancer therapy. The therapies may be administered in any suitable manner known in the art. For example, the first and second
 20 cancer treatment may be administered sequentially (at different times) or concurrently (at the same time). In some aspects, the first and second cancer treatments are administered in a separate composition. In some aspects, the first and second cancer treatments are in the same composition.

[0169] Aspects of the disclosure relate to compositions and methods comprising therapeutic
 25 compositions. The different therapies may be administered in one composition or in more than one composition, such as 2 compositions, 3 compositions, or 4 compositions. Various combinations of the agents may be employed.

[0170] The therapeutic compositions of the disclosure may be administered by the same route of administration or by different routes of administration. In some aspects, the cancer
 30 therapy is administered intravenously, intramuscularly, subcutaneously, topically, orally, transdermally, intraperitoneally, intraorbitally, by implantation, by inhalation, intrathecally, intraventricularly, or intranasally. In some aspects, the antibiotic is administered intravenously, intramuscularly, subcutaneously, topically, orally, transdermally, intraperitoneally,

intraorbitally, by implantation, by inhalation, intrathecally, intraventricularly, or intranasally. The appropriate dosage may be determined based on the type of disease to be treated, severity and course of the disease, the clinical condition of the individual, the individual's clinical history and response to the treatment, and the discretion of the attending physician.

5 **[0171]** The treatments may include various “unit doses.” Unit dose is defined as containing a predetermined-quantity of the therapeutic composition. The quantity to be administered, and the particular route and formulation, is within the skill of determination of those in the clinical arts. A unit dose need not be administered as a single injection but may comprise continuous infusion over a set period of time. In some aspects, a unit dose comprises a single administrable
10 dose.

[0172] Precise amounts of the therapeutic composition also depend on the judgment of the practitioner and are peculiar to each individual. Factors affecting dose include physical and clinical state of the patient, the route of administration, the intended goal of treatment (alleviation of symptoms versus cure) and the potency, stability and toxicity of the particular
15 therapeutic substance or other therapies a subject may be undergoing.

X. Kits

[0173] Certain aspects of the present invention also concern kits containing compositions of the disclosure or compositions to implement methods of the invention. In some aspects, kits can be used to evaluate one or more biomarkers or HLA types. In certain aspects, a kit contains,
20 contains at least or contains at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 100, 500, 1,000 or more probes, primers or primer sets, synthetic molecules or inhibitors, or any value or range and combination derivable therein.

[0174] Kits may comprise components, which may be individually packaged or placed in a
25 container, such as a tube, bottle, vial, syringe, or other suitable container means.

[0175] Individual components may also be provided in a kit in concentrated amounts; in some aspects, a component is provided individually in the same concentration as it would be in a solution with other components. Concentrations of components may be provided as 1x, 2x, 5x, 10x, or 20x or more.

30 **[0176]** In certain aspects, negative and/or positive control nucleic acids, probes, and inhibitors are included in some kit aspects. In addition, a kit may include a sample that is a negative or positive control for methylation of one or more biomarkers.

[0177] It is contemplated that any method or composition described herein can be implemented with respect to any other method or composition described herein and that different aspects may be combined. The claims originally filed are contemplated to cover claims that are multiply dependent on any filed claim or combination of filed claims.

5 XI. Examples

[0178] The following examples are included to demonstrate preferred embodiments of the disclosure. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the disclosure, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the disclosure.

Example 1: Design and testing of EGFR-specific T-cell Receptors

[0179] HLA-A*3101 transduced H1975 cells were pulsed with EGFR(L858R) peptide (HVKITDFGR – SEQ ID NO:85) and co-cultured with sorted, TCR-transduced T cells at the indicated E:T ratio for 4 hours. T-cell Killing activity was determined by measuring Cr51 release in the supernatant. The TCR-mediated cytotoxicity for clones 1 and 2 are shown in FIG. 1. H1975-A*3101 or HLA-A*3303-transduced 293 target cells were pulsed with different amounts of HVKITDFGR (SEQ ID NO:85) peptide. 1×10^5 target and TCR-transduced effector cells were seeded in each well and incubated overnight. The spots # in FIG. 3 is the number of IFN-gamma secreting T cells in response to peptide mutant EGFR peptide recognition. PBMCs were transduced with virus expressing the TCRs, and TCR-transduced tetramer positive T cells were sorted. Next, HLA*A33:03 293 cells were pulsed with HVKITDFGR (SEQ ID NO:85) peptide and labeled with Cr51. FIGS. 4-5 show the specific lysis of target cells incubated with effector (EGFR TCR-specific T cells) at different E:T ratios for four hours. Cr51 release was measured. 293 cells stably expressing HLA-A*3301 and either wild-type (WT) or L858R-mutated EGFR genes were co-cultured with different ratios of sorted TCR-T cells expressing TCR33-32H. After 4 hours of incubation, released Cr51 activity was measured (FIG. 6). As shown in FIG. 6, TCR33-32H can recognize and kill 293 cells transduced to express the whole EGFR-L858R gene. This experiment demonstrates natural processing and presentation of the HVKITDFGR (SEQ ID NO:85) Neoantigen peptide by tumor cells. Comparing the killing activity of different EGFR-A33 TCR clones. Normal PBMC were transduced with one of 3 different EGFR-A33 TCRs

(TCR32H, TCR37-3 and TCR33-4). Tetramer and CD8 double-positive T cells were co-cultured with 293 cells stably expressing WT or L858R-mutated EGFR genes. killing activity at different E:T ratios was compared by Cr51 release assay (FIG. 7). These experiments collectively demonstrate that the EGFR neoantigen-specific TCRs that were cloned and expressed can endow T cells with the capacity to recognize, secrete interferon-gamma, and kill HLA-A*3301 or A*3303-expressing tumor target cells that have been pulsed with cognate peptide antigen HVKITDFGR (SEQ ID NO:85), or that have been transduced to express full length EGFR(L858R) protein, but not wild-type EGFR. Figure 7 demonstrates that the neoantigen peptide HVKITDFGR (SEQ ID NO:85) can be naturally processed and presented on HLA-A*3101 and A*3303 from the full-length mutated EGFR protein.

* * *

[0180] All of the methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

WHAT IS CLAIMED IS:

1. A polypeptide comprising an antigen binding variable region comprising a CDR3 comprising the amino acid sequence of SEQ ID NO:8 or an amino acid sequence with at least 80% sequence identity to SEQ ID NO:8.
- 5 2. The polypeptide of claim 1, wherein the variable region comprises a CDR1, CDR2, and/or CDR3.
3. The polypeptide of claim 2, wherein the variable region comprises a CDR1 and/or CDR2 with the amino acid sequence of SEQ ID NO:6 and 7, respectively, or with an amino acid sequence that is at least 80% sequence identity to SEQ ID NO:6 and SEQ ID NO:7,
10 respectively.
4. The polypeptide of any one of claims 1-3, wherein the variable region comprises an amino acid sequence or SEQ ID NO:4 or an amino acid sequence with at least 70% sequence identity to SEQ ID NO:4.
5. The polypeptide of any one of claims 1-4, wherein the polypeptide comprises a T cell
15 receptor beta (TCR-b) variable region.
6. The polypeptide of claim 5, wherein the polypeptide comprises a TCR-b variable and constant region.
7. The polypeptide of any one of claims 1-6, wherein the polypeptide further comprises a signal peptide.
- 20 8. The polypeptide of claim 7, wherein the signal peptide comprises the amino acid sequence of SEQ ID NO:5 or an amino acid sequence with at least 80% identity to SEQ ID NO:5.
9. A polypeptide comprising an antigen binding variable region comprising a CDR3 comprising the amino acid sequence of SEQ ID NO:14 or an amino acid sequence with at least
25 80% sequence identity to SEQ ID NO:14.
10. The polypeptide of claim 9, wherein the variable region comprises a CDR1, CDR2, and/or CDR3.
11. The polypeptide of claim 10, wherein the variable region comprises a CDR1 and/or CDR2 with the amino acid sequence of SEQ ID NO:12 and SEQ ID NO:13, respectively, or
30 with at least 80% sequence identity to SEQ ID NO:12 and SEQ ID NO:13, respectively.
12. The polypeptide of any one of claims 9-11, wherein the variable region comprises the amino acid sequence of SEQ ID NO:10 or an amino acid sequence with at least 70% sequence identity to SEQ ID NO:10.

13. The polypeptide of any one of claims 9-12, wherein the polypeptide comprises a T cell receptor alpha (TCR-a) variable region.
14. The polypeptide of claim 13, wherein the polypeptide comprises a TCR-a variable and constant region.
- 5 15. The polypeptide of any one of claims 9-14, wherein the polypeptide further comprises a signal peptide.
16. The polypeptide of claim 15, wherein the signal peptide comprises the amino acid sequence of SEQ ID NO:11 or an amino acid sequence with at least 80% identity to SEQ ID NO:11.
- 10 17. A polypeptide comprising an antigen binding variable region comprising a CDR3 comprising the amino acid sequence of SEQ ID NO:22 or an amino acid sequence with at least 80% sequence identity to SEQ ID NO:22.
18. The polypeptide of claim 17, wherein the variable region comprises a CDR1, CDR2, and/or CDR3.
- 15 19. The polypeptide of claim 18, wherein the variable region comprises a CDR1 and/or CDR2 with the amino acid sequence of SEQ ID NO:20 and 21, respectively, or with an amino acid sequence that is at least 80% sequence identity to SEQ ID NO:20 and SEQ ID NO:21, respectively.
- 20 20. The polypeptide of any one of claims 17-19, wherein the variable region comprises an amino acid sequence or SEQ ID NO:18 or an amino acid sequence with at least 70% sequence identity to SEQ ID NO:18.
21. The polypeptide of any one of claims 17-20, wherein the polypeptide comprises a T cell receptor alpha (TCR-a) variable region.
22. The polypeptide of claim 21, wherein the polypeptide comprises a TCR-a variable and
25 constant region.
23. The polypeptide of any one of claims 17-22, wherein the polypeptide further comprises a signal peptide.
24. The polypeptide of claim 23, wherein the signal peptide comprises the amino acid sequence of SEQ ID NO:19 or an amino acid sequence with at least 80% identity to SEQ ID
30 NO:19.
25. A polypeptide comprising an antigen binding variable region comprising a CDR3 comprising the amino acid sequence of SEQ ID NO:28 or an amino acid sequence with at least 80% sequence identity to SEQ ID NO:28.

26. The polypeptide of claim 25, wherein the variable region comprises a CDR1, CDR2, and/or CDR3.
27. The polypeptide of claim 26, wherein the variable region comprises a CDR1 and/or CDR2 with the amino acid sequence of SEQ ID NO:26 and SEQ ID NO:27, respectively, or
5 with at least 80% sequence identity to SEQ ID NO:26 and SEQ ID NO:27, respectively.
28. The polypeptide of any one of claims 25-27, wherein the variable region comprises the amino acid sequence of SEQ ID NO:24 or an amino acid sequence with at least 70% sequence identity to SEQ ID NO:24.
29. The polypeptide of any one of claims 25-28, wherein the polypeptide comprises a T
10 cell receptor beta (TCR-b) variable region.
30. The polypeptide of claim 29, wherein the polypeptide comprises a TCR-b variable and constant region.
31. The polypeptide of any one of claims 25-28, wherein the polypeptide further comprises a signal peptide.
- 15 32. The polypeptide of claim 31, wherein the signal peptide comprises the amino acid sequence of SEQ ID NO:25 or an amino acid sequence with at least 80% identity to SEQ ID NO:25.
33. A polypeptide comprising an antigen binding variable region comprising a CDR3 comprising the amino acid sequence of SEQ ID NO:36 or an amino acid sequence with at least
20 80% sequence identity to SEQ ID NO:36.
34. The polypeptide of claim 33, wherein the variable region comprises a CDR1, CDR2, and/or CDR3.
35. The polypeptide of claim 34, wherein the variable region comprises a CDR1 and/or CDR2 with the amino acid sequence of SEQ ID NO:34 and 35, respectively, or with an amino
25 acid sequence that is at least 80% sequence identity to SEQ ID NO:34 and SEQ ID NO:35, respectively.
36. The polypeptide of any one of claims 33-35, wherein the variable region comprises an amino acid sequence or SEQ ID NO:32 or an amino acid sequence with at least 70% sequence identity to SEQ ID NO:32.
- 30 37. The polypeptide of any one of claims 33-36, wherein the polypeptide comprises a T cell receptor alpha (TCR-a) variable region.
38. The polypeptide of claim 37, wherein the polypeptide comprises a TCR-a variable and constant region.

39. The polypeptide of any one of claims 33-38, wherein the polypeptide further comprises a signal peptide.

40. The polypeptide of claim 39, wherein the signal peptide comprises the amino acid sequence of SEQ ID NO:33 or an amino acid sequence with at least 80% identity to SEQ ID

5 NO:33.

41. A polypeptide comprising an antigen binding variable region comprising a CDR3 comprising the amino acid sequence of SEQ ID NO:42 or an amino acid sequence with at least 80% sequence identity to SEQ ID NO:42.

42. The polypeptide of claim 41, wherein the variable region comprises a CDR1, CDR2, and/or CDR3.

43. The polypeptide of claim 42, wherein the variable region comprises a CDR1 and/or CDR2 with the amino acid sequence of SEQ ID NO:40 and SEQ ID NO:41, respectively, or with at least 80% sequence identity to SEQ ID NO:40 and SEQ ID NO:41, respectively.

44. The polypeptide of any one of claims 41-43, wherein the variable region comprises the amino acid sequence of SEQ ID NO:38 or an amino acid sequence with at least 70% sequence identity to SEQ ID NO:38.

45. The polypeptide of any one of claims 40-44, wherein the polypeptide comprises a T cell receptor beta (TCR-b) variable region.

46. The polypeptide of claim 45, wherein the polypeptide comprises a TCR-b variable and constant region.

47. The polypeptide of any one of claims 40-46, wherein the polypeptide further comprises a signal peptide.

48. The polypeptide of claim 47, wherein the signal peptide comprises the amino acid sequence of SEQ ID NO:39 or an amino acid sequence with at least 80% identity to SEQ ID NO:39.

49. A polypeptide comprising an antigen binding variable region comprising a CDR3 comprising the amino acid sequence of SEQ ID NO:50 or an amino acid sequence with at least 80% sequence identity to SEQ ID NO:50.

50. The polypeptide of claim 49, wherein the variable region comprises a CDR1, CDR2, and/or CDR3.

51. The polypeptide of claim 50, wherein the variable region comprises a CDR1 and/or CDR2 with the amino acid sequence of SEQ ID NO:48 and 49, respectively, or with an amino acid sequence that is at least 80% sequence identity to SEQ ID NO:48 and SEQ ID NO:49, respectively.

52. The polypeptide of any one of claims 49-51, wherein the variable region comprises an amino acid sequence or SEQ ID NO:46 or an amino acid sequence with at least 70% sequence identity to SEQ ID NO:46.
53. The polypeptide of any one of claims 49-52, wherein the polypeptide comprises a T cell receptor alpha (TCR-a) variable region.
54. The polypeptide of claim 53, wherein the polypeptide comprises a TCR-a variable and constant region.
55. The polypeptide of any one of claims 49-54, wherein the polypeptide further comprises a signal peptide.
56. The polypeptide of claim 55, wherein the signal peptide comprises the amino acid sequence of SEQ ID NO:47 or an amino acid sequence with at least 80% identity to SEQ ID NO:47.
57. A polypeptide comprising an antigen binding variable region comprising a CDR3 comprising the amino acid sequence of SEQ ID NO:56 or an amino acid sequence with at least 80% sequence identity to SEQ ID NO:56.
58. The polypeptide of claim 57, wherein the variable region comprises a CDR1, CDR2, and/or CDR3.
59. The polypeptide of claim 58, wherein the variable region comprises a CDR1 and/or CDR2 with the amino acid sequence of SEQ ID NO:54 and SEQ ID NO:55, respectively, or with at least 80% sequence identity to SEQ ID NO:54 and SEQ ID NO:55, respectively.
60. The polypeptide of any one of claims 57-59, wherein the variable region comprises the amino acid sequence of SEQ ID NO:52 or an amino acid sequence with at least 70% sequence identity to SEQ ID NO:52.
61. The polypeptide of any one of claims 56-60, wherein the polypeptide comprises a T cell receptor beta (TCR-b) variable region.
62. The polypeptide of claim 61, wherein the polypeptide comprises a TCR-b variable and constant region.
63. The polypeptide of any one of claims 56-62, wherein the polypeptide further comprises a signal peptide.
64. The polypeptide of claim 63, wherein the signal peptide comprises the amino acid sequence of SEQ ID NO:53 or an amino acid sequence with at least 80% identity to SEQ ID NO:53.

65. A polypeptide comprising an antigen binding variable region comprising a CDR3 comprising the amino acid sequence of SEQ ID NO:64 or an amino acid sequence with at least 80% sequence identity to SEQ ID NO:64.
66. The polypeptide of claim 65, wherein the variable region comprises a CDR1, CDR2, and/or CDR3.
67. The polypeptide of claim 66, wherein the variable region comprises a CDR1 and/or CDR2 with the amino acid sequence of SEQ ID NO:62 and 63, respectively, or with an amino acid sequence that is at least 80% sequence identity to SEQ ID NO:62 and SEQ ID NO:63, respectively.
68. The polypeptide of any one of claims 65-67, wherein the variable region comprises an amino acid sequence or SEQ ID NO:60 or an amino acid sequence with at least 70% sequence identity to SEQ ID NO:60.
69. The polypeptide of any one of claims 65-68, wherein the polypeptide comprises a T cell receptor alpha (TCR-a) variable region.
70. The polypeptide of claim 69, wherein the polypeptide comprises a TCR-a variable and constant region.
71. The polypeptide of any one of claims 65-70, wherein the polypeptide further comprises a signal peptide.
72. The polypeptide of claim 71, wherein the signal peptide comprises the amino acid sequence of SEQ ID NO:61 or an amino acid sequence with at least 80% identity to SEQ ID NO:61.
73. A polypeptide comprising an antigen binding variable region comprising a CDR3 comprising the amino acid sequence of SEQ ID NO:70 or an amino acid sequence with at least 80% sequence identity to SEQ ID NO:70.
74. The polypeptide of claim 73, wherein the variable region comprises a CDR1, CDR2, and/or CDR3.
75. The polypeptide of claim 74, wherein the variable region comprises a CDR1 and/or CDR2 with the amino acid sequence of SEQ ID NO:68 and SEQ ID NO:69, respectively, or with at least 80% sequence identity to SEQ ID NO:68 and SEQ ID NO:69, respectively.
76. The polypeptide of any one of claims 73-75, wherein the variable region comprises the amino acid sequence of SEQ ID NO:66 or an amino acid sequence with at least 70% sequence identity to SEQ ID NO:66.
77. The polypeptide of any one of claims 72-76, wherein the polypeptide comprises a T cell receptor beta (TCR-b) variable region.

78. The polypeptide of claim 77, wherein the polypeptide comprises a TCR-b variable and constant region.
79. The polypeptide of any one of claims 72-78, wherein the polypeptide further comprises a signal peptide.
- 5 80. The polypeptide of claim 79, wherein the signal peptide comprises the amino acid sequence of SEQ ID NO:67 or an amino acid sequence with at least 80% identity to SEQ ID NO:67.
81. A polypeptide comprising an antigen binding variable region comprising a CDR3 comprising the amino acid sequence of SEQ ID NO:78 or an amino acid sequence with at least
- 10 80% sequence identity to SEQ ID NO:78.
82. The polypeptide of claim 81, wherein the variable region comprises a CDR1, CDR2, and/or CDR3.
83. The polypeptide of claim 82, wherein the variable region comprises a CDR1 and/or CDR2 with the amino acid sequence of SEQ ID NO:76 and 77, respectively, or with an amino
- 15 acid sequence that is at least 80% sequence identity to SEQ ID NO:76 and SEQ ID NO:77, respectively.
84. The polypeptide of any one of claims 81-83, wherein the variable region comprises an amino acid sequence or SEQ ID NO:74 or an amino acid sequence with at least 70% sequence identity to SEQ ID NO:74.
- 20 85. The polypeptide of any one of claims 81-84, wherein the polypeptide comprises a T cell receptor alpha (TCR-a) variable region.
86. The polypeptide of claim 85, wherein the polypeptide comprises a TCR-a variable and constant region.
87. The polypeptide of any one of claims 81-86, wherein the polypeptide further comprises
- 25 a signal peptide.
88. The polypeptide of claim 87, wherein the signal peptide comprises the amino acid sequence of SEQ ID NO:75 or an amino acid sequence with at least 80% identity to SEQ ID NO:75.
89. A polypeptide comprising an antigen binding variable region comprising a CDR3
- 30 comprising the amino acid sequence of SEQ ID NO:84 or an amino acid sequence with at least 80% sequence identity to SEQ ID NO:84.
90. The polypeptide of claim 89, wherein the variable region comprises a CDR1, CDR2, and/or CDR3.

91. The polypeptide of claim 90, wherein the variable region comprises a CDR1 and/or CDR2 with the amino acid sequence of SEQ ID NO:82 and SEQ ID NO:83, respectively, or with at least 80% sequence identity to SEQ ID NO:82 and SEQ ID NO:83, respectively.
92. The polypeptide of any one of claims 89-91, wherein the variable region comprises the amino acid sequence of SEQ ID NO:80 or an amino acid sequence with at least 70% sequence identity to SEQ ID NO:80.
93. The polypeptide of any one of claims 88-92, wherein the polypeptide comprises a T cell receptor beta (TCR-b) variable region.
94. The polypeptide of claim 93, wherein the polypeptide comprises a TCR-b variable and constant region.
95. The polypeptide of any one of claims 88-94, wherein the polypeptide further comprises a signal peptide.
96. The polypeptide of claim 95, wherein the signal peptide comprises the amino acid sequence of SEQ ID NO:81 or an amino acid sequence with at least 80% identity to SEQ ID NO:81.
97. A nucleic acid encoding for the polypeptide of any one of claims 1-96.
98. An engineered T-cell Receptor (TCR) comprising a TCR-b polypeptide and a TCR-a polypeptide, wherein the TCR-b polypeptide comprises a CDR3 with the amino acid sequence of SEQ ID NO:8 or an amino acid sequence with at least 80% sequence identity to SEQ ID NO:8 and the TCR-a polypeptide comprises a CDR3 with the amino acid sequence of SEQ ID NO:14 or an amino acid sequence with at least 80% sequence identity to SEQ ID NO:14.
99. The TCR of claim 98, wherein the TCR comprises a TCR-b polypeptide comprising a variable region comprising CDR1, CDR2, and CDR3 and a TCR-a polypeptide comprising a variable region comprising CDR1, CDR2, and CDR3.
100. The TCR of claim 99, wherein the TCR-b polypeptide comprises a CDR1 having the amino acid sequence of SEQ ID NO:6 or having an amino acid sequence with at least 80% sequence identity to SEQ ID NO:6 and/or the TCR-a polypeptide comprises a CDR1 having the amino acid sequence of SEQ ID NO:12 or having an amino acid sequence with at least 80% sequence identity to SEQ ID NO:12.
101. The TCR of claim 99 or 100, wherein the TCR-b polypeptide comprises a CDR2 having the amino acid sequence of SEQ ID NO:7 or having an amino acid sequence with at least 80% sequence identity to SEQ ID NO:7 and/or the TCR-a polypeptide comprises a CDR2 having the amino acid sequence of SEQ ID NO:13 or having an amino acid sequence with at least 80% sequence identity to SEQ ID NO:13.

102. The TCR of any one of claims 99-101, wherein the CDR1, CDR2, and CDR3 of the TCR-b polypeptide comprise the amino acid sequence of SEQ ID NO: 6, 7, and 8, respectively and wherein the CDR1, CDR3, and CDR3 of the TCR-a polypeptide comprise the amino acid sequence of SEQ ID NO:12, 13, and 14, respectively.

5 103. The TCR of any one of claims 99-102, wherein the TCR-b polypeptide comprises the amino acid sequence of SEQ ID NO:4 or an amino acid sequence with at least 70% sequence identity to SEQ ID NO:4 and the TCR-a polypeptide comprises the amino acid sequence of SEQ ID NO:10 or an amino acid sequence with at least 70% sequence identity to SEQ ID NO:10.

10 104. An engineered T-cell Receptor (TCR) comprising a TCR-a polypeptide and a TCR-b polypeptide, wherein the TCR-a polypeptide comprises a CDR3 with the amino acid sequence of SEQ ID NO:22 or an amino acid sequence with at least 80% sequence identity to SEQ ID NO:22 and the TCR-b polypeptide comprises a CDR3 with the amino acid sequence of SEQ ID NO:28 or an amino acid sequence with at least 80% sequence identity to SEQ ID NO:28.

15 105. The TCR of claim 104, wherein the TCR comprises a TCR-b polypeptide comprising a variable region comprising CDR1, CDR2, and CDR3 and a TCR-a polypeptide comprising a variable region comprising CDR1, CDR2, and CDR3.

106. The TCR of claim 105, wherein the TCR-a polypeptide comprises a CDR1 having the amino acid sequence of SEQ ID NO:20 or having an amino acid sequence with at least 80% sequence identity to SEQ ID NO:20 and/or the TCR-b polypeptide comprises a CDR1 having the amino acid sequence of SEQ ID NO:26 or having an amino acid sequence with at least 80% sequence identity to SEQ ID NO:26.

20 107. The TCR of claim 105 or 106, wherein the TCR-a polypeptide comprises a CDR2 having the amino acid sequence of SEQ ID NO:21 or having an amino acid sequence with at least 80% sequence identity to SEQ ID NO:21 and/or the TCR-b polypeptide comprises a CDR2 having the amino acid sequence of SEQ ID NO:27 or having an amino acid sequence with at least 80% sequence identity to SEQ ID NO:27.

25 108. The TCR of any one of claims 105-107, wherein the CDR1, CDR2, and CDR3 of the TCR-a polypeptide comprise the amino acid sequence of SEQ ID NO:20, 21, and 22, respectively and wherein the CDR1, CDR3, and CDR3 of the TCR-b polypeptide comprise the amino acid sequence of SEQ ID NO:26, 27, and 28, respectively.

30 109. The TCR of any one of claims 105-108, wherein the TCR-a polypeptide comprises the amino acid sequence of SEQ ID NO:18 or an amino acid sequence with at least 70% sequence identity to SEQ ID NO:18 and the TCR-b polypeptide comprises the amino acid sequence of

SEQ ID NO:24 or an amino acid sequence with at least 70% sequence identity to SEQ ID NO:24.

110. An engineered T-cell Receptor (TCR) comprising a TCR-a polypeptide and a TCR-b polypeptide, wherein the TCR-a polypeptide comprises a CDR3 with the amino acid sequence of SEQ ID NO:36 or an amino acid sequence with at least 80% sequence identity to SEQ ID NO:36 and the TCR-b polypeptide comprises a CDR3 with the amino acid sequence of SEQ ID NO:42 or an amino acid sequence with at least 80% sequence identity to SEQ ID NO:42.

111. The TCR of claim 110, wherein the TCR comprises a TCR-b polypeptide comprising a variable region comprising CDR1, CDR2, and CDR3 and a TCR-a polypeptide comprising a variable region comprising CDR1, CDR2, and CDR3.

112. The TCR of claim 111, wherein the TCR-a polypeptide comprises a CDR1 having the amino acid sequence of SEQ ID NO:34 or having an amino acid sequence with at least 80% sequence identity to SEQ ID NO:34 and/or the TCR-b polypeptide comprises a CDR1 having the amino acid sequence of SEQ ID NO:40 or having an amino acid sequence with at least 80% sequence identity to SEQ ID NO:40.

113. The TCR of claim 111 or 112, wherein the TCR-a polypeptide comprises a CDR2 having the amino acid sequence of SEQ ID NO:35 or having an amino acid sequence with at least 80% sequence identity to SEQ ID NO:35 and/or the TCR-b polypeptide comprises a CDR2 having the amino acid sequence of SEQ ID NO:41 or having an amino acid sequence with at least 80% sequence identity to SEQ ID NO:41.

114. The TCR of any one of claims 111-113, wherein the CDR1, CDR2, and CDR3 of the TCR-a polypeptide comprise the amino acid sequence of SEQ ID NO:34, 35, and 36, respectively and wherein the CDR1, CDR3, and CDR3 of the TCR-b polypeptide comprise the amino acid sequence of SEQ ID NO:40, 41, and 42, respectively.

115. The TCR of any one of claims 111-114, wherein the TCR-a polypeptide comprises the amino acid sequence of SEQ ID NO:32 or an amino acid sequence with at least 70% sequence identity to SEQ ID NO:32 and the TCR-b polypeptide comprises the amino acid sequence of SEQ ID NO:38 or an amino acid sequence with at least 70% sequence identity to SEQ ID NO:38.

116. An engineered T-cell Receptor (TCR) comprising a TCR-a polypeptide and a TCR-b polypeptide, wherein the TCR-a polypeptide comprises a CDR3 with the amino acid sequence of SEQ ID NO:50 or an amino acid sequence with at least 80% sequence identity to SEQ ID NO:50 and the TCR-b polypeptide comprises a CDR3 with the amino acid sequence of SEQ ID NO:56 or an amino acid sequence with at least 80% sequence identity to SEQ ID NO:56.

117. The TCR of claim 116, wherein the TCR comprises a TCR-b polypeptide comprising a variable region comprising CDR1, CDR2, and CDR3 and a TCR-a polypeptide comprising a variable region comprising CDR1, CDR2, and CDR3.

118. The TCR of claim 117, wherein the TCR-a polypeptide comprises a CDR1 having the amino acid sequence of SEQ ID NO:48 or having an amino acid sequence with at least 80% sequence identity to SEQ ID NO:48 and/or the TCR-b polypeptide comprises a CDR1 having the amino acid sequence of SEQ ID NO:54 or having an amino acid sequence with at least 80% sequence identity to SEQ ID NO:54.

119. The TCR of claim 117 or 118, wherein the TCR-a polypeptide comprises a CDR2 having the amino acid sequence of SEQ ID NO:49 or having an amino acid sequence with at least 80% sequence identity to SEQ ID NO:49 and/or the TCR-b polypeptide comprises a CDR2 having the amino acid sequence of SEQ ID NO:55 or having an amino acid sequence with at least 80% sequence identity to SEQ ID NO:55.

120. The TCR of any one of claims 117-119, wherein the CDR1, CDR2, and CDR3 of the TCR-a polypeptide comprise the amino acid sequence of SEQ ID NO:48, 49, and 50, respectively and wherein the CDR1, CDR3, and CDR3 of the TCR-b polypeptide comprise the amino acid sequence of SEQ ID NO:54, 55, and 56, respectively.

121. The TCR of any one of claims 117-120, wherein the TCR-a polypeptide comprises the amino acid sequence of SEQ ID NO:46 or an amino acid sequence with at least 70% sequence identity to SEQ ID NO:46 and the TCR-b polypeptide comprises the amino acid sequence of SEQ ID NO:52 or an amino acid sequence with at least 70% sequence identity to SEQ ID NO:52.

122. An engineered T-cell Receptor (TCR) comprising a TCR-a polypeptide and a TCR-b polypeptide, wherein the TCR-a polypeptide comprises a CDR3 with the amino acid sequence of SEQ ID NO:64 or an amino acid sequence with at least 80% sequence identity to SEQ ID NO:64 and the TCR-b polypeptide comprises a CDR3 with the amino acid sequence of SEQ ID NO:70 or an amino acid sequence with at least 80% sequence identity to SEQ ID NO:70.

123. The TCR of claim 122, wherein the TCR comprises a TCR-b polypeptide comprising a variable region comprising CDR1, CDR2, and CDR3 and a TCR-a polypeptide comprising a variable region comprising CDR1, CDR2, and CDR3.

124. The TCR of claim 123, wherein the TCR-a polypeptide comprises a CDR1 having the amino acid sequence of SEQ ID NO:62 or having an amino acid sequence with at least 80% sequence identity to SEQ ID NO:62 and/or the TCR-b polypeptide comprises a CDR1 having

the amino acid sequence of SEQ ID NO:68 or having an amino acid sequence with at least 80% sequence identity to SEQ ID NO:68.

125. The TCR of claim 123 or 124, wherein the TCR-a polypeptide comprises a CDR2 having the amino acid sequence of SEQ ID NO:63 or having an amino acid sequence with at least 80% sequence identity to SEQ ID NO:63 and/or the TCR-b polypeptide comprises a CDR2 having the amino acid sequence of SEQ ID NO:69 or having an amino acid sequence with at least 80% sequence identity to SEQ ID NO:69.

126. The TCR of any one of claims 123-125, wherein the CDR1, CDR2, and CDR3 of the TCR-a polypeptide comprise the amino acid sequence of SEQ ID NO:62, 63, and 64, respectively and wherein the CDR1, CDR3, and CDR3 of the TCR-b polypeptide comprise the amino acid sequence of SEQ ID NO:68, 69, and 70, respectively.

127. The TCR of any one of claims 123-126, wherein the TCR-a polypeptide comprises the amino acid sequence of SEQ ID NO:60 or an amino acid sequence with at least 70% sequence identity to SEQ ID NO:60 and the TCR-b polypeptide comprises the amino acid sequence of SEQ ID NO:66 or an amino acid sequence with at least 70% sequence identity to SEQ ID NO:66.

128. An engineered T-cell Receptor (TCR) comprising a TCR-a polypeptide and a TCR-b polypeptide, wherein the TCR-a polypeptide comprises a CDR3 with the amino acid sequence of SEQ ID NO:78 or an amino acid sequence with at least 80% sequence identity to SEQ ID NO:78 and the TCR-b polypeptide comprises a CDR3 with the amino acid sequence of SEQ ID NO:84 or an amino acid sequence with at least 80% sequence identity to SEQ ID NO:84.

129. The TCR of claim 128, wherein the TCR comprises a TCR-b polypeptide comprising a variable region comprising CDR1, CDR2, and CDR3 and a TCR-a polypeptide comprising a variable region comprising CDR1, CDR2, and CDR3.

130. The TCR of claim 129, wherein the TCR-a polypeptide comprises a CDR1 having the amino acid sequence of SEQ ID NO:76 or having an amino acid sequence with at least 80% sequence identity to SEQ ID NO:76 and/or the TCR-b polypeptide comprises a CDR1 having the amino acid sequence of SEQ ID NO:82 or having an amino acid sequence with at least 80% sequence identity to SEQ ID NO:82.

131. The TCR of claim 129 or 130, wherein the TCR-a polypeptide comprises a CDR2 having the amino acid sequence of SEQ ID NO:77 or having an amino acid sequence with at least 80% sequence identity to SEQ ID NO:77 and/or the TCR-b polypeptide comprises a CDR2 having the amino acid sequence of SEQ ID NO:83 or having an amino acid sequence with at least 80% sequence identity to SEQ ID NO:83.

132. The TCR of any one of claims 129-131, wherein the CDR1, CDR2, and CDR3 of the TCR-a polypeptide comprise the amino acid sequence of SEQ ID NO:76, 77, and 78, respectively and wherein the CDR1, CDR3, and CDR3 of the TCR-b polypeptide comprise the amino acid sequence of SEQ ID NO:82, 83, and 84, respectively.

5 133. The TCR of any one of claims 129-132, wherein the TCR-a polypeptide comprises the amino acid sequence of SEQ ID NO:74 or an amino acid sequence with at least 70% sequence identity to SEQ ID NO:74 and the TCR-b polypeptide comprises the amino acid sequence of SEQ ID NO:80 or an amino acid sequence with at least 70% sequence identity to SEQ ID NO:80.

10 134. The TCR of any one of claims 98-133, wherein the TCR comprises a modification or is chimeric.

135. The TCR of any one of claims 98-134, wherein the TCR-b polypeptide and TCR-a polypeptide are operably linked.

15 136. The TCR of claim 135, wherein the TCR-b polypeptide and TCR-a polypeptide are operably linked through a peptide bond.

137. The TCR of claim 136, wherein the TCR-b polypeptide and TCR-a polypeptide are on the same polypeptide and wherein the TCR-b is amino-proximal to the TCR-a.

138. The TCR of claim 136, wherein the TCR-b polypeptide and TCR-a polypeptide are on the same polypeptide and wherein the TCR-a is amino-proximal to the TCR-b.

20 139. The TCR of any one of claims 98-138, wherein the TCR is a single-chain TCR.

140. A fusion protein comprising the TCR of any one of claims 98-139 and a CD3 binding region.

25 141. The fusion protein of claim 140, wherein the CD3 binding region comprises a CD3-specific fragment antigen binding (Fab), single chain variable fragment (scFv), single domain antibody, or single chain antibody.

142. The TCR of any one of claims 98-137 or the fusion protein of claim 140 or 141, wherein the TCR or fusion protein is conjugated to a detection or therapeutic agent.

143. The TCR or fusion protein of claim 142, wherein the agent comprises a fluorescent molecule, radiative molecule, or toxin.

30 144. A nucleic acid encoding a TCR-b polypeptide comprising a CDR3 having the amino acid sequence of SEQ ID NO:8 or an amino acid sequence with at least 80% sequence identity to SEQ ID NO:8 and/or a TCR-a polypeptide comprising a CDR3 having the amino acid sequence of SEQ ID NO:14 or an amino acid sequence with at least 80% sequence identity to SEQ ID NO:14.

145. The nucleic acid of claim 144, wherein the nucleic acid encodes for a TCR-b polypeptide comprising a CDR1, CDR2, and CDR3 and/or a TCR-a polypeptide comprising a CDR1, CDR2, and CDR3.

5 146. The nucleic acid of claim 145, wherein the nucleic acid encodes for a TCR-b comprising a CDR1 having the amino acid sequence of SEQ ID NO:6 or an amino acid sequence with at least 80% sequence identity to SEQ ID NO:6 and/or a TCR-a comprising a CDR1 having the amino acid sequence of SEQ ID NO:12 or an amino acid sequence with at least 80% sequence identity to SEQ ID NO:12.

10 147. The nucleic acid of claim 145 or 146, wherein the nucleic acid encodes for a TCR-b comprising a CDR2 having the amino acid sequence of SEQ ID NO:7 or an amino acid sequence with at least 80% sequence identity to SEQ ID NO:7 and/or a TCR-a comprising a CDR2 having the amino acid sequence of SEQ ID NO:13 or an amino acid sequence with at least 80% sequence identity to SEQ ID NO:13.

15 148. The nucleic acid of any one of claims 144-147, wherein the nucleic acid encodes a TCR-b variable region having the amino acid sequence of SEQ ID NO:4 or an amino acid sequence with at least 70% sequence identity to SEQ ID NO:4 and/or a TCR-a variable region having the amino acid sequence of SEQ ID NO:10 or an amino acid sequence with at least 70% sequence identity to SEQ ID NO:10.

20 149. The nucleic acid of claim 148, wherein the nucleic acid encodes a TCR-b polypeptide chain having the amino acid sequence of SEQ ID NO:3 or an amino acid sequence with at least 70% sequence identity to SEQ ID NO:3 and/or a TCR-a polypeptide chain having the amino acid sequence of SEQ ID NO:9 or an amino acid sequence with at least 70% sequence identity to SEQ ID NO:9.

25 150. The nucleic acid of claim 149, wherein the nucleic acid comprises SEQ ID NO:1 or a fragment thereof.

151. A nucleic acid encoding a TCR-a polypeptide comprising a CDR3 having the amino acid sequence of SEQ ID NO:22 or an amino acid sequence with at least 80% sequence identity to SEQ ID NO:22 and/or a TCR-b polypeptide comprising a CDR3 having the amino acid sequence of SEQ ID NO:28 or an amino acid sequence with at least 80% sequence identity to
30 SEQ ID NO:28.

152. The nucleic acid of claim 151, wherein the nucleic acid encodes for a TCR-a polypeptide comprising a CDR1, CDR2, and CDR3 and/or a TCR-b polypeptide comprising a CDR1, CDR2, and CDR3.

153. The nucleic acid of claim 152, wherein the nucleic acid encodes for a TCR-a comprising a CDR1 having the amino acid sequence of SEQ ID NO:20 or an amino acid sequence with at least 80% sequence identity to SEQ ID NO:20 and/or a TCR-b comprising a CDR1 having the amino acid sequence of SEQ ID NO:26 or an amino acid sequence with at least 80% sequence identity to SEQ ID NO:26.

154. The nucleic acid of claim 152 or 153, wherein the nucleic acid encodes for a TCR-a comprising a CDR2 having the amino acid sequence of SEQ ID NO:21 or an amino acid sequence with at least 80% sequence identity to SEQ ID NO:21 and/or a TCR-b comprising a CDR2 having the amino acid sequence of SEQ ID NO:27 or an amino acid sequence with at least 80% sequence identity to SEQ ID NO:27.

155. The nucleic acid of any one of claims 151-154, wherein the nucleic acid encodes a TCR-a variable region having the amino acid sequence of SEQ ID NO:18 or an amino acid sequence with at least 70% sequence identity to SEQ ID NO:18 and/or a TCR-b variable region having the amino acid sequence of SEQ ID NO:24 or an amino acid sequence with at least 70% sequence identity to SEQ ID NO:24.

156. The nucleic acid of claim 155, wherein the nucleic acid encodes a TCR-a polypeptide chain having the amino acid sequence of SEQ ID NO:17 or an amino acid sequence with at least 70% sequence identity to SEQ ID NO:17 and/or a TCR-b polypeptide chain having the amino acid sequence of SEQ ID NO:23 or an amino acid sequence with at least 70% sequence identity to SEQ ID NO:23.

157. The nucleic acid of claim 156, wherein the nucleic acid comprises SEQ ID NO:15 or a fragment thereof.

158. A nucleic acid encoding a TCR-a polypeptide comprising a CDR3 having the amino acid sequence of SEQ ID NO:36 or an amino acid sequence with at least 80% sequence identity to SEQ ID NO:36 and/or a TCR-b polypeptide comprising a CDR3 having the amino acid sequence of SEQ ID NO:42 or an amino acid sequence with at least 80% sequence identity to SEQ ID NO:42.

159. The nucleic acid of claim 158, wherein the nucleic acid encodes for a TCR-a polypeptide comprising a CDR1, CDR2, and CDR3 and/or a TCR-b polypeptide comprising a CDR1, CDR2, and CDR3.

160. The nucleic acid of claim 159, wherein the nucleic acid encodes for a TCR-a comprising a CDR1 having the amino acid sequence of SEQ ID NO:34 or an amino acid sequence with at least 80% sequence identity to SEQ ID NO:34 and/or a TCR-b comprising a CDR1 having the

amino acid sequence of SEQ ID NO:40 or an amino acid sequence with at least 80% sequence identity to SEQ ID NO:40.

161. The nucleic acid of claim 159 or 160, wherein the nucleic acid encodes for a TCR-a comprising a CDR2 having the amino acid sequence of SEQ ID NO:35 or an amino acid sequence with at least 80% sequence identity to SEQ ID NO:35 and/or a TCR-b comprising a CDR2 having the amino acid sequence of SEQ ID NO:41 or an amino acid sequence with at least 80% sequence identity to SEQ ID NO:41.

162. The nucleic acid of any one of claims 158-161, wherein the nucleic acid encodes a TCR-a variable region having the amino acid sequence of SEQ ID NO:32 or an amino acid sequence with at least 70% sequence identity to SEQ ID NO:32 and/or a TCR-b variable region having the amino acid sequence of SEQ ID NO:38 or an amino acid sequence with at least 70% sequence identity to SEQ ID NO:38.

163. The nucleic acid of claim 162, wherein the nucleic acid encodes a TCR-a polypeptide chain having the amino acid sequence of SEQ ID NO:31 or an amino acid sequence with at least 70% sequence identity to SEQ ID NO:31 and/or a TCR-b polypeptide chain having the amino acid sequence of SEQ ID NO:37 or an amino acid sequence with at least 70% sequence identity to SEQ ID NO:37.

164. The nucleic acid of claim 163, wherein the nucleic acid comprises SEQ ID NO:29 or a fragment thereof.

165. A nucleic acid encoding a TCR-a polypeptide comprising a CDR3 having the amino acid sequence of SEQ ID NO:50 or an amino acid sequence with at least 80% sequence identity to SEQ ID NO:50 and/or a TCR-b polypeptide comprising a CDR3 having the amino acid sequence of SEQ ID NO:56 or an amino acid sequence with at least 80% sequence identity to SEQ ID NO:56.

166. The nucleic acid of claim 165, wherein the nucleic acid encodes for a TCR-a polypeptide comprising a CDR1, CDR2, and CDR3 and/or a TCR-b polypeptide comprising a CDR1, CDR2, and CDR3.

167. The nucleic acid of claim 166, wherein the nucleic acid encodes for a TCR-a comprising a CDR1 having the amino acid sequence of SEQ ID NO:48 or an amino acid sequence with at least 80% sequence identity to SEQ ID NO:48 and/or a TCR-b comprising a CDR1 having the amino acid sequence of SEQ ID NO:54 or an amino acid sequence with at least 80% sequence identity to SEQ ID NO:54.

168. The nucleic acid of claim 166 or 167, wherein the nucleic acid encodes for a TCR-a comprising a CDR2 having the amino acid sequence of SEQ ID NO:49 or an amino acid

sequence with at least 80% sequence identity to SEQ ID NO:49 and/or a TCR-b comprising a CDR2 having the amino acid sequence of SEQ ID NO:55 or an amino acid sequence with at least 80% sequence identity to SEQ ID NO:55.

169. The nucleic acid of any one of claims 165-168, wherein the nucleic acid encodes a TCR-a variable region having the amino acid sequence of SEQ ID NO:46 or an amino acid sequence with at least 70% sequence identity to SEQ ID NO:46 and/or a TCR-b variable region having the amino acid sequence of SEQ ID NO:52 or an amino acid sequence with at least 70% sequence identity to SEQ ID NO:52.

170. The nucleic acid of claim 169, wherein the nucleic acid encodes a TCR-a polypeptide chain having the amino acid sequence of SEQ ID NO:45 or an amino acid sequence with at least 70% sequence identity to SEQ ID NO:45 and/or a TCR-b polypeptide chain having the amino acid sequence of SEQ ID NO:51 or an amino acid sequence with at least 70% sequence identity to SEQ ID NO:51.

171. The nucleic acid of claim 170, wherein the nucleic acid comprises SEQ ID NO:43 or a fragment thereof.

172. A nucleic acid encoding a TCR-a polypeptide comprising a CDR3 having the amino acid sequence of SEQ ID NO:64 or an amino acid sequence with at least 80% sequence identity to SEQ ID NO:64 and/or a TCR-b polypeptide comprising a CDR3 having the amino acid sequence of SEQ ID NO:70 or an amino acid sequence with at least 80% sequence identity to SEQ ID NO:70.

173. The nucleic acid of claim 172, wherein the nucleic acid encodes for a TCR-a polypeptide comprising a CDR1, CDR2, and CDR3 and/or a TCR-b polypeptide comprising a CDR1, CDR2, and CDR3.

174. The nucleic acid of claim 173, wherein the nucleic acid encodes for a TCR-a comprising a CDR1 having the amino acid sequence of SEQ ID NO:62 or an amino acid sequence with at least 80% sequence identity to SEQ ID NO:62 and/or a TCR-b comprising a CDR1 having the amino acid sequence of SEQ ID NO:68 or an amino acid sequence with at least 80% sequence identity to SEQ ID NO:68.

175. The nucleic acid of claim 173 or 174, wherein the nucleic acid encodes for a TCR-a comprising a CDR2 having the amino acid sequence of SEQ ID NO:63 or an amino acid sequence with at least 80% sequence identity to SEQ ID NO:63 and/or a TCR-b comprising a CDR2 having the amino acid sequence of SEQ ID NO:69 or an amino acid sequence with at least 80% sequence identity to SEQ ID NO:69.

176. The nucleic acid of any one of claims 172-175, wherein the nucleic acid encodes a TCR-a variable region having the amino acid sequence of SEQ ID NO:60 or an amino acid sequence with at least 70% sequence identity to SEQ ID NO:60 and/or a TCR-b variable region having the amino acid sequence of SEQ ID NO:66 or an amino acid sequence with at least 70% sequence identity to SEQ ID NO:66.

177. The nucleic acid of claim 176, wherein the nucleic acid encodes a TCR-a polypeptide chain having the amino acid sequence of SEQ ID NO:59 or an amino acid sequence with at least 70% sequence identity to SEQ ID NO:59 and/or a TCR-b polypeptide chain having the amino acid sequence of SEQ ID NO:65 or an amino acid sequence with at least 70% sequence identity to SEQ ID NO:65.

178. The nucleic acid of claim 177, wherein the nucleic acid comprises SEQ ID NO:57 or a fragment thereof.

179. A nucleic acid encoding a TCR-a polypeptide comprising a CDR3 having the amino acid sequence of SEQ ID NO:78 or an amino acid sequence with at least 80% sequence identity to SEQ ID NO:78 and/or a TCR-b polypeptide comprising a CDR3 having the amino acid sequence of SEQ ID NO:84 or an amino acid sequence with at least 80% sequence identity to SEQ ID NO:84.

180. The nucleic acid of claim 179, wherein the nucleic acid encodes for a TCR-a polypeptide comprising a CDR1, CDR2, and CDR3 and/or a TCR-b polypeptide comprising a CDR1, CDR2, and CDR3.

181. The nucleic acid of claim 180, wherein the nucleic acid encodes for a TCR-a comprising a CDR1 having the amino acid sequence of SEQ ID NO:76 or an amino acid sequence with at least 80% sequence identity to SEQ ID NO:76 and/or a TCR-b comprising a CDR1 having the amino acid sequence of SEQ ID NO:82 or an amino acid sequence with at least 80% sequence identity to SEQ ID NO:82.

182. The nucleic acid of claim 180 or 181, wherein the nucleic acid encodes for a TCR-a comprising a CDR2 having the amino acid sequence of SEQ ID NO:77 or an amino acid sequence with at least 80% sequence identity to SEQ ID NO:77 and/or a TCR-b comprising a CDR2 having the amino acid sequence of SEQ ID NO:83 or an amino acid sequence with at least 80% sequence identity to SEQ ID NO:83.

183. The nucleic acid of any one of claims 179-182, wherein the nucleic acid encodes a TCR-a variable region having the amino acid sequence of SEQ ID NO:74 or an amino acid sequence with at least 70% sequence identity to SEQ ID NO:74 and/or a TCR-b variable region

having the amino acid sequence of SEQ ID NO:80 or an amino acid sequence with at least 70% sequence identity to SEQ ID NO:80.

184. The nucleic acid of claim 183, wherein the nucleic acid encodes a TCR-a polypeptide chain having the amino acid sequence of SEQ ID NO:73 or an amino acid sequence with at least 70% sequence identity to SEQ ID NO:73 and/or a TCR-b polypeptide chain having the amino acid sequence of SEQ ID NO:79 or an amino acid sequence with at least 70% sequence identity to SEQ ID NO:79.

185. The nucleic acid of claim 184, wherein the nucleic acid comprises SEQ ID NO:71 or a fragment thereof.

186. The nucleic acid of any one of claims 144-185, wherein the nucleic acid comprises a TCR-a (TRA) and TCR-b (TRB) gene.

187. The nucleic acid of any one of claims 144-186, wherein the nucleic acid is polycistronic.

188. The nucleic acid of claim 187, wherein the nucleic acid comprises an internal ribosome entry site (IRES) or a 2A cleavable linker.

189. The nucleic acid(s) of any one of claims 144-188, wherein the nucleic acid comprises a cDNA encoding the TCR-a and/or TCR-b genes.

190. The nucleic acid(s) of any one of claims 144-189, wherein the nucleic acid further encodes for a polypeptide comprising a CD3 binding region.

191. The nucleic acid of claim 190, wherein the CD3 binding region comprises a CD3-specific fragment antigen binding (Fab), single chain variable fragment (scFv), single domain antibody, or single chain antibody.

192. A nucleic acid vector comprising the nucleic acid(s) of any one of claims 97 or 144-191.

193. The vector of claim 192, wherein the vector comprises a promoter that directs the expression of the nucleic acid.

194. The vector of claim 193, wherein the promoter comprises a murine stem cell virus (MSCV) promoter.

195. The vector of any one of claims 192-194, wherein the vector comprises the TCR-a and TCR-b genes.

196. A cell comprising the polypeptide of any one of claims 1-96, TCR of any one of claims 98-139, 142, or 143, the fusion protein of any one of claims 140-143, the nucleic acid(s) of any one of claims 97 or 144-191, or the vector of any one of claims 192-195.

197. The cell of claim 196, wherein the cell comprises a stem cell, a progenitor cell, an immune cell, or a natural killer (NK) cell.

198. The cell of claim 197, wherein the cell comprises a hematopoietic stem or progenitor cell, a T cell, a cell differentiated from mesenchymal stem cells (MSCs) or an induced pluripotent stem cell (iPSC).

199. The cell of claim 197 or 198, wherein the cell is isolated or derived from peripheral blood mononuclear cell (PBMCs).

200. The cell of claim 198 or 199, wherein the T cell comprises a cytotoxic T lymphocyte (CTL), a CD8⁺ T cell, a CD4⁺ T cell, an invariant NK T (iNKT) cell, a gamma-delta T cell, a NKT cell, or a regulatory T cell.

201. The cell of any one of claims 196-200, wherein the cell is isolated from a cancer patient.

202. A composition comprising the polypeptide of any one of claims 1-96, TCR of any one of claims 98-139, 142, or 143, the fusion protein of any one of claims 140-143, the nucleic acid(s) of any one of claims 97 or 144-191, or the vector of any one of claims 192-195, or the cell of any one of claims 196-201.

203. The composition of claim 202, wherein the composition has been determined to be serum-free, mycoplasma-free, endotoxin-free, and/or sterile.

204. A method of making an engineered cell comprising transferring the nucleic acid of any one of claims 97 or 144-191 or the vector of any one of claims 192-195 into a cell.

205. The method of claim 204, wherein the method further comprises culturing the cell in media, incubating the cell at conditions that allow for the division of the cell, screening the cell, and/or freezing the cell.

206. A method for treating cancer in a subject comprising administering the composition of claim 202 or 203 or the cells of any one of claims 196-201 to a subject in need thereof.

207. The method of claim 206, wherein the cancer comprises lung cancer.

208. The method of claim 206 or 207, wherein the subject has previously been treated for the cancer.

209. The method of claim 208, wherein the subject has been determined to be resistant to the previous treatment.

210. The method of any one of claims 206-209, wherein the method further comprises the administration of an additional therapy.

211. The method of any one of claims 206-210, wherein the cancer comprises stage I, II, III, or IV cancer.

212. The method of any one of claims 206-211, wherein the cancer comprises metastatic and/or recurrent cancer.

213. The method of any one of claims 206-212, wherein the subject is and/or has been determined to be HLA-A31 or HLA-A33 positive.

5 214. The method of any one of claims 206-213, wherein the subject harbors an EGFR L858R mutation.

215. The method of any one of claims 206-214, wherein the cancer comprises an EGFR L858R positive cancer.

10 216. The method of any one of claims 206-215, wherein the lung cancer comprises lung adenocarcinoma, non-small cell lung carcinoma, squamous cell lung carcinoma, or small cell lung carcinoma.

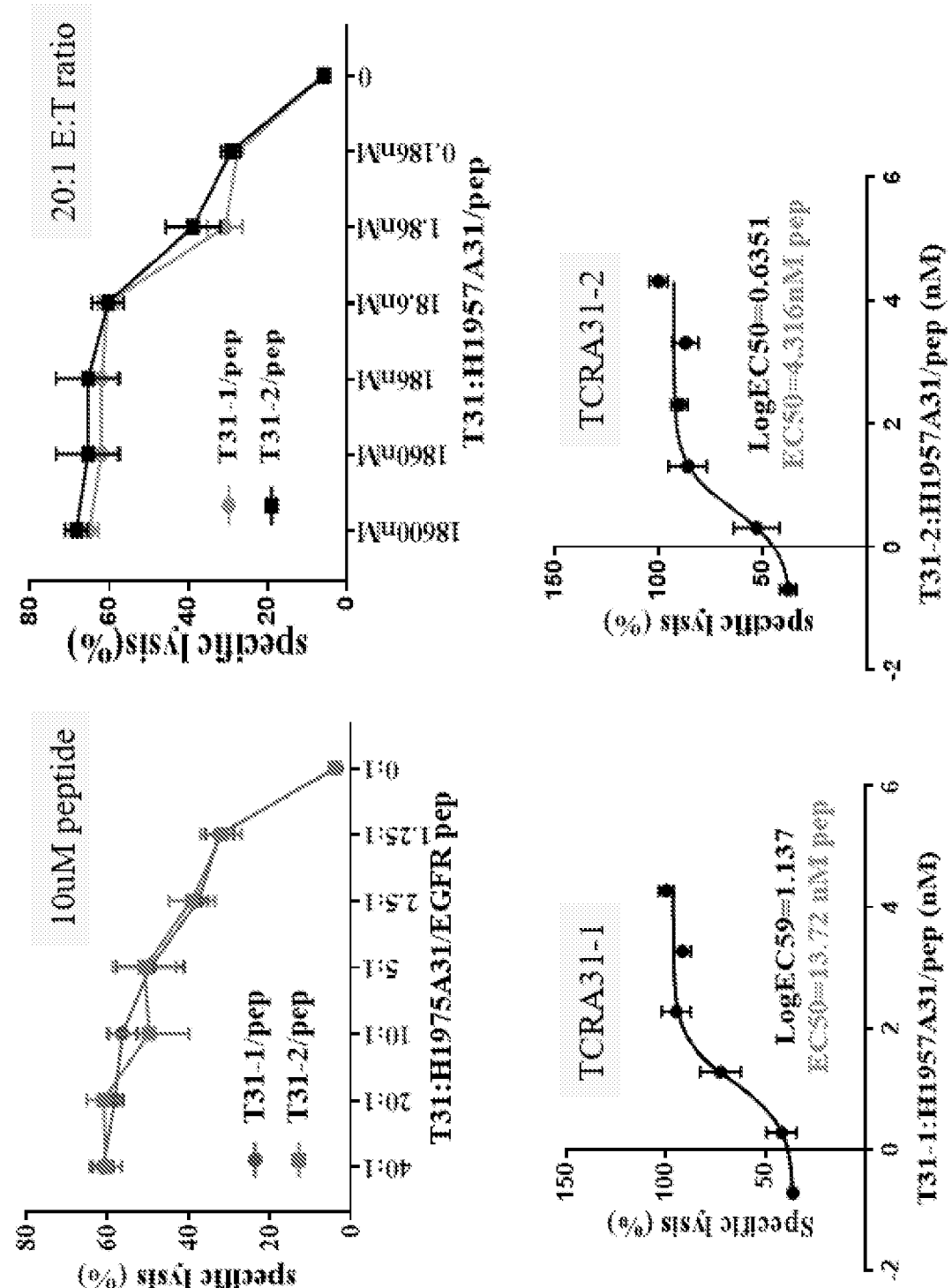
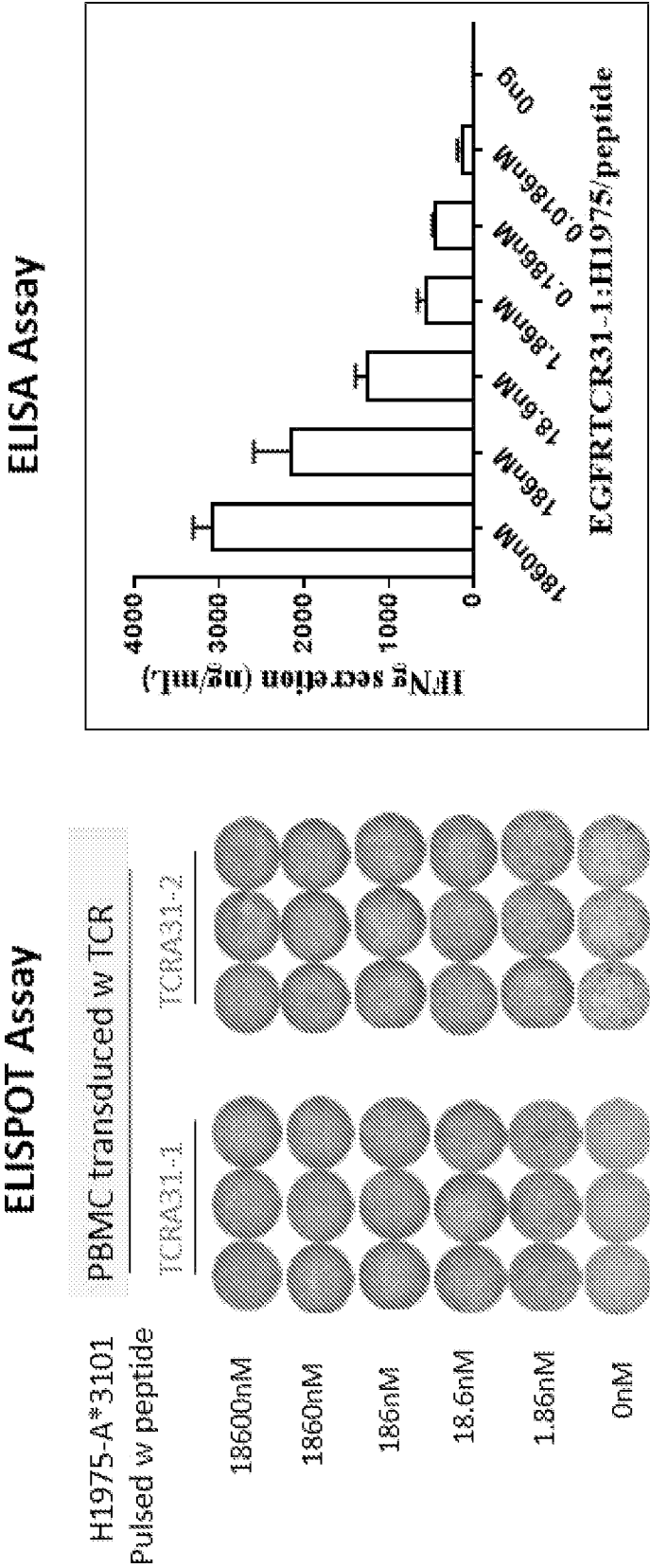


FIG. 1



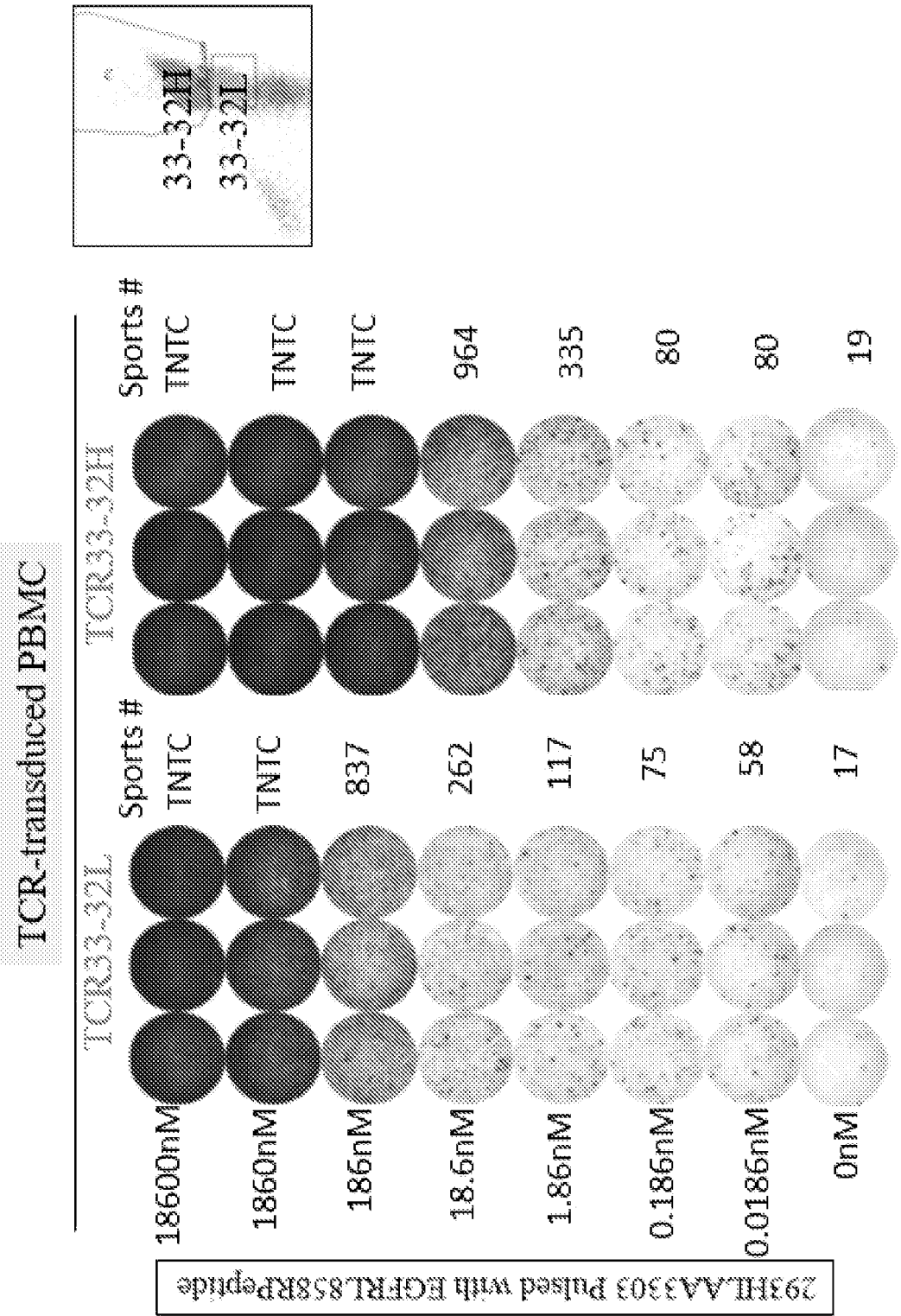


FIG. 3

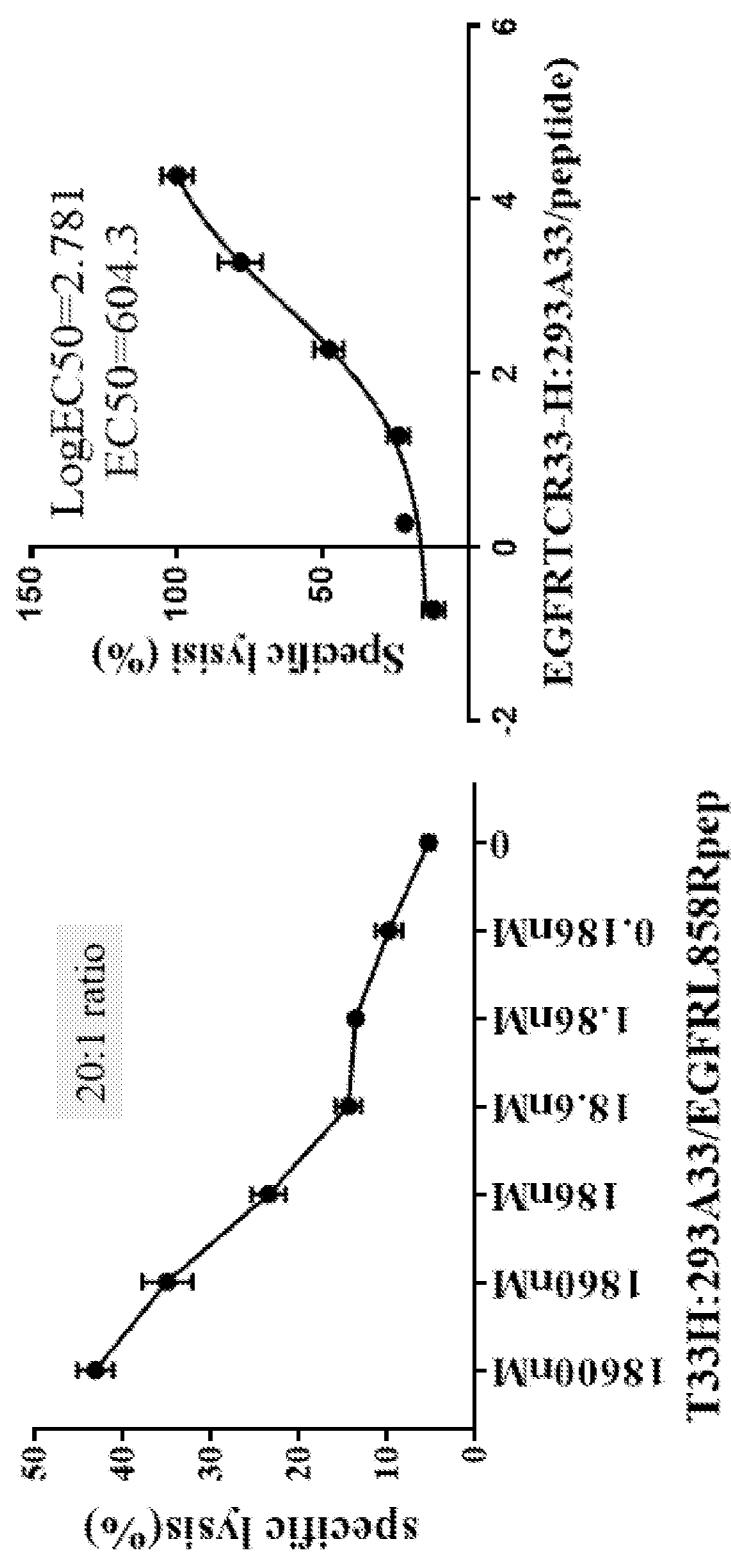


FIG. 4

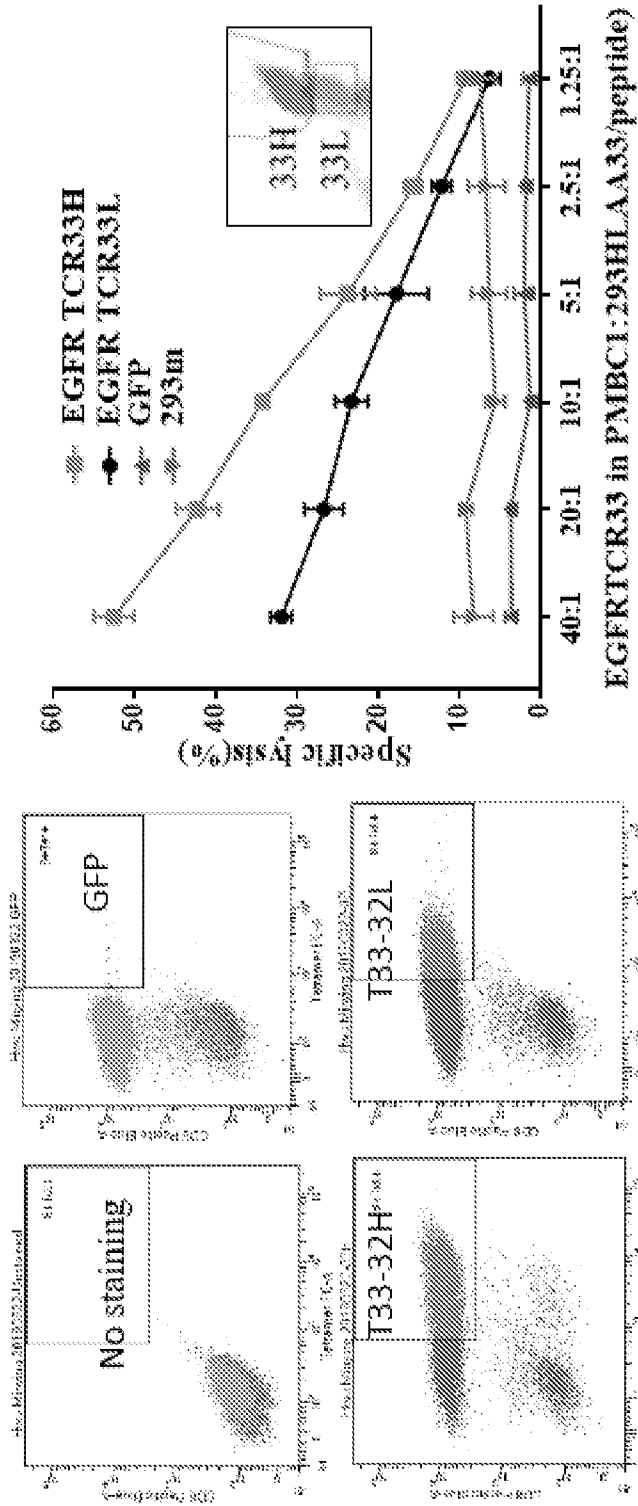


FIG. 5

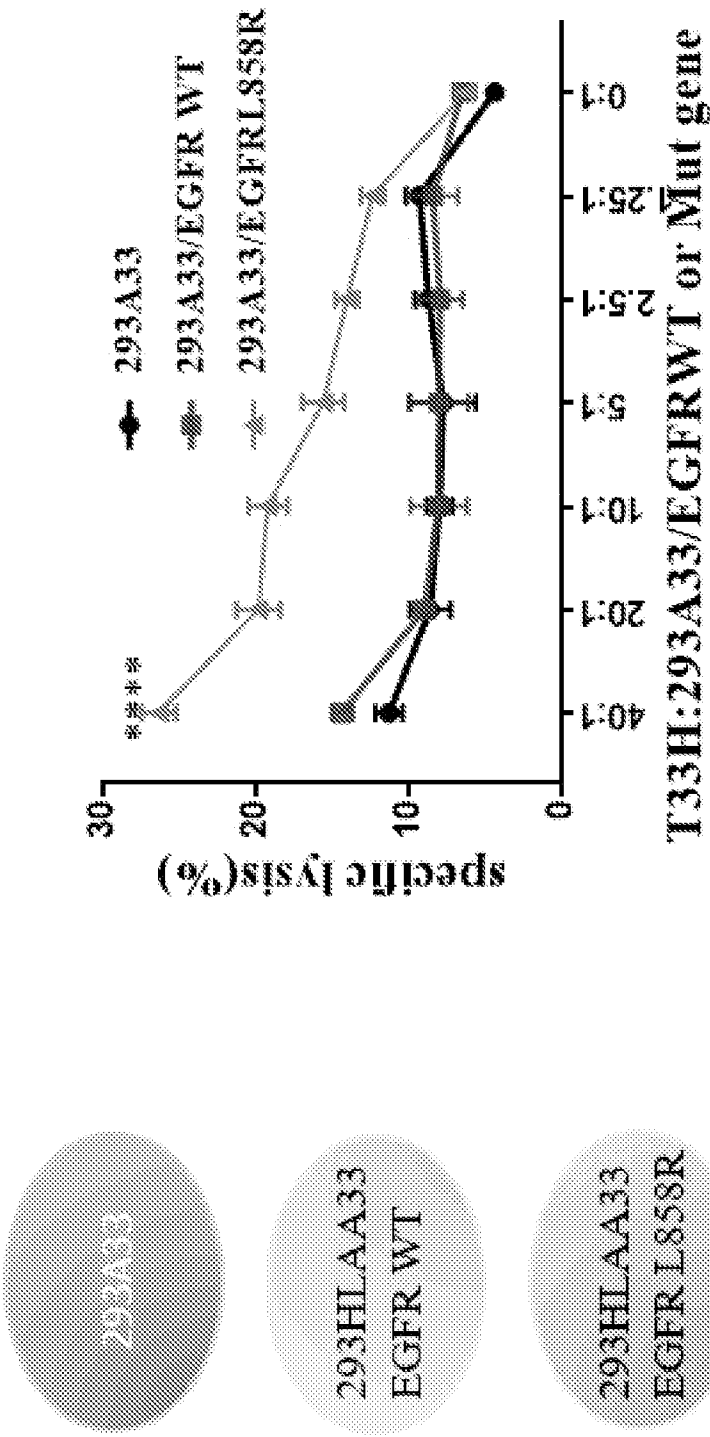


FIG. 6

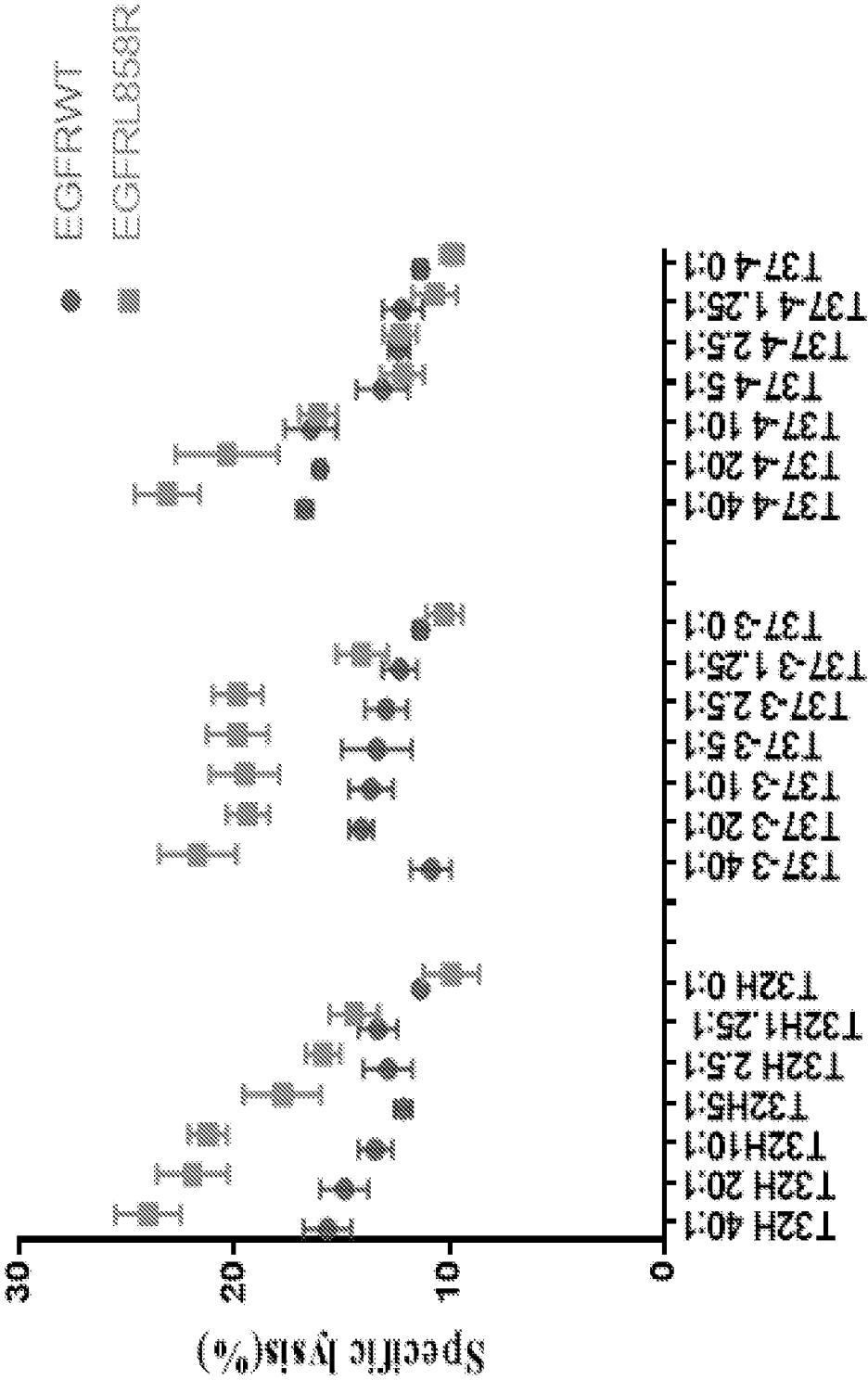


FIG. 7

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2021/058019

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 35/17; A61K 39/395; C07K 14/705 (2022.01)

CPC - A61K 35/17; C07K 14/7051; C07K 2317/565; C07K 2319/03; C07K 2319/33 (2022.02)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

see Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

see Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

see Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2019/036688 A1 (GRITSTONE ONCOLOGY INC.) 21 February 2019 (21.02.2019) entire document	1-4, 144-147
A	US 2019/0225692 A1 (JUNO THERAPEUTICS INC.) 25 July 2019 (25.07.2019) entire document	1-4, 144-147
A	US 2020/0281973 A1 (NOVARTIS AG et al) 10 September 2020 (10.09.2020) entire document	1-4, 144-147
A	US 2016/0289760 A1 (REPertoire GENESIS INCORPORATION) 06 October 2016 (06.10.2016) entire document	1-4, 144-147

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"D" document cited by the applicant in the international application

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

28 February 2022

Date of mailing of the international search report

MAR 09 2022

Name and mailing address of the ISA/US

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2021/058019

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

a. ☒ forming part of the international application as filed:

☒ in the form of an Annex C/ST.25 text file.

☐ on paper or in the form of an image file.

b. ☐ furnished together with the international application under PCT Rule 13ter.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

☐ in the form of an Annex C/ST.25 text file (Rule 13ter.1(a)).

☐ on paper or in the form of an image file (Rule 13ter.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

SEQ ID NOs: 4, 6-8, 10, and 12-14 were searched.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2021/058019

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Claims 5-8, 13-16, 21-24, 29-32, 37-40, 45-48, 53-56, 61-64, 69-72, 77-80, 85-88, 93-97, 102, 103, 108, 109, 114, 115, 120, 121, 126, 127, 132-143, 148-150, 155-157, 162-164, 169-171, 176-178, and 183-216 +

3. ☒ Claims Nos.: See claims above
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See extra sheet(s).

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-4, 144-147

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2021/058019

Continued from Box No. III Observations where unity of invention is lacking

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees need to be paid.

Group I+: claims 1-4, 9-12, 17-20, 25-28, 33-36, 41-44, 49-52, 57-60, 65-68, 73-76, 81-84, 89-92, 98-101, 104-107, 110-113, 116-119, 122-125, 128-131, 144-147, 151-154, 158-161, 165-168, 172-175, and 179-182 are drawn to polypeptide comprising an antigen binding variable regions, and engineered T-cell Receptors (TCRs) comprising the same, and nucleic acids encoding the same.

The first invention of Group I+ is restricted to a polypeptide comprising an antigen binding variable region, where the variable region is selected to be SEQ ID NO:4 which further comprises CDR1 (SEQ ID NO:6, CDR2 (SEQ ID NO:7), CDR3 (SEQ ID NO:8), and engineered T-cell Receptors (TCRs) comprising the same, and nucleic acids encoding the same. It is believed that claims 1-4 and 144-147 read on this first named invention and thus these claims will be searched without fee to the extent that they read on SEQ ID NOs:4, 6, 7, and 8.

Applicant is invited to elect additional variable region polypeptides, and their respective corresponding SEQ ID NO to be searched in a specific combination by paying additional fee for each set of election. An exemplary election would be a polypeptide comprising an antigen binding variable region, where the variable region is selected to be SEQ ID NO:10 CDR1 (SEQ ID NO:12, CDR2 (SEQ ID NO:13), CDR3 (SEQ ID NO:14), and engineered T-cell Receptors (TCRs) comprising the same, and nucleic acids encoding the same. Additional variable region polypeptides, and their respective corresponding SEQ ID NO will be searched upon the payment of additional fees. Applicants must specify the claims that read on any additional elected inventions. Applicants must further indicate, if applicable, the claims which read on the first named invention if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined.

The inventions listed in Groups I+ do not relate to a single general inventive concept under PCT Rule 13.1, because under PCT Rule 13.2 they lack the same or corresponding special technical features for the following reasons:

The Groups I+ formulas do not share a significant structural element responsible for CD3 binding, requiring the selection of alternative antigen binding variable regions where "a polypeptide comprising an antigen binding variable region comprising a CDR3 comprising the amino acid sequence of SEQ ID NO:8 or an amino acid sequence with at least 80% sequence identity to SEQ ID NO:8," and "a polypeptide comprising an antigen binding variable region comprising a CDR3 comprising the amino acid sequence of SEQ ID NO: 14 or an amino acid sequence with at least 80% sequence identity to SEQ ID NO:14" and "a polypeptide comprising an antigen binding variable region comprising a CDR3 comprising the amino acid sequence of SEQ ID NO:22 or an amino acid sequence with at least 80% sequence identity to SEQ ID NO:22" and "a polypeptide comprising an antigen binding variable region comprising a CDR3 comprising the amino acid sequence of SEQ ID NO:28 or an amino acid sequence with at least 80% sequence identity to SEQ ID NO:28."

Additionally, even if Groups I+ were considered to share the technical features of a polypeptide comprising an antigen binding variable region comprising a CDR3; an engineered T-cell Receptor (TCR) comprising a TCR-b polypeptide and a TCR-a polypeptide, wherein the TCR-b polypeptide comprises a CDR3; a nucleic acid encoding a TCR-b polypeptide comprising a CDR3; a nucleic acid encoding a TCR-a polypeptide comprising a CDR3. However, these shared technical features do not represent a contribution over the prior art.

Specifically, US 2020/0281973 A1 to Novartis Ag et al. discloses a polypeptide (CAR includes a polypeptide, Para. [0011]) comprising an antigen binding variable region comprising a CDR3 (chimeric antigen receptor that includes an antigen binding domain... a heavy chain complementary determining region 3 (HC CDR3), Para. [0016]); an engineered T-cell Receptor (TCR) (an immune effector cell (e.g., T cell) engineered to express a first chimeric antigen receptor (CAR), Para. [0076]) comprising a TCR-b polypeptide and a TCR-a polypeptide (the variable domain of a TCR alpha chain, Para. [0407]), wherein the TCR-b polypeptide (variable domain of a TCR beta chain, Para. [0407]) comprises CDR3 (chimeric antigen receptor that includes an antigen binding domain... a heavy chain complementary determining region 3 (HC CDR3), Para. [0016]), and the TCR-a polypeptide comprises CDR3 (chimeric antigen receptor that includes an antigen binding domain... a heavy chain complementary determining region 3 (HC CDR3), Para. [0016]) a nucleic acid encoding a TCR-b polypeptide and a TCR-a polypeptide (nucleic acid construct for bicistronic expression of a B-cell antigen CAR, Para. [0074]; the variable domain of a TCR alpha chain ... variable domain of a TCR beta chain, Para. [0407]), wherein the TCR-b polypeptide comprises CDR3, and the TCR-a polypeptide comprises CDR3 (chimeric antigen receptor that includes an antigen binding domain... a heavy chain complementary determining region 3 (HC CDR3), Para. [0016]).

The inventions listed in Groups I+ therefore lack unity under Rule 13 because they do not share a same or corresponding special technical features.