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(54) Title: ENGINEERED T CELL RECEPTORS 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 cancer testis (CT) antigens of the MAGE-A4 antigen. Accordingly, aspects of the disclosure relate to an engineered T-cell Receptors (TCRs), nucleic acids encoding the TCRs, and cells comprising the nucleic acids and TCRs. Also provided are compositions comprising the cells, nucleic acids, or engineered TCRs of the disclosure, methods of making the cells and methods of using the embodiments of the disclosure for therapeutic treatments.



**ENGINEERED T CELL RECEPTORS AND METHODS OF USE****BACKGROUND OF THE INVENTION**

[0001] This application is claims benefit of priority of U.S. Provisional Application No. 5 63/026,445, filed May 18, 2020, which is hereby incorporated by reference in its entirety.

**I. Field of the Invention**

[0002] This invention relates to the field of cancer therapy.

**II. Background**

[0003] Adoptive T-cell therapy is one potentially powerful treatment for cancer that 10 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 15 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 20 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 25 class II MHC protein is also expressed from 3 gene regions: HLA-DR, HLA-DP, and HLA-DQ.) There is a need for the engineering of TCRs that are directed to cancer-specific antigens and useful for the treatment of cancer.

**SUMMARY OF THE INVENTION**

[0004] This disclosure provides for engineered T cell Receptors (TCRs), cells comprising 30 the TCRs, and methods of making and using the TCRs. The current disclosure relates to TCRs that specifically recognize cancer testis (CT) antigen of the MAGE-A4 (MA4-230,

GVYDGREHTV (SEQ ID NO:35)) and (MA4-286, KVLEHVVRV (SEQ ID NO:36)). Accordingly, aspects of the disclosure relate to a polypeptide comprising a TCR alpha (TCR-a) variable region comprising a CDR3 comprising an amino acid sequence with at least 80% sequence identity to LVVEGNRDDKII (SEQ ID NO:9). Other aspects relate to a polypeptide comprising a TCR beta (TCR-b) variable region comprising a CDR3 comprising an amino acid sequence with at least 80% sequence identity to ASPPTPSSYNEQF (SEQ ID NO:16). In other polypeptide aspects, the disclosure relates to a polypeptide comprising a TCR alpha (TCR-a) variable region comprising a CDR3 comprising an amino acid sequence with at least 80% sequence identity to AVGSMDSNYQLI (SEQ ID NO:25). Further aspects relate to a polypeptide comprising a TCR beta (TCR-b) variable region comprising a CDR3 comprising an amino acid sequence with at least 80% sequence identity to ASSQERTWPYNEQF (SEQ ID NO:30).

**[0005]** The disclosure also provides for T-cell receptors (TCR) and engineered TCRs, such as a TCR comprising a TCR-a polypeptide and a TCR-b polypeptide, wherein the TCR-a polypeptide comprises a CDR3 comprising an amino acid sequence with at least 80% sequence identity to LVVEGNRDDKII (SEQ ID NO:9) and the TCR-b polypeptide comprises a CDR3 comprising an amino acid sequence with at least 80% sequence identity to ASPPTPSSYNEQF (SEQ ID NO:16). A further aspect relates to an engineered TCR comprising a TCR-a polypeptide and a TCR-b polypeptide, wherein the TCR-a polypeptide comprises a CDR3 comprising an amino acid sequence with at least 80% sequence identity to AVGSMDSNYQLI (SEQ ID NO:25) and the TCR-b polypeptide comprises a CDR3 comprising an amino acid sequence with at least 80% sequence identity to ASSQERTWPYNEQF (SEQ ID NO:30).

**[0006]** 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. The disclosure also relates to a method for reducing tumor size or volume in a subject comprising administering a polypeptide, composition, cell, nucleic acid, or engineered TCR to a subject in need thereof. Methods of the disclosure also relate to stimulating an immune response against cancer cells in a subject by administering a polypeptide, composition, cell, nucleic acid, or

engineered TCR to a subject in need thereof. Aspects of the disclosure provide for treating or preventing a cancer in a subject by administering a polypeptide, composition, cell, nucleic acid, or engineered TCR to a subject in need thereof. The polypeptide, composition, cell, nucleic acid, or engineered TCR of the disclosure may also be used in a method for increasing overall survival, reducing rate of recurrence, and/or increasing recurrence free survival in a subject having cancer.

**[0007]** 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.

**[0008]** In some aspects, the polypeptide of the disclosure or the TCR-a polypeptide comprises a CDR 3 comprising an amino acid sequence with 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 LVVEG NRDDKII (SEQ ID NO:9). In some aspects, the TCR-a polypeptide comprises a CDR 3 comprising an amino acid sequence of SEQ ID NO:9.

In some aspects, the polypeptide of the disclosure or the TCR-b polypeptide comprises a CDR3 comprising an amino acid sequence with 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 ASPPTPSSYNEQF (SEQ ID NO:16). In some aspects, the polypeptide of the disclosure or the TCR-b polypeptide comprises a CDR3 comprising an amino acid

sequence of SEQ ID NO:16. In some aspects, the engineered TCR comprises a CDR 3 comprising an amino acid sequence with 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 LVVEG NRDDKII (SEQ ID NO:9) and a TCR-b polypeptide comprising

a CDR3 comprising an amino acid sequence with 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 ASPPTPSSYNEQF (SEQ ID NO:16). In some aspects, the engineered TCR comprises a CDR 3 comprising an amino acid of SEQ ID NO:9 and a TCR-b polypeptide comprising a CDR3 comprising an amino acid sequence of SEQ ID NO:16. In some aspects, the polypeptide of the disclosure or the TCR-a polypeptide comprises a CDR 3

comprising an amino acid sequence with 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 AVGSMDSNYQLI (SEQ ID NO:25). In some aspects, the polypeptide of the disclosure or the TCR-a polypeptide comprises a CDR 3 comprising the amino acid sequence of SEQ ID NO:25. In some aspects, the polypeptide of the disclosure or the TCR-b polypeptide comprises a CDR3 comprising an amino acid sequence with 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 ASSQERTWPYNEQF (SEQ ID NO:30). In some aspects, the polypeptide of the disclosure or the TCR-b polypeptide comprises a CDR3 comprising the amino acid sequence of SEQ ID NO:30. In some aspects, the engineered TCR comprises a TCR-a polypeptide comprising a CDR 3 comprising an amino acid sequence with 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 AVGSMDSNYQLI (SEQ ID NO:25) and a TCR-b polypeptide comprising a CDR3 comprising an amino acid sequence with 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 ASSQERTWPYNEQF (SEQ ID NO:30). In some aspects, the engineered TCR comprises a TCR-a polypeptide comprising a CDR 3 comprising an amino acid sequence of SEQ ID NO:25 and a TCR-b polypeptide comprising a CDR3 comprising the amino acid sequence of SEQ ID NO:30.

**[0009]** In some aspects, the polypeptide comprises a variable region comprising a CDR1, CDR2, and CDR3 from a TCR-a polypeptide or a TCR-b polypeptide. In some aspects, the TCR-a variable region comprises a CDR1 with at least 80% sequence identity to NIATNDY (SEQ ID NO:7). In some aspects, the TCR-a variable region comprises a CDR1 with 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 NIATNDY (SEQ ID NO:7). In some aspects, the TCR-a variable region comprises a CDR2 with at least 80% sequence identity to GYKTK (SEQ ID NO:8). In some aspects, the TCR-a variable region comprises a CDR2 with 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 GYKTK (SEQ ID NO:8). In some aspects, the TCR-a variable region comprises a CDR1 comprising the amino acid sequence of SEQ ID NO:7 and/or a CDR2 comprising the amino acid sequence of SEQ ID NO:8. In some aspects, the TCR-a variable region comprises an amino acid sequence with at least 70% sequence identity to SEQ ID NO:5. In some aspects, the TCR-a variable region

comprises an amino acid sequence with 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:5. In some aspects, the TCR-a variable region comprises the amino acid sequence of SEQ ID NO:5. In some aspects, the TCR-b variable region comprises a CDR1 with at least 80% sequence identity to MNHEY (SEQ ID NO:14). In some aspects, the TCR-b variable region comprises a CDR1 with 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 MNHEY (SEQ ID NO:14). In some aspects, the TCR-b variable region comprises a CDR2 with at least 80% sequence identity to SVGEGT (SEQ ID NO:15). In some aspects, the TCR-b variable region comprises a CDR2 with 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 SVGEGT (SEQ ID NO:15). In some aspects, the TCR-b variable region comprises a CDR1 comprising the amino acid sequence of SEQ ID NO:14 and/or a CDR2 comprising the amino acid sequence of SEQ ID NO:15. In some aspects, the TCR-b variable region comprises an amino acid sequence with at least 70% sequence identity to SEQ ID NO:12. In some aspects, the TCR-b variable region comprises an amino acid sequence with 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:12. In some aspects, the TCR-b variable region comprises the amino acid sequence of SEQ ID NO:12. In some aspects, the TCR-a variable region comprises a CDR1 with at least 80% sequence identity to TTLSN (SEQ ID NO:23). In some aspects, the TCR-a variable region comprises a CDR1 with 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 TTLSN (SEQ ID NO:23). In some aspects, the TCR-a variable region comprises a CDR2 with at least 80% sequence identity to LVKSGEV (SEQ ID NO:24). In some aspects, the TCR-a variable region comprises a CDR2 with 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 LVKSGEV (SEQ ID NO:24). In some aspects, the TCR-a variable region comprises a CDR1 comprising the amino acid sequence of SEQ ID NO:23 and/or a CDR2 comprising the amino acid sequence of SEQ ID NO:24. In some aspects, the TCR-a variable region comprises an amino acid sequence with at least 70% sequence identity to SEQ ID NO:21. In some aspects, the TCR-a variable region comprises an amino acid sequence with 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:21. In some aspects, the TCR-a variable region comprises the amino acid sequence of SEQ ID NO:21. In some aspects, the TCR-b variable region comprises a CDR1 with at least 80% sequence identity to MNHEY (SEQ ID NO:14). In some aspects, the TCR-b variable region comprises a CDR1 with 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 MNHEY (SEQ ID NO:14). In some aspects, the TCR-b variable region comprises a CDR2 with at least 80% sequence identity to SVGEGT (SEQ ID NO:15). In some aspects, the TCR-b variable region comprises a CDR2 with 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 SVGEGT (SEQ ID NO:15). In some aspects, the TCR-b variable region comprises a CDR1 comprising the amino acid sequence of SEQ ID NO:14 and/or a CDR2 comprising the amino acid sequence of SEQ ID NO:15. In some aspects, the TCR-b variable region comprises an amino acid sequence with at least 70% sequence identity to SEQ ID NO:28. In some aspects, the TCR-b variable region comprises an amino acid sequence with 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:28. In some aspects, the TCR-a variable region comprises the amino acid sequence of SEQ ID NO:28.

**[0010]** In some aspects, the polypeptide or TCR comprises a variable region comprising a CDR1 with 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:7 and/or comprises a CDR1 with at least, at most, or exactly 1, 2, 3, 4, 5, 6, or 7 (or any derivable range therein) amino acids that are immediately adjacent to (i.e. flanking) CDR1 in the variable region of SEQ ID NO:5. In some aspects, the polypeptide or TCR comprises a variable region comprising a CDR2 with 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:8 and/or comprises a CDR2 with at least, at most, or exactly 1, 2, 3, 4, 5, 6, or 7 (or any derivable range therein) amino acids that are immediately adjacent to (i.e. flanking) CDR2 in the variable region of SEQ ID NO:5. In some aspects, the polypeptide or TCR comprises a variable region comprising a CDR3 with 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/or comprises a CDR3 with at least, at most, or exactly 1, 2, 3, 4, 5, 6, or 7 (or any derivable range therein) amino acids that are immediately adjacent to (i.e. flanking) CDR3 in the variable region of SEQ ID NO:5. In some aspects, the

polypeptide or TCR comprises a variable region comprising a CDR1 with 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:14 and/or comprises a CDR1 with at least, at most, or exactly 1, 2, 3, 4, 5, 6, or 7 (or any derivable range therein) amino acids that are immediately adjacent to (i.e. flanking) CDR1 in the variable region of SEQ ID NO:12. In some aspects, the polypeptide or TCR comprises a variable region comprising a CDR2 with 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:15 and/or comprises a CDR2 with at least, at most, or exactly 1, 2, 3, 4, 5, 6, or 7 (or any derivable range therein) amino acids that are immediately adjacent to (i.e. flanking) CDR2 in the variable region of SEQ ID NO:12. In some aspects, the polypeptide or TCR comprises a variable region comprising a CDR3 with 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:16 and/or comprises a CDR3 with at least, at most, or exactly 1, 2, 3, 4, 5, 6, or 7 (or any derivable range therein) amino acids that are immediately adjacent to (i.e. flanking) CDR3 in the variable region of SEQ ID NO:12.

**[0011]** In some aspects, the polypeptide or TCR comprises a variable region comprising a CDR1 with 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 and/or comprises a CDR1 with at least, at most, or exactly 1, 2, 3, 4, 5, 6, or 7 (or any derivable range therein) amino acids that are immediately adjacent to (i.e. flanking) CDR1 in the variable region of SEQ ID NO:21. In some aspects, the polypeptide or TCR comprises a variable region comprising a CDR2 with 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:24 and/or comprises a CDR2 with at least, at most, or exactly 1, 2, 3, 4, 5, 6, or 7 (or any derivable range therein) amino acids that are immediately adjacent to (i.e. flanking) CDR2 in the variable region of SEQ ID NO:21. In some aspects, the polypeptide or TCR comprises a variable region comprising a CDR3 with 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:25 and/or comprises a CDR3 with at least, at most, or exactly 1, 2, 3, 4, 5, 6, or 7 (or any derivable range therein) amino acids that are immediately adjacent to (i.e. flanking) CDR3 in the variable region of SEQ ID NO:21. In some aspects, the polypeptide or TCR comprises a variable region comprising a CDR1 with 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:14 and/or comprises a CDR1 with at least, at most, or exactly 1, 2, 3, 4, 5, 6, or 7 (or any derivable range therein) amino acids that are immediately adjacent to (i.e. flanking) CDR1 in the variable region of SEQ ID NO:28. In some aspects, the polypeptide or TCR comprises a variable region comprising a CDR2 with 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:15 and/or comprises a CDR2 with at least, at most, or exactly 1, 2, 3, 4, 5, 6, or 7 (or any derivable range therein) amino acids that are immediately adjacent to (i.e. flanking) CDR2 in the variable region of SEQ ID NO:28. In some aspects, the polypeptide or TCR comprises a variable region comprising a CDR3 with 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:30 and/or comprises a CDR3 with at least, at most, or exactly 1, 2, 3, 4, 5, 6, or 7 (or any derivable range therein) amino acids that are immediately adjacent to (i.e. flanking) CDR3 in the variable region of SEQ ID NO:28.

15 **[0012]** In some aspects, the TCR comprises a TCR-a polypeptide comprising a variable region comprising CDR1, CDR2, and CDR3 and a TCR-b polypeptide comprising a variable region comprising CDR1, CDR2, and CDR3. In some aspects, the TCR-a polypeptide comprises a CDR1 with at least 80% sequence identity to SEQ ID NO:7 and/or the TCR-b polypeptide comprises a CDR1 with at least 80% sequence identity to SEQ ID NO:14. In some aspects, the TCR-a polypeptide comprises a CDR1 with 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:7 and/or the TCR-b polypeptide comprises a CDR1 with 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:14. In some aspects, the TCR-a polypeptide comprises a CDR1 comprising the amino acid sequence of SEQ ID NO:7 and the TCR-b polypeptide comprises a CDR1 comprising the amino acid sequence of SEQ ID NO:14. In some aspects, the TCR-a polypeptide comprises a CDR2 with at least 80% sequence identity to SEQ ID NO:8 and the TCR-b polypeptide comprises a CDR2 with at least 80% sequence identity to SEQ ID NO:15. In some aspects, the TCR-a polypeptide comprises a CDR2 with 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:8 and the TCR-b polypeptide comprises a CDR2 with 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:15. In some

aspects, the TCR-a polypeptide comprises a CDR2 comprising the amino acid sequence of SEQ ID NO:8 and the TCR-b polypeptide comprises a CDR2 comprising the amino acid sequence of SEQ ID NO:15. In some aspects, the CDR1, CDR2, and CDR3 of the TCR-a polypeptide comprise the amino acid sequence of SEQ ID NO: 7, 8, and 9, respectively and wherein the CDR1, CDR3, and CDR3 of the TCR-b polypeptide comprise the amino acid sequence of SEQ ID NO:14, 15, and 16, respectively. In some aspects, the TCR-a variable region comprises an amino acid sequence with at least 70% sequence identity to SEQ ID NO:5 and the TCR-b variable region comprises an amino acid sequence with at least 70% sequence identity to SEQ ID NO:12. In some aspects, the TCR-a variable region comprises an amino acid sequence with 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:5 and the TCR-b variable region comprises an amino acid sequence with 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:12. In some aspects, the TCR-a polypeptide comprises an amino acid sequence with at least 70% sequence identity to SEQ ID NO:3 and the TCR-b polypeptide comprises an amino acid sequence with at least 70% sequence identity to SEQ ID NO:10. In some aspects, the TCR-a polypeptide comprises an amino acid sequence with 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 and the TCR-b polypeptide comprises an amino acid sequence with 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:10.

**[0013]** In some aspects, the TCR-a polypeptide comprises a CDR1 with at least 80% sequence identity to SEQ ID NO:23 and/or the TCR-b polypeptide comprises a CDR1 with at least 80% sequence identity to SEQ ID NO:14. In some aspects, the TCR-a polypeptide comprises a CDR1 with 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 and/or the TCR-b polypeptide comprises a CDR1 with 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:14. In some aspects, the TCR-a polypeptide comprises a CDR1 comprising the amino acid sequence of SEQ ID NO:23 and the TCR-b polypeptide comprises a CDR1 comprising the amino acid sequence of SEQ ID NO:14. In some aspects, the TCR-a polypeptide comprises a CDR2 with at least 80% sequence identity to SEQ ID NO:24 and the TCR-b polypeptide comprises a CDR2 with at least 80% sequence

identity to SEQ ID NO:15. In some aspects, the TCR-a polypeptide comprises a CDR2 with 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:24 and the TCR-b polypeptide comprises a CDR2 with 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:15. In some aspects, the TCR-a polypeptide comprises a CDR2 comprising the amino acid sequence of SEQ ID NO:24 and the TCR-b polypeptide comprises a CDR2 comprising the amino acid sequence of SEQ ID NO:15. In some aspects, the CDR1, CDR2, and CDR3 of the TCR-a polypeptide comprise the amino acid sequence of SEQ ID NO: 23, 24, and 25, respectively and wherein the CDR1, CDR3, and CDR3 of the TCR-b polypeptide comprise the amino acid sequence of SEQ ID NO:14, 15, and 30, respectively. In some aspects, the TCR-a variable region comprises an amino acid sequence with at least 70% sequence identity to SEQ ID NO:21 and the TCR-b variable region comprises an amino acid sequence with at least 70% sequence identity to SEQ ID NO:28. . In some aspects, the TCR-a variable region comprises an amino acid sequence with 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:21 and the TCR-b variable region comprises an amino acid sequence with 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:28. In some aspects, the TCR-a polypeptide comprises an amino acid sequence with at least 70% sequence identity to SEQ ID NO:19 and the TCR-b polypeptide comprises an amino acid sequence with at least 70% sequence identity to SEQ ID NO:26. In some aspects, the TCR-a polypeptide comprises an amino acid sequence with 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:19 and the TCR-b polypeptide comprises an amino acid sequence with 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:26.

**[0014]** 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.

**[0015]** 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. 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. In some aspects, the TCR may be further defined as a single chain TCR.

**[0016]** 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; 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 SEQ ID NOS:7-9, 14-16, 23-25, and 30. In some aspects, the CDR comprises a substitution at amino acid 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, or 30. The amino acid 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, or 30 of SEQ ID NOS:7-9, 14-16, 23-25, and 30 may be substituted with alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, or valine.

**[0017]** 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.

**[0018]** On some aspects, the disclosure relates to a nucleic acid encoding a TCR-a polypeptide comprising a CDR3 comprising an amino acid sequence with at least 80% sequence identity to LVVEGNRDDKII (SEQ ID NO:9) and the TCR-b polypeptide comprises a CDR3 comprising an amino acid sequence with at least 80% sequence identity to ASPPTPSSYNEQF (SEQ ID NO:16). In some aspects, the nucleic acid encodes a TCR-a polypeptide comprising a CDR3 comprising an amino acid sequence with 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 LVVEG NRDDKII (SEQ ID NO:9) and the TCR-b polypeptide comprises a CDR3 comprising an amino acid sequence with 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 ASPPTPSSYNEQF (SEQ ID NO:16). In some aspects, 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. In some aspects, the nucleic acid encodes for a TCR-a comprising a CDR1 with at least 80% sequence identity to SEQ ID NO:7 and/or a TCR-b comprising a CDR1 with at least 80% sequence identity to SEQ ID NO:14. In some aspects, the nucleic acid encodes for a TCR-a comprising a CDR1 with 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:7 and/or a TCR-b comprising a CDR1 with 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:14. In some aspects, the nucleic acid encodes for a TCR-a comprising a CDR2 with at least 80% sequence identity to SEQ ID NO:8 and a TCR-b comprising a CDR2 with at least 80% sequence identity to SEQ ID NO:15. In some aspects, the nucleic acid encodes for a TCR-a comprising a CDR2 with 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:8 and a TCR-b comprising a CDR2 with 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:15. In some aspects, the nucleic acid encodes a TCR-a variable region comprising an amino acid sequence with at least 70% sequence identity to SEQ ID NO:5 and/or a TCR-b variable region comprising an amino acid sequence with at least 70% sequence identity to SEQ ID NO:12. In some aspects, the nucleic acid encodes a TCR-a variable region comprising an amino acid sequence with 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:5 and/or a TCR-b variable region comprising an amino acid sequence with 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:12. In some aspects, the nucleic acid encodes a TCR-a polypeptide chain comprising an amino acid sequence with at least 70% sequence identity to SEQ ID NO:3 and/or a TCR-b chain comprising an amino acid sequence with at least 70% sequence identity to SEQ ID NO:10. In some aspects, the nucleic

acid encodes a TCR-a polypeptide chain comprising an amino acid sequence with 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 and/or a TCR-b chain comprising an amino acid sequence with 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:10. In some aspects, the nucleic acid comprises SEQ ID NO:1 and/or SEQ ID NO:2. In some aspects, the nucleic acid comprises a nucleic acid sequence with 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:1 and/or with 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:2.

**[0019]** In some aspects, the disclosure relates to a nucleic acid encoding a TCR-a polypeptide comprising a CDR3 comprising an amino acid sequence with at least 80% sequence identity to AVGSMDSNYQLI (SEQ ID NO:25) and the TCR-b polypeptide comprises a CDR3 comprising an amino acid sequence with at least 80% sequence identity to ASSQERTWPYNEQF (SEQ ID NO:30). In some aspects, the nucleic acid encoding a TCR-a polypeptide comprises a CDR3 comprising an amino acid sequence with 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 AVGSMDSNYQLI (SEQ ID NO:25) and the TCR-b polypeptide comprises a CDR3 comprising an amino acid sequence with 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 ASSQERTWPYNEQF (SEQ ID NO:30). In some aspects, 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. In some aspects, the nucleic acid encodes for a TCR-a comprising a CDR1 with at least 80% sequence identity to SEQ ID NO:23 and/or a TCR-b comprising a CDR1 with at least 80% sequence identity to SEQ ID NO:14. In some aspects, the nucleic acid encodes for a TCR-a comprising a CDR1 with 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 and/or a TCR-b comprising a CDR1 with 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:14. In some aspects, the nucleic acid encodes for a TCR-a comprising a CDR2 with at least 80% sequence identity to SEQ ID NO:24 and a

TCR-b comprising a CDR2 with at least 80% sequence identity to SEQ ID NO:15. In some aspects, the nucleic acid encodes for a TCR-a comprising a CDR2 with 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:24 and a TCR-b comprising a CDR2 with 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:15. In some aspects, the nucleic acid encodes a TCR-a variable region comprising an amino acid sequence with at least 70% sequence identity to SEQ ID NO:21 and/or a TCR-b variable region comprising an amino acid sequence with at least 70% sequence identity to SEQ ID NO:28. In some aspects, the nucleic acid encodes a TCR-a variable region comprising an amino acid sequence with 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:21 and/or a TCR-b variable region comprising an amino acid sequence with 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:28. In some aspects, the nucleic acid encodes a TCR-a polypeptide chain comprising an amino acid sequence with at least 70% sequence identity to SEQ ID NO:19 and/or a TCR-b chain comprising an amino acid sequence with at least 70% sequence identity to SEQ ID NO:26. In some aspects, the nucleic acid encodes a TCR-a polypeptide chain comprising an amino acid sequence with 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:19 and/or a TCR-b chain comprising an amino acid sequence with 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:26. In some aspects, the nucleic acid comprises SEQ ID NO:17 and/or SEQ ID NO:18. In some aspects, the nucleic acid comprises a nucleic acid sequence with 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/or with 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:18. 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 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.

**[0020]** In some aspects, the vector comprises both of the TCR-a and TCR-b genes. In some 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 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<sup>+</sup> T cell, a CD4<sup>+</sup> 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 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).

**[0021]** 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 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.

**[0022]** In some aspects, the subject has been diagnosed with cancer, such as a cancer described herein. In some aspects, the cancer comprises a solid tumor. In some aspects, the subject has previously been treated for the cancer. In some aspects, 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 cancer comprises lung cancer. 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 cancer comprises a MAGE-A4<sup>+</sup> cancer. In some aspects, the subject has been determined to have MAGE-A4<sup>+</sup> cancerous or precancerous cells. In some aspects, the subject has been determined to be HLA-A\*0201 positive.

**[0023]** 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.

**[0024]** 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 testis antigen such as MAGE-A4.

**[0025]** 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.

**[0026]** 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.

**[0027]** 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.”

**[0028]** 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.

**[0029]** 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.

**[0030]** 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 described in the context of the term “comprising” may also be implemented in the context of the term “consisting of” or “consisting essentially of.”

[0031] 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.

5 [0032] 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 discussed with respect to one aspect of the disclosure applies to other aspects of the disclosure as well and vice versa.

[0033] It is specifically contemplated that any limitation discussed with respect to one  
10 embodiment of the invention may apply to any other embodiment 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  
15 elsewhere in the application, such as in the Summary of Invention, Detailed Description of the Embodiments, Claims, and description of Figure Legends.

[0034] 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 of the invention,  
20 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**

[0035] The following drawings form part of the present specification and are included to  
25 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.

[0036] **FIG. 1.** MAGE-A4 expression in normal tissue. The GTEx dataset shows that MAGE-A4 only expresses in Testis tissues, but not any other normal tissues.

30 [0037] **FIG. 2.** MAGE-A4 expression in tumor tissue. The TCGA dataset shows that MAGE-A4 highly expresses in several solid cancers, such as lung cancer, esophagus carcinoma, head and neck cancer, ovarian cancer, and melanoma.

[0038] **FIG. 3.** MA4-230 GGYDGREHTV (SEQ ID NO:35) CTL generation. Representative generation of MAGE-A4 specific T cell products from dendritic cell-T cell (DC-T) co-culturing system with healthy donor's PBMC. Small CD8+/Tetramer+ population was observed in 3 wells of one 48 well plate after 2 stimulations using MA4-230 peptide pulsed DC. The 3 positive wells were sorted separately using tetramer guided sorting technology and underwent 1 or 2 rounds of expansions with rapid expansion protocol (REP). CD8 and tetramer staining of the final products is shown.

[0039] **FIG. 4A-D.** Functional avidity of MAGE-A4 specific T cells. **A.** 3 MAGE-A4 CTL cell lines lysed T2 cells pulsed with various concentrations of MA4-230 peptide with an effector to target (E:T) ratio of 20:1. **B.** 3 MAGE-A4 CTL cell lines lysed MAGE-A4 expressing lung cancer cell line H2023 (MAGE-A4+, HLA-A2+) at various E:T ratios. Another lung cell line H522 (MAGE-A4-, HLA-A2+) was used as the negative control. **C.** 3 MAGE-A4 CTL cell lines lysed MAGE-A4 expressing and HLA-A2 forced expressing tumor cell line H1299-A2 (MAGE-A4+, HLA-A2+) at various E:T ratios. The parental cell line H1299 (MAGE-A4+, HLA-A2-) was used as the negative control. **D.** 3 MAGE-A4 CTL cell lines lysed the tumor cell line H1395 (MAGE-A4+, HLA-A2+) and H1355 (MAGE-A4-, HLA-A2+).

[0040] **FIG. 5.** MA4-230 H1 TCR-T generation. Whole length TCR from MA4-230 H1 CTL line was introduced into allogenic PBMC using a retroviral vector. Following the infection, the CD8+ tetramer+ population was sorted and expanded. High purity of TCR-T was obtained following the sorting and expansion.

[0041] **FIG. 6A-E.** Functional avidity of MA4-230 H1 TCR-T. **A.** MA4-230 H1 TCR-T lysed T2 cells pulsed with various concentrations of MA4-230 peptide with an effector to target (E:T) ratio of 20:1. **B.** MA4-230 H1 TCR-T lysed MAGE-A4 expressing lung cancer cell line H2023 (MAGE-A4+, HLA-A2+); another lung cancer cell line H522 (MAGE-A4-, HLA-A2+) was used as the negative control. **C.** MA4-230 H1 TCR-T lysed MAGE-A4 expressing and HLA-A2 forced expressing tumor cell line H1299-A2 (MAGE-A4+, HLA-A2+) at various E:T ratios. The parental cell line H1299 (MAGE-A4+, HLA-A2-) was used as the negative control. **D, E.** MA4-230 H1 TCR-T lysed the tumor cell lines A375 (MAGE-A4+, HLA-A2+) and H1395 (MAGE-A4+, HLA-A2+) at various E:T ratios.

[0042] **FIG. 7A-B.** Functional detection of MA4-230 H1 TCR-T with intracellular cytokine staining (ICS) assay. The MA4-230 H1 TCR-T was co-cultured with T2 pulsed with MA4-230 peptide/M26 peptide, and tumor cell lines, A375 (MAGE-A4+, HLA-A2+), H522 (MAGE-A4-, HLA-A2+), H1395 (MAGE-A4+, HLA-A2+), and H2023 (MAGE-A4+, HLA-

A2+) at E:T=10:1 ratio. PMA plus Ionomycine treated TCR-T was used as a positive control. Only DMSO vehicle treatment was as negative control. After overnight co-culturing, the TCR pathway down-stream activated marker, **A.** IFN- $\gamma$  and CD137, and **B.** CD69, and TNF- $\alpha$  were detected with ICS assay. After co-culturing with T2 pulsed with MA4-230 peptide, A375, H1395, H2023, the level of CD137, CD69, IFN- $\gamma$  and TNF- $\alpha$  of TCR-T were significantly enhanced compared with negative control.

**[0043] FIG. 8.** MA4-286 KVLEHVVRV (SEQ ID NO:36) CTL generation. Representative generation of MAGE-A4 specific T cell products from dendritic cell-T cell (DC-T) co-culturing system with healthy donor's PBMC. Small CD8+/Tetramer+ population was observed in 2 wells of one 48 well plate after 2 stimulations using MA4-286 peptide pulsed DC. The 2 positive wells were sorted separately using tetramer guided sorting technology and underwent 1 or 2 rounds of expansions with rapid expansion protocol (REP). CD8 and tetramer staining of the final products is shown.

**[0044] FIG. 9A-D.** Functional assay of MA4-286 CTL/Functional avidity of MAGE-A4 specific T cells. **A.** 3 MAGE-A4 CTL cell line lysis of T2 cells pulsed with various concentrations of MA4-286 peptide with an effector to target (E:T) ratio of 20:1. **B.** 3 MAGE-A4 CTL cell line lysis of MAGE-A4 expressing lung cancer cell line H2023 (MAGE-A4+, HLA-A2+) at various E:T ratios. Another lung cell line H522 (MAGE-A4-, HLA-A2+) was used as the negative control. **C.** 3 MAGE-A4 CTL cell line lysis of MAGE-A4 expressing and HLA-A2 forced expressing tumor cell line H1299-A2 (MAGE-A4+, HLA-A2+) at various E:T ratios. The parental cell line H1299 (MAGE-A4+, HLA-A2-) was used as the negative control. **D.** 3 MAGE-A4 CTL cell line lysis of the tumor cell lines H1395 (MAGE-A4+, HLA-A2+) and H1355 (MAGE-A4-, HLA-A2+).

**[0045] FIG. 10.** MA4-286 F1 TCR-T generation. Whole length TCR from MA4-286 F1 CTL line was introduced into allogenic PBMC using a retroviral vector. After infection, the CD8+tetramer+ population were sorting and expanded. A high purity of TCR-T was obtained after sorting and expansion.

**[0046] FIG. 11A-F.** MA4-286 F1 TCR-T functional assay/Functional avidity of MA4-286 F1 TCR-T. **A.** MA4-286 F1 TCR-T lysis of T2 cells pulsed with various concentrations of MA4-286 peptide with an effector to target (E:T) ratio of 20:1. **B.** MA4-286 F1 TCR-T lysis of MAGE-A4 expressing lung cancer cell line H2023 (MAGE-A4+, HLA-A2+); another lung cancer cell line, H522 (MAGE-A4-, HLA-A2+), was used as the negative control. **C.** MA4-286 F1 TCR-T lysis of MAGE-A4 expressing and HLA-A2 forced expressing tumor cell line, H1299-A2 (MAGE-A4+, HLA-A2+), at various E:T ratios. The parental cell line, H1299

(MAGE-A4+, HLA-A2-), was used as the negative control. **D, E, F.** MA4-286 F1 TCR-T lysis of the more tumor cell lines: A375 (MAGE-A4+, HLA-A2+), H1395 (MAGE-A4+, HLA-A2+), and H1755 (MAGE-A4+, HLA-A2+) at various E:T ratios.

**[0047] FIG. 12A-B.** Functional detection of MA4-286 F1 TCR-T with intracellular cytokine staining (ICS) assay. The MA4-286 F1 TCR-T was co-cultured with T2 pulsed with MA4-286 peptide/M26 peptide, tumor cell line, A375 (MAGE-A4+, HLA-A2+), H522 (MAGE-A4-, HLA-A2+), H1395 (MAGE-A4+, HLA-A2+), H2023 (MAGE-A4+, HLA-A2+) at E:T=10:1 ratio. PMA plus Ionomycine treated TCR-T was as positive control. Only DMSO vehicle treatment was as negative control. After overnight co-culturing, the TCR pathway down-stream activated marker **A.** CD137 and IFN- $\gamma$ ; and **B.** CD69 and TNF- $\alpha$  were detected with ICS assay. After co-culturing with T2 pulsed with MA4-286 peptide, A375, H1395, H2023, the level of CD137, CD69, IFN- $\gamma$  and TNF- $\alpha$  of TCR-T were significantly enhanced compared with negative control.

## **DETAILED DESCRIPTION OF THE INVENTION**

### **I. Engineered T Cell Receptors**

**[0048]** T-cell receptors comprise two different polypeptide chains, termed the T-cell receptor  $\alpha$  (TCR $\alpha$ ) and  $\beta$  (TCR $\beta$ ) chains, linked by a disulfide bond. These  $\alpha$ : $\beta$  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  $\gamma$  and  $\delta$ . Both types of T-cell receptor differ from the membrane-bound immunoglobulin that serves 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.

**[0049]** 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 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.

**[0050]** 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 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 $\alpha$  domain, where the fold is unlike that of any other immunoglobulin-like domain. The half of the domain that is juxtaposed with the C $\beta$  domain forms a  $\beta$  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  $\alpha$  helix. The intramolecular disulfide bond, which in immunoglobulin-like domains normally joins two  $\beta$  strands, in a C $\alpha$  domain joins a  $\beta$  strand to this segment of  $\alpha$  helix.

**[0051]** 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 $\alpha$  and C $\beta$  domains is distinctive in being assisted by carbohydrate, with a sugar group from the C $\alpha$  domain making a number of hydrogen bonds to the C $\beta$  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 $\alpha$  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  $\beta$  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 $\beta$  CDR2 loop in two of the seven V $\beta$  domains whose structures are known. As yet, the crystallographic structures of seven T-cell receptors have been solved to this level of resolution.

**[0052]** 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.

**[0053]** 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.

[0054] In some aspects the engineered TCRs of the disclosure comprise a variable as shown below:

Description	Sequence	SEQ ID NO:
<p><b>MA4-230 H1</b>  <b>TCR sequence;</b>  <b>Alpha chain</b>  <b>(TRAV04*01F)</b></p>	<p>ATGAGGCAAGTGGCGAGAGTGATCGTGTTCCCTGACCCTGA                      GTACTTTGAGCCTTGCTAAGACCACCCAGCCCATCTCCAT                      GGACTCATATGAAGGACAAGAAGTGAACATAACCTGTAG                      CCACAACAACATTGCTACAAATGATTATATCACGTGGTAC                      CAACAGTTTCCCAGCCAAGGACCACGATTTATTATTCAAG                      GATACAAGACAAAAGTTACAAACGAAGTGGCCTCCCTGTT                      TATCCCTGCCGACAGAAAGTCCAGCACTCTGAGCCTGCCC                      CGGGTTTCCCTGAGCGACACTGCTGTGTACTACTGCCTCGT                      GGTCGAGGGGAACAGAGATGACAAGATCATCTTTGGAAA                      AGGGACACGACTTCATATTCTCCCAATATCCAGAACCCT                      GACCCTGCCGTGTACCAGCTGAGAGACTCTAAATCCAGTG                      ACAAGTCTGTCTGCCTATTCACCGATTTTGATTCTCAAACA                      AATGTGTACAAAGTAAGGATTCTGATGTGTATATCACAG                      ACAAACACTGTGCTAGACATGAGGTCTATGGACTTCAAGAG                      CAACAGTGCTGTGGCCTGGAGCAACAAATCTGACTTTGCA                      TGTGCAAACGCCTTCAACAACAGCATTATCCAGAAGACA                      CCTTCTTCCCCAGCCCAGAAAGTTCCTGTGATGTCAAGCTG                      GTCGAGAAAAGCTTTGAAACAGATACGAACCTAACTTTC                      AAAACCTGTCAGTGATTGGGTTCCGAATCCTCCTCCTGAA                      AGTGGCCGGGTTTAATCTGCTCATGACGCTGCGGCTGTGG                      TCCAGCTAA</p>	<p>1</p>
<p><b>MA4-230 H1</b>  <b>TCR sequence;</b>  <b>Beta chain</b>  <b>(TRBV06-2*01F, or TRBV06-3*01F)</b></p>	<p>ATGAGCCTCGGGTCTCCTGTGCTGTGGGGCCTTTTCTCTCCT                      GTGGGCAGGTCCAGTGAATGCTGGTGTCACTCAGACCCCA                      AAATTCCGGGTCTGAAGACAGGACAGAGCATGACACTG                      CTGTGTGCCAGGATATGAACCATGAATACATGTACTGGT                      ATCGACAAGACCCAGGCATGGGGCTGAGGCTGATTCATTA                      CTCAGTTGGTGAGGGTACAACCTGCCAAAGGAGAGGTCCCT                      GATGGCTACAATGTCTCCAGATTAACAAAACAGAAATTTCC                      TGCTGGGGTTGGAGTCGGCTGCTCCCTCCCAAACATCTGT                      GTAATTCTGTGCCAGCCCCCAACGCCCTCCTCCTACAATG                      AGCAGTTCTTCGGGCCAGGGACACGGCTCACCGTGCTAGA                      GGACCTGAAAACGTGTTCCACCCGAGGTCGCTGTGTTT</p>	<p>2</p>

	GAGCCATCAGAAGCAGAGATCTCCCACACCCAAAAGGCC AACTGGTGTGCCTGGCCACAGGCTTCTTCCCTGACCACG TGGAGCTGAGCTGGTGGGTGAATGGGAAGGAGGTGCACA GTGGGGTTCAGCACGGACCCGCAGCCCCTCAAGGAGCAGC CCGCCCTCAATGACTCCAGATACTGCCTGAGCAGCCGCCT GAGGGTCTCGGCCACCTTCTGGCAGAACCCCCGCAACCAC TTCCGCTGTCAAGTCCAGTTCTACGGGCTCTCGGAGAATG ACGAGTGGACCCAGGATAGGGCCAAACCCGTCACCCAGA TCGTCAGCGCCGAGGCCTGGGGTAGAGCAGACTGTGGCTT TACCTCGGTGTCCTACCAGCAAGGGGTCCTGTCTGCCACC ATCCTCTATGAGATCCTGCTAGGGAAGGCCACCCTGTATG CTGTGCTGGTCAGCGCCCTTGTGTTGATGGCCATGGTCAA GAGAAAGGATTTCTAA	
<b>MA4-230 H1 TCR sequence; Alpha chain (TRAV04*01F)</b>	MRQVARVIVFLLSTLSLAKTTQPISMDSYEGQEVNITCSHN NIATNDYITWYQQFPSQGPRFIIQGYKTKVTNEVASLFIPADR KSSTLSLPRVSLSDTAVYYCLVVEG NRDDKIIFGKGTRLHILP NIQNPDPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDV YITDKTVLDMRSMDFKSNSAVAWSNKSDFACANAFNNSIPE DTFPSPPESSCDVKLVEKSFETDTNLFQNL SVIGFRILLKVA GFNLLMTLRLWSS	3
Signal Peptide	MRQVARVIVFLLSTLS	4
alpha - V region	LAKTTQPISMDSYEGQEVNITCSHN NIATNDYITWYQQFPSQ GPRFIIQGYKTKVTNEVASLFIPADRKSSTLSLPRVSLSDTAVY YCLVVEG NRDDKIIFGKGTRLHILP	5
alpha - C region	NIQNPDPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDV YITDKTVLDMRSMDFKSNSAVAWSNKSDFACANAFNNSIPE DTFPSPPESSCDVKLVEKSFETDTNLFQNL SVIGFRILLKVA GFNLLMTLRLWSS	6
CDR1 - alpha	NIATNDY	7
CDR2 - alpha	GYKTK	8
CDR3 - alpha	LVVEG NRDDKII	9
<b>MA4-230 H1 TCR sequence; Beta chain (TRBV06-</b>	MSLGLLCCGAFSLLWAGPVNAGVTQTPKFRVLKTGQSM TLL CAQDMNHEYMYWYRQDPGMGLRLIHYSV GEGTTAKGEVP DGYNVSRLLKKNFLLGLESAAPSQTSVYFCASPPTSSYNEQ FFGPGTRLTVLEDLKNVFPPEVA VFEPSEAEISHTQKATLVCL ATGFFPDHVELS WWWNGKEVHSGVSTDPQPLKEQPALNDSR	10

<b>2*01F, or TRBV06-3*01F)</b>	YCLSSRLRVSATFWQNPRNHFRCQVQFYGLSENDEWTQDRA KPVTQIVSAEAWGRADCGFTSVSYQQGVLSATILYEILLGKA TLYAVLVSALVLMAMVKRKDF	
Signal Peptide	MSLGLLCCGAFSLLWAGPV	11
beta - V region	NAGVTQTPKFRVLKTGQSMTELLCAQDMNHEYMYWYRQDP GMGLRLIHYSVGEGETTAKGEVPDGYNVSRLLKQNFLLGLES AAPSQTSVYFCASPPTPSSYNEQFFGPGTRLTVL	12
beta - C region	EDLKNVFPPEVAVFEPSEAEISHTQKATLVCLATGFFPDHVEL SWWVNGKEVHSGVSTDPQPLKEQPALNDSRYCLSSRLRVSA TFWQNPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEA WGRADCGFTSVSYQQGVLSATILYEILLGKATLYAVLVSALV LMAMVKRKDF	13
CDR1 - beta	MNHEY	14
CDR2 - beta	SVGEGT	15
CDR3 - beta	ASPPTPSSYNEQF	16
<b>MA4-286 F1 TCR sequence; Alpha chain (TRAV25*01F)</b>	ATGAAGAGGGAGAGGGAGATGCTACTCATCACATCAATG TTGGTCTTATGGATGCAATTGTCACAGGTGAATGGACAAC AGGTAATGCAAATTCCTCAGTACCAGCATGTACAAGAAGG AGAGGACTTCACCACGTACTGCAATTCCTCAACTACTTTA AGCAATATACAGTGGTATAAGCAAAGGCCTGGTGGACATC CCGTTTTTTTGATACAGTTAGTGAAGAGTGGAGAAGTGAA GAAGCAGAAAAGACTGACATTTTCAGTTTGGAGAAGCAAA AAAGAACAGCTCCCTGCACATCACAGCCACCCAGACTACA GATGTAGGAACCTACTTCTGTGCAGTGGGAAGCATGGATA GCAACTATCAGTTAATCTGGGGCGCTGGGACCAAGCTAAT TATAAAGCCAGATATCCAGAACCCTGACCCTGCCGTGTAC CAGCTGAGAGACTCTAAATCCAGTGACAAGTCTGTCTGCC TATTCACCGATTTTGTATTCTCAAACAAATGTGTACAAAAGT AAGGATTCTGATGTGTATATCACAGACAAAAGTGTGCTAG ACATGAGGTCTATGGACTTCAAGAGCAACAGTGCTGTGGC CTGGAGCAACAAATCTGACTTTGCATGTGCAAACGCCTTC AACAAACAGCATTATTCCAGAAGACACCTTCTTCCCCAGCC CAGAAAGTTCCTGTGATGTCAAGCTGGTCGAGAAAAGCTT TGAAACAGATACGAACCTAACTTTCAAACCTGTCAGTG ATTGGGTTCCGAATCCTCCTCCTGAAAGTGGCCGGGTTTA ATCTGCTCATGACGCTGCGGCTGTGGTCCAGCTAA	17

<p><b>MA4-286 F1</b> <b>TCR sequence;</b> <b>Beta chain</b> <b>(TRBV06-2*01F, or TRBV06-3*01F)</b></p>	<p>ATGAGCCTCGGGCTCCTGTGCTGTGGGGCCTTTTCTCTCCT GTGGGCAGGTCCAGTGAATGCTGGTGTCACTCAGACCCCA AAATTCCGGGTCTGAAGACAGGACAGAGCATGACACTG CTGTGTGCCCAGGATATGAACCATGAATACATGTACTGGT ATCGACAAGACCCAGGCATGGGGCTGAGGCTGATTCATTA CTCAGTTGGTGAGGGTACAACCTGCCAAAGGAGAGGTCCCT GATGGCTACAATGTCTCCAGATTAACAAAAACAGAATTTCC TGCTGGGGTTGGAGTCGGCTGCTCCCTCCCAAACATCTGT GTACTTCTGTGCCAGCAGCCAGGAACGGACCTGGCCCTAC AATGAGCAGTTCTTCGGGCCAGGGACACGGCTCACCGTGC TAGAGGACCTGAAAAACGTGTTCCCACCCGAGGTGCTGT GTTTGAGCCATCAGAAGCAGAGATCTCCACACCCAAAAG GCCACACTGGTGTGCTGGCCACAGGCTTCTTCCCTGACC ACGTGGAGCTGAGCTGGTGGGTGAATGGGAAGGAGGTGC ACAGTGGGGTCAGCACGGACCCGCAGCCCCTCAAGGAGC AGCCCGCCCTCAATGACTCCAGATACTGCCTGAGCAGCCG CCTGAGGGTCTCGGCCACCTTCTGGCAGAACCCCGCAAC CACTTCCGCTGTCAAGTCCAGTTCTACGGGCTCTCGGAGA ATGACGAGTGGACCCAGGATAGGGCCAAACCCGTCACCC AGATCGTCAGCGCCGAGGCCTGGGGTAGAGCAGACTGTG GCTTTACCTCGGTGTCCTACCAGCAAGGGGTCTGTCTGCC ACCATCCTCTATGAGATCCTGCTAGGGAAGGCCACCCTGT ATGCTGTGCTGGTCAGCGCCCTTGTGTTGATGGCCATGGTC AAGAGAAAGGATTTCTAA</p>	<p>18</p>
<p><b>MA4-286 F1</b> <b>TCR sequence;</b> <b>Alpha chain</b> <b>(TRAV25*01F)</b></p>	<p>MKREREMLLITSMLVLWMQLSQVNGQQVMQIPQYQHVQEG EDFTTYCNSSTTLSNIQWYKQRPGGHPVFLIQLVKSGEVKKQ KRLTFQFGEAKKNSSLHITATQTTDVGTYFCAVGSMDSNYQ LIWGAGTKLIKPDIQNPDPVYQLRDSKSSDKSVCLFTDFDS QTNVSQSKDSVYITDKTVLDMRSMDFKSNSAVAWSNKSDF ACANAFNNSIIPEDTFFPSPPESSCDVKLVEKSFETDTNLFQNL SVIGFRILLKLVAGFNLLMTLRLWSS</p>	<p>19</p>
<p>Signal Peptide</p>	<p>MKREREMLLITSMLVLWMQLSQVN</p>	<p>20</p>
<p>alpha - V region</p>	<p>GQQVMQIPQYQHVQEGEDFTTYCNSSTTLSNIQWYKQRPGG HPVFLIQLVKSGEVKKQKRLTFQFGEAKKNSSLHITATQTTD VGTYFCAVGSMDSNYQLIWGAGTKLIKP</p>	<p>21</p>

alpha - C region	DIQNPDPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDV YITDKTVLDMRSMDFKSNSAVAWSNKSDFACANAFNNSIPE DTFFPSPRESSCDVKLVEKSFETDTNLFQNLVIGFRILLKVA GFNLLMTLRLWSS	22
CDR1 - alpha	TTLSN	23
CDR2 - alpha	LVKSGEV	24
CDR3 - alpha	AVGSMDSNYQLI	25
<b>MA4-286 F1 TCR sequence; Beta chain (TRBV06- 2*01F, or TRBV06-3*01F)</b>	MSLGLLCCGAFSLLWAGPVNAGVTQTPKFRVLKTGQSMTLL CAQDMNHEYMYWYRQDPGMGLRLIHYSVGEGTTAKGEVP DGYNVSRLLKQNFLLGLESAAAPSQTSVYFCASSQERTWPYN EQFFGPGTRLTVLEDLKNVFPPEVAVFEPSEAEISHTQKATLV CLATGFFPDHVELSWWVNGKEVHSGVSTDPQPLKEQPALND SRYCLSSRLRVSAATFWQNPRNHFRQCQVQFYGLSENDEWTQD RAKPVTQIVSAEAWGRADCGFTSVSYQQGVLSATILYEILLG KATLYAVLVSALVLMAMVKKRDF	26
Signal Peptide	MSLGLLCCGAFSLLWAGPV	27
beta - V region	NAGVTQTPKFRVLKTGQSMTLLCAQDMNHEYMYWYRQDP GMGLRLIHYSVGEGTTAKGEVPDGYNVSRLLKQNFLLGLES AAAPSQTSVYFCASSQERTWPYNEQFFGPGTRLTVL	28
beta - C region	EDLKNVFPPEVAVFEPSEAEISHTQKATLVCLATGFFPDHVEL SWWVNGKEVHSGVSTDPQPLKEQPALNDSRYCLSSRLRVSA TFWQNPRNHFRQCQVQFYGLSENDEWTQDRAKPVTQIVSAEA WGRADCGFTSVSYQQGVLSATILYEILLGKATLYAVLVSALV LMAMVKKRDF	29
CDR1 - beta	MNHEY	14
CDR2 - beta	SVGEGT	15
CDR3 - beta	ASSQERTWPYNEQF	30

[0055] The following table relates to features of the TCR-a aspects:

<b>Result summary:</b>	<b>Productive TRA rearranged sequence</b> (no stop codon and in-frame junction)		
V-GENE and allele	<u>Homsap</u> <u>TRAV4*01 F</u>	score = 1315	identity = <b>100.00%</b> (264/264 nt)
J-GENE and allele	<u>Homsap</u> <u>TRAJ30*01 F</u>	score = 276	identity = 98.25% (56/57 nt)
FR-IMGT lengths, CDR-IMGT lengths and AA JUNCTION	[25.17.34.11]	[7.5.12]	CLVVEGNRDDKIIF (SEQ ID NO:31)

<b>Result summary:</b>	<b>Productive TRA rearranged sequence</b> (no stop codon and in-frame junction)		
V-GENE and allele	<u>Homsap TRAV25*01 F</u>	score = 1330	identity = <b>100.00%</b> (267/267 nt)
J-GENE and allele	<u>Homsap TRAJ33*01 F</u>	score = 285	identity = 100.00% (57/57 nt)
FR-IMGT lengths, CDR-IMGT lengths and AA JUNCTION	[26.17.34.11]	[5.7.12]	CAVGSMDSNYQLIW (SEQ ID NO:32)

[0056] The following table relates to features of the TCR-b aspects:

<b>Result summary:</b>	<b>Productive TRB rearranged sequence</b> (no stop codon and in-frame junction)		
V-GENE and allele	<u>Homsap TRBV6-2*01 F, or Homsap TRBV6-3*01 F</u>	score = 1360	identity = <b>100.00%</b> (273/273 nt)
J-GENE and allele	<u>Homsap TRBJ2-1*01 F</u>	score = 250	identity = 100.00% (50/50 nt)
D-GENE and allele by IMGT/JunctionAnalysis	No results	-	
FR-IMGT lengths, CDR-IMGT lengths and AA JUNCTION	[26.17.37.10]	[5.6.13]	CASPPTPSSYNEQFF (SEQ ID NO:33)

<b>Result summary:</b>	<b>Productive TRB rearranged sequence</b> (no stop codon and in-frame junction)		
V-GENE and allele	<u>Homsap TRBV6-2*01 F, or Homsap TRBV6-3*01 F</u>	score = 1360	identity = <b>100.00%</b> (273/273 nt)
J-GENE and allele	<u>Homsap TRBJ2-1*01 F</u>	score = 232	identity = 96.00% (48/50 nt)
D-GENE and allele by IMGT/JunctionAnalysis	<u>Homsap TRBD1*01 F</u>	D-REGION is in reading frame 1	
FR-IMGT lengths, CDR-IMGT lengths and AA JUNCTION	[26.17.37.10]	[5.6.14]	CASSQERTWPYNEQFF (SEQ ID NO:34)

5 **II. Proteinaceous Compositions**

[0057] 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.

5 [0058] 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.

15 [0059] 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, 25 300, 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.).

**[0060]** 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:1-36. 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.

**[0061]** The polypeptides, proteins, or polynucleotides encoding such polypeptides or proteins of the disclosure may include a substitute at amino acid 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, or 315 of one of SEQ ID NOS:3-16 or 19-36. The substitution at amino acid 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, or 315 of SEQ ID NOS:3-16 or 19-36 may be a substitution with a with alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, or valine.

**[0062]** 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,  
5 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251,  
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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)  
10 of SEQ ID NOS:1-36.

**[0063]** In some aspects, the protein, polypeptide, or nucleic acid may comprise 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,  
15 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,  
20 181, 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,  
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25 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294,  
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314, 315, 316, 317, 318, 319, or 320 (or any derivable range therein) contiguous amino acids  
or nucleic acids of SEQ ID NOS:1-36.

**[0064]** In some aspects, the polypeptide, protein, or nucleic acid may comprise at least, at  
30 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,  
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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,  
5 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213,  
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10 290, 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:1-36 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%,  
15 94%, 95%, 96%, 97%, 98%, 99%, or 100% (or any derivable range therein) similar, identical,  
or homologous to one of SEQ ID NOS:1-36.

**[0065]** 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,  
20 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,  
25 160, 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,  
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30 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273,  
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15 616, 617, 618, 619, 620, 621, 622, 623, 624, 625, 626, 627, 628, 629, 630, 631, 632, 633, 634,  
635, 636, 637, 638, 639, 640, 641, 642, 643, 644, 645, 646, 647, 648, 649, 650, 651, 652, 653,  
654, 655, 656, 657, 658, 659, 660, 661, 662, 663, 664, 665, 666, 667, 668, 669, 670, 671, 672,  
673, 674, 675, 676, 677, 678, 679, 680, 681, 682, 683, 684, 685, 686, 687, 688, 689, 690, 691,  
692, 693, 694, 695, 696, 697, 698, 699, 700, 701, 702, 703, 704, 705, 706, 707, 708, 709, 710,  
20 711, 712, 713, 714, 715, 716, 717, 718, 719, 720, 721, 722, 723, 724, 725, 726, 727, 728, 729,  
730, 731, 732, 733, 734, 735, 736, 737, 738, 739, 740, 741, 742, 743, 744, 745, 746, 747, 748,  
749, 750, 751, 752, 753, 754, 755, 756, 757, 758, 759, 760, 761, 762, 763, 764, 765, 766, 767,  
768, 769, 770, 771, 772, 773, 774, 775, 776, 777, 778, 779, 780, 781, 782, 783, 784, 785, 786,  
787, 788, 789, 790, 791, 792, 793, 794, 795, 796, 797, 798, 799, 800, 801, 802, 803, 804, 805,  
25 806, 807, 808, 809, 810, 811, 812, 813, 814, 815, 816, 817, 818, 819, 820, 821, 822, 823, 824,  
825, 826, 827, 828, 829, 830, 831, 832, 833, 834, 835, 836, 837, 838, 839, 840, 841, 842, 843,  
844, 845, 846, 847, 848, 849, 850, 851, 852, 853, 854, 855, 856, 857, 858, 859, 860, 861, 862,  
863, 864, 865, 866, 867, 868, 869, 870, 871, 872, 873, 874, 875, 876, 877, 878, 879, 880, 881,  
882, 883, 884, 885, 886, 887, 888, 889, 890, 891, 892, 893, 894, 895, 896, 897, 898, 899, 900,  
30 901, 902, 903, 904, 905, 906, 907, 908, 909, 910, 911, 912, 913, 914, 915, 916, 917, 918, 919,  
920, 921, 922, 923, 924, 925, 926, 927, 928, 929, 930, 931, 932, 933, 934, 935, 936, 937, 938,  
939, 940, 941, 942, 943, 944, 945, 946, 947, 948, 949, or 950 of any of SEQ ID NOS:1-36 and  
comprising at least, at most, or exactly 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,

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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,  
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931, 932, 933, 934, 935, 936, 937, 938, 939, 940, 941, 942, 943, 944, 945, 946, 947, 948, 949,  
or 950 (or any derivable range therein) contiguous amino acids or nucleotides of any of SEQ  
15 ID NOS:1-36.

**[0066]** 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/](http://ncbi.nlm.nih.gov/))  
20 and The Universal Protein Resource (UniProt; on the World Wide Web at [uniprot.org](http://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.

**[0067]** 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  
25 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).

**[0068]** 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  
30 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.

5 **[0069]** 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.

**[0070]** Amino acid sequence variants of the disclosure can be substitutional, insertional, or  
10 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%,  
15 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.

**[0071]** 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,  
20 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.

25 **[0072]** 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.

**[0073]** Insertional mutants typically involve the addition of amino acid residues at a non-  
30 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.

**[0074]** 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.

**[0075]** 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.

**[0076]** 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.

**[0077]** 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).

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

**[0078]** 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.  
20 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);  
25 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  $\pm 2$  are included, in other aspects, those which are within  $\pm 1$  are included, and in still other aspects, those within  
30  $\pm 0.5$  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.

[0079] 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.

[0080] 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](http://expasy.org/proteomics/protein%20structure).

[0081] 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

[0082] 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).

**[0083]** 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. 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.

**[0084]** 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 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 protein.

**[0085]** 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 (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.

[0086] 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.

#### 20           A.     **Hybridization**

[0087] 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 25 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 30 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.

[0088] 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**

[0089] 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 embodiment, one or more particular amino acid residues are changed using, for example, a site-directed mutagenesis protocol. In another embodiment, 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.

[0090] 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**

[0091] 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.

[0092] In another embodiment, 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 chains of the antibody of interest. In an even more preferred aspect, the oligonucleotides encode all or part of one or more of the CDRs or TCRs.

5 [0093] 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.

#### 15 **IV. Polypeptide Expression**

[0094] 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**

[0095] 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**

25 [0096] 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, 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.

[0097] 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 VH or VL sequence 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

[0098] 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

[0099] 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

**[0100]** 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- $\kappa$ 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.

[0101] 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

[0102] The nucleic acid molecule encoding either or both of the entire heavy and light chains of an antibody 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

[0103] 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

[0104] Aspects of the disclosure may include administration of immune checkpoint inhibitors, which are further described below.

## a. PD-1, PDL1, and PDL2 inhibitors

**[0105]** 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  
5 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.

**[0106]** Alternative names for “PD-1” include CD279 and SLEB2. Alternative names for  
10 “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.

**[0107]** 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  
15 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 antibody, an antigen binding fragment thereof, an immunoadhesin, a fusion protein, or  
20 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 herein by reference.

**[0108]** In some aspects, the PD-1 inhibitor is an anti-PD-1 antibody (e.g., a human antibody,  
25 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 extracellular or PD-1 binding portion of PDL1 or PDL2 fused to a constant region (e.g., an Fc  
30 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.

**[0109]** 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.

**[0110]** 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

**[0111]** 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.

[0112] 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.

[0113] 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.

[0114] 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).

[0115] 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

[0116] 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

[0117] 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.

[0118] 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).

[0119] 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.

[0120] 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.

[0121] 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

[0122] 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.

[0123] 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.

10 [0124] Exemplary CAR-T therapies include Tisagenlecleucel (Kymriah) and Axicabtagene ciloleucel (Yescarta). In some aspects, the CAR-T therapy targets CD19.

### 5. Cytokine therapy

[0125] 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.

15 [0126] 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 $\alpha$  and IFN $\beta$ ), type II (IFN $\gamma$ ) and type III (IFN $\lambda$ ).

20 [0127] Interleukins have an array of immune system effects. IL-2 is an exemplary interleukin cytokine therapy.

### 6. Adoptive T-cell therapy

[0128] 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.

30 [0129] 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

5 [0130] 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, chlorozoticin, streptozocin) and triazines (e.g.,  
10 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  
15 mitoxanthrone), enzymes (e.g., L-asparaginase), and biological response modifiers (e.g., Interferon- $\alpha$ ), 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.

20 [0131] 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  
25 efficacious doses used in clinical applications including about 15 mg/m<sup>2</sup> to about 20 mg/m<sup>2</sup> 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  
30 alone.

[0132] Other suitable chemotherapeutic agents include antimicrotubule agents, e.g., Paclitaxel ("Taxol") and doxorubicin hydrochloride ("doxorubicin"). The combination of an Egr-1 promoter/TNF $\alpha$  construct delivered via an adenoviral vector and doxorubicin was

determined to be effective in overcoming resistance to chemotherapy and/or TNF- $\alpha$ , which suggests that combination treatment with the construct and doxorubicin overcomes resistance to both doxorubicin and TNF- $\alpha$ .

5 [0133] Doxorubicin is absorbed poorly and is preferably administered intravenously. In certain aspects, appropriate intravenous doses for an adult include about 60 mg/m<sup>2</sup> to about 75 mg/m<sup>2</sup> at about 21-day intervals or about 25 mg/m<sup>2</sup> to about 30 mg/m<sup>2</sup> on each of 2 or 3 successive days repeated at about 3 week to about 4 week intervals or about 20 mg/m<sup>2</sup> 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  
10 combined with other myelopoietic suppressant drugs.

[0134] 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 (HN<sub>2</sub>), cyclophosphamide and/or ifosfamide, melphalan (L-sarcosine), and chlorambucil. Cyclophosphamide (CYTOXAN®) is available from Mead Johnson and  
15 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  
20 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.

[0135] Additional suitable chemotherapeutic agents include pyrimidine analogs, such as cytarabine (cytosine arabinoside), 5-fluorouracil (fluorouracil; 5-FU) and floxuridine (fluorodeoxyuridine; FudR). 5-FU may be administered to a subject in a dosage of anywhere between  
25 about 7.5 to about 1000 mg/m<sup>2</sup>. 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.

[0136] Gemcitabine diphosphate (GEMZAR®, Eli Lilly & Co., “gemcitabine”), another  
30 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.

[0137] 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

[0138] 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

[0139] 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).

[0140] 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

[0141] 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.

10 [0142] 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.

[0143] 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.

**[0144]** 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.).

**[0145]** 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.

**[0146]** 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.

**[0147]** 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.

**[0148]** 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; Upešlacis et al., 1995; Price, 1995).

**[0149]** 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),

**[0150]** 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

[0151] 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 [0152] 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,  $\alpha$ 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.

[0153] 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).

[0154] 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).

[0155] 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-I-thyronine). . In specific aspects, one or more of these may be explicitly excluded.

[0156] 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 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, 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 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-I-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 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 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 following: corticosterone, D-Galactose, ethanolamine, glutathione, L-carnitine, linoleic acid, linolenic acid, progesterone, putrescine, sodium selenite, or triiodo-I-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.

**[0157]** 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.

**[0158]** 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.

**[0159]** 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.

**[0160]** 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.

**[0161]** 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,  $\alpha\beta 6$  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 $\alpha$ , GD2, G250/CAIX, GD3, Glypican-3 (GPC3), Her2, IL-13R $\alpha$ 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,  $\beta$ -catenin, BRCA1/2, CML66, Fibronectin, MART-2, TGF- $\beta$ 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**

[0162] 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.

[0163] 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.

[0164] 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 **[0165]** 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.

**[0166]** 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.

**[0167]** The cancers amenable for treatment include, but are not limited to, tumors of all types, locations, sizes, and characteristics. In some aspects, the cancer comprises a solid tumor. In some aspects, the methods relate to reducing tumor volume or treating cancers that are recurrent and/or metastatic. The methods and compositions of the disclosure are suitable for  
20 treating, for example, pancreatic cancer, colon cancer, acute myeloid leukemia, adrenocortical carcinoma, AIDS-related cancers, AIDS-related lymphoma, anal cancer, appendix cancer, astrocytoma, childhood cerebellar or cerebral basal cell carcinoma, bile duct cancer, extrahepatic bladder cancer, bone cancer, osteosarcoma/malignant fibrous histiocytoma, brainstem glioma, brain tumor, cerebellar astrocytoma brain tumor, cerebral  
25 astrocytoma/malignant glioma brain tumor, ependymoma brain tumor, medulloblastoma brain tumor, supratentorial primitive neuroectodermal tumors brain tumor, visual pathway and hypothalamic glioma, breast cancer, lymphoid cancer, bronchial adenomas/carcinoids, tracheal cancer, lung cancer, Burkitt lymphoma, carcinoid tumor, childhood carcinoid tumor, gastrointestinal carcinoma of unknown primary, central nervous system lymphoma, primary  
30 cerebellar astrocytoma, childhood cerebral astrocytoma/malignant glioma, childhood cervical cancer, childhood cancers, chronic lymphocytic leukemia, chronic myelogenous leukemia, chronic myeloproliferative disorders, cutaneous T-cell lymphoma, desmoplastic small round cell tumor, endometrial cancer, ependymoma, esophageal cancer, Ewing's, childhood  
extragonadal Germ cell tumor, extrahepatic bile duct cancer, eye Cancer, intraocular melanoma

eye Cancer, retinoblastoma, gallbladder cancer, gastric (stomach) cancer, gastrointestinal carcinoid tumor, gastrointestinal stromal tumor (GIST), germ cell tumor: extracranial, extragonadal, or ovarian, gestational trophoblastic tumor, glioma of the brain stem, glioma, childhood cerebral astrocytoma, childhood visual pathway and hypothalamic glioma, gastric  
5 carcinoid, hairy cell leukemia, head and neck cancer, heart cancer, hepatocellular (liver) cancer, Hodgkin lymphoma, hypopharyngeal cancer, hypothalamic and visual pathway glioma, childhood intraocular melanoma, islet cell carcinoma (endocrine pancreas), kaposi sarcoma, kidney cancer (renal cell cancer), laryngeal cancer, leukemia, acute lymphoblastic (also called acute lymphocytic leukemia) leukemia, acute myeloid (also called acute myelogenous  
10 leukemia) leukemia, chronic lymphocytic (also called chronic lymphocytic leukemia) leukemia, chronic myelogenous (also called chronic myeloid leukemia) leukemia, hairy cell lip and oral cavity cancer, liposarcoma, liver cancer (primary), non-small cell lung cancer, small cell lung cancer, lymphomas, AIDS-related lymphoma, Burkitt lymphoma, cutaneous T-cell lymphoma, Hodgkin lymphoma, Non-Hodgkin (an old classification of all lymphomas except  
15 Hodgkin's) lymphoma, primary central nervous system lymphoma, Waldenstrom macroglobulinemia, malignant fibrous histiocytoma of bone/osteosarcoma, childhood medulloblastoma, melanoma, intraocular (eye) melanoma, merkel cell carcinoma, adult malignant mesothelioma, childhood mesothelioma, metastatic squamous neck cancer, mouth cancer, multiple endocrine neoplasia syndrome, multiple myeloma/plasma cell neoplasm,  
20 mycosis fungoides, myelodysplastic syndromes, myelodysplastic/myeloproliferative diseases, chronic myelogenous leukemia, adult acute myeloid leukemia, childhood acute myeloid leukemia, multiple myeloma, chronic myeloproliferative disorders, nasal cavity and paranasal sinus cancer, nasopharyngeal carcinoma, neuroblastoma, oral cancer, oropharyngeal cancer, osteosarcoma/malignant, fibrous histiocytoma of bone, ovarian cancer, ovarian epithelial  
25 cancer (surface epithelial-stromal tumor), ovarian germ cell tumor, ovarian low malignant potential tumor, pancreatic cancer, islet cell paranasal sinus and nasal cavity cancer, parathyroid cancer, penile cancer, pharyngeal cancer, pheochromocytoma, pineal astrocytoma, pineal germinoma, pineoblastoma and supratentorial primitive neuroectodermal tumors, childhood pituitary adenoma, plasma cell neoplasia/multiple myeloma, pleuropulmonary  
30 blastoma, primary central nervous system lymphoma, prostate cancer, rectal cancer, renal cell carcinoma (kidney cancer), renal pelvis and ureter transitional cell cancer, retinoblastoma, rhabdomyosarcoma, childhood Salivary gland cancer Sarcoma, Ewing family of tumors, Kaposi sarcoma, soft tissue sarcoma, uterine sezary syndrome sarcoma, skin cancer (nonmelanoma), skin cancer (melanoma), skin carcinoma, Merkel cell small cell lung cancer,

small intestine cancer, soft tissue sarcoma, squamous cell carcinoma, squamous neck cancer with occult primary, metastatic stomach cancer, supratentorial primitive neuroectodermal tumor, childhood T-cell lymphoma, testicular cancer, throat cancer, thymoma, childhood thymoma, thymic carcinoma, thyroid cancer, urethral cancer, uterine cancer, endometrial  
5 uterine sarcoma, vaginal cancer, visual pathway and hypothalamic glioma, childhood vulvar cancer, and wilms tumor (kidney cancer).

## **X. Kits**

[0168] 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  
10 can be used to evaluate one or more biomarkers or HLA types. In certain aspects, a kit contains, 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.

15 [0169] Kits may comprise components, which may be individually packaged or placed in a container, such as a tube, bottle, vial, syringe, or other suitable container means.

[0170] 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,  
20 2x, 5x, 10x, or 20x or more.

[0171] 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.

[0172] It is contemplated that any method or composition described herein can be  
25 implemented with respect to any other method or composition described herein and that different embodiments 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.

## **XI. EXAMPLES**

[0173] The following examples are given for the purpose of illustrating various  
30 embodiments of the invention and are not meant to limit the present invention in any fashion. One skilled in the art will appreciate readily that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those objects, ends and advantages inherent herein. The present examples, along with the methods described

herein are presently representative of preferred embodiments, are exemplary, and are not intended as limitations on the scope of the invention. Changes therein and other uses which are encompassed within the spirit of the invention as defined by the scope of the claims will occur to those skilled in the art.

### 5 **Example 1: TCR Evaluation.**

**[0174]** The GTEx dataset shows that MAGE-A4 only expresses in Testis tissues, but not any other normal tissues (FIG. 1). MAGE-A4 is highly expresses in several solid cancers, such as lung cancer, esophagus carcinoma, head and neck cancer, ovarian cancer, and melanoma (FIG. 2). Dendritic cells were co-cultured with T cells from healthy donor's peripheral blood mononuclear cells (PBMCs). A small CD8+/Tetramer+ population was observed in 3 wells of 10 one 48 well plate after two stimulations using MA4-230 peptide-pulsed DCs. The 3 positive wells were sorted separately using tetramer guided sorting technology and underwent 1 or 2 rounds of expansions with rapid expansion protocol (REP). CD8 and tetramer staining of the final products is shown in FIG. 3.

**[0175]** The MAGE-A4 CTL cell lines were found to lyse various MAGE-A4 expressing cells, including T2 cells pulsed with various concentrations of MA4-230 peptide and with an effector to target (E:T) ratio of 20:1 (FIG. 4A), MAGE-A4 expressing lung cancer cell line H2023 (MAGE-A4+, HLA-A2+) at various E:T ratios (the lung cell line H522 (MAGE-A4-, HLA-A2+) was used as the negative control) (FIG. 4B), HLA-A2 forced expressing tumor cell 20 line H1299-A2 (MAGE-A4+, HLA-A2+) at various E:T ratios (parental cell line H1299 (MAGE-A4+, HLA-A2-) was used as the negative control) (FIG. 4C), and the tumor cell lines H1395 (MAGE-A4+, HLA-A2+) and H1355 (MAGE-A4-, HLA-A2+) (FIG. 4D).

**[0176]** The TCRs from MA4-230 CTL line were sequenced and cloned. Whole length TCR from MA4-230 H1 CTL line was introduced into allogenic PBMC using a retroviral vector. 25 Following the infection, the CD8+ tetramer+ population was sorted and expanded. High purity of TCR-T was obtained following the sorting and expansion (FIG. 5). MA4-230 H1 TCR-T cells were found to lyse MAGE-A4-expressing cells (FIG. 6). The MA4-230 H1 TCR-T was co-cultured with T2 pulsed with MA4-230 peptide/M26 peptide, and tumor cell lines, A375 (MAGE-A4+, HLA-A2+), H522 (MAGE-A4-, HLA-A2+), H1395 (MAGE-A4+, HLA-A2+), 30 and H2023 (MAGE-A4+, HLA-A2+) at E:T=10:1 ratio. PMA plus Ionomycin treated TCR-T was used as a positive control. Only DMSO vehicle treatment was used as a negative control. After overnight co-culturing, the TCR pathway down-stream activated markers, IFN- $\gamma$  and CD137 (FIG. 7A), and CD69, and TNF- $\alpha$  (FIG. 7B) were detected with an ICS assay. After

co-culturing with T2 pulsed with MA4-230 peptide, A375, H1395, H2023, the level of CD137, CD69, IFN- $\gamma$  and TNF- $\alpha$  of TCR-T were significantly enhanced compared with negative control.

[0177] MA4-286 CTL cells were generated. FIG. 8 shows a representative generation of MAGE-A4 specific T cell products from dendritic cell-T cell (DC-T) co-culturing system with healthy donor's PBMC. Small CD8+/Tetramer+ population was observed in two wells of one 48 well plate after 2 stimulations using MA4-286 peptide pulsed DC. The two positive wells were sorted separately using tetramer guided sorting technology and underwent 1 or 2 rounds of expansions with rapid expansion protocol (REP). CD8 and tetramer staining of the final products is shown. The MAGE-A4 CTL cell lines lysed T2 cells pulsed with various concentrations of MA4-286 peptide with an effector to target (E:T) ratio of 20:1 (FIG. 9A), MAGE-A4 expressing lung cancer cell line H2023 (MAGE-A4+, HLA-A2+) at various E:T ratios (the lung cell line H522 (MAGE-A4-, HLA-A2+) was used as the negative control) (FIG. 9B), MAGE-A4 expressing and HLA-A2 forced expressing tumor cell line H1299-A2 (MAGE-A4+, HLA-A2+) at various E:T ratios (the parental cell line H1299 (MAGE-A4+, HLA-A2-) was used as the negative control) (FIG. 9C), and the tumor cell lines H1395 (MAGE-A4+, HLA-A2+) and H1355 (MAGE-A4-, HLA-A2+) (FIG. 9D). The TCRs were sequenced and cloned. Whole length TCR from MA4-286 F1 CTL line was introduced into allogenic PBMC using a retroviral vector. After infection, the CD8+tetramer+ population were sorting and expanded. A high purity of TCR-T was obtained after sorting and expansion (FIG. 10). The MA4-286 F1 TCR-T cells were found to lyse various MAGE-A4 expressing cells (FIG. 11). The MA4-286 F1 TCR-T was co-cultured with T2 pulsed with MA4-286 peptide/M26 peptide, tumor cell line, A375 (MAGE-A4+, HLA-A2+), H522 (MAGE-A4-, HLA-A2+), H1395 (MAGE-A4+, HLA-A2+), H2023 (MAGE-A4+, HLA-A2+) at E:T=10:1 ratio. PMA plus Ionomycine treated TCR-T was as positive control. Only DMSO vehicle treatment was as negative control. After overnight co-culturing, the TCR pathway downstream activated marker CD137 and IFN- $\gamma$  (FIG. 12A); and CD69 and TNF- $\alpha$  (FIG. 12B) were detected with ICS assay. After co-culturing with T2 pulsed with MA4-286 peptide, A375, H1395, H2023, the level of CD137, CD69, IFN- $\gamma$  and TNF- $\alpha$  of TCR-T were significantly enhanced compared with negative control.

\* \* \*

[0178] 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 a TCR alpha (TCR-a) variable region comprising a CDR3 comprising an amino acid sequence with at least 80% sequence identity to LVVEGNRDDKII (SEQ ID NO:9).
- 5 2. The polypeptide of claim 1, wherein the CDR3 comprises the amino acid sequence of SEQ ID NO:9.
3. The polypeptide of claim 1 or 2, wherein the TCR-a variable region comprises a CDR1, CDR2, and CDR3.
4. The polypeptide of claim 3, wherein the TCR-a variable region comprises a CDR1 with  
10 at least 80% sequence identity to NIATNDY (SEQ ID NO:7).
5. The polypeptide of claim 3 or 4, wherein the TCR-a variable region comprises a CDR2 with at least 80% sequence identity to GYKTK (SEQ ID NO:8).
6. The polypeptide of claim 4 or 5, wherein the TCR-a variable region comprises a CDR1 comprising the amino acid sequence of SEQ ID NO:7 and/or a CDR2 comprising the amino  
15 acid sequence of SEQ ID NO:8.
7. The polypeptide of any one of claims 1-6, wherein the TCR-a variable region comprises an amino acid sequence with at least 70% sequence identity to SEQ ID NO:5.
8. The polypeptide of claim 7, wherein the TCR-a variable region comprises the amino acid sequence of SEQ ID NO:5.
- 20 9. A polypeptide comprising a TCR beta (TCR-b) variable region comprising a CDR3 comprising an amino acid sequence with at least 80% sequence identity to ASPPTPSSYNEQF (SEQ ID NO:16).
10. The polypeptide of claim 9, wherein the CDR3 comprises the amino acid sequence of SEQ ID NO:16.
- 25 11. The polypeptide of claim 9 or 10, wherein the TCR-b variable region comprises a CDR1, CDR2, and CDR3.
12. The polypeptide of claim 11, wherein the TCR-b variable region comprises a CDR1 with at least 80% sequence identity to MNHEY (SEQ ID NO:14).
13. The polypeptide of claim 11 or 12, wherein the TCR-b variable region comprises a  
30 CDR2 with at least 80% sequence identity to SVGEGT (SEQ ID NO:15).
14. The polypeptide of claim 12 or 13, wherein the TCR-b variable region comprises a CDR1 comprising the amino acid sequence of SEQ ID NO:14 and/or a CDR2 comprising the amino acid sequence of SEQ ID NO:15.

15. The polypeptide of any one of claims 9-14, wherein the TCR-b variable region comprises an amino acid sequence with at least 70% sequence identity to SEQ ID NO:12.
16. The polypeptide of claim 15, wherein the TCR-b variable region comprises the amino acid sequence of SEQ ID NO:12.
- 5 17. A polypeptide comprising a TCR-a variable region comprising a CDR3 comprising an amino acid sequence with at least 80% sequence identity to AVGSMDSNYQLI (SEQ ID NO:25).
18. The polypeptide of claim 17, wherein the CDR3 comprises the amino acid sequence of SEQ ID NO:25.
- 10 19. The polypeptide of claim 17 or 18, wherein the TCR-a variable region comprises a CDR1, CDR2, and CDR3.
20. The polypeptide of claim 19, wherein the TCR-a variable region comprises a CDR1 with at least 80% sequence identity to TTLSN (SEQ ID NO:23).
21. The polypeptide of claim 19 or 20, wherein the TCR-a variable region comprises a  
15 CDR2 with at least 80% sequence identity to LVKSGEV (SEQ ID NO:24).
22. The polypeptide of claim 20 or 21, wherein the TCR-a variable region comprises a CDR1 comprising the amino acid sequence of SEQ ID NO:23 and/or a CDR2 comprising the amino acid sequence of SEQ ID NO:24.
23. The polypeptide of any one of claims 17-22, wherein the TCR-a variable region  
20 comprises an amino acid sequence with at least 70% sequence identity to SEQ ID NO:21.
24. The polypeptide of claim 23, wherein the TCR-a variable region comprises the amino acid sequence of SEQ ID NO:21.
25. A polypeptide comprising a TCR-b variable region comprising a CDR3 comprising an amino acid sequence with at least 80% sequence identity to ASSQERTWPYNEQF (SEQ ID  
25 NO:30).
26. The polypeptide of claim 25, wherein the CDR3 comprises the amino acid sequence of SEQ ID NO:30.
27. The polypeptide of claim 25 or 26, wherein the TCR-b variable region comprises a CDR1, CDR2, and CDR3.
- 30 28. The polypeptide of claim 27, wherein the TCR-b variable region comprises a CDR1 with at least 80% sequence identity to MNHEY (SEQ ID NO:14).
29. The polypeptide of claim 27 or 28, wherein the TCR-b variable region comprises a CDR2 with at least 80% sequence identity to SVGEGT (SEQ ID NO:15).

30. The polypeptide of claim 28 or 29, wherein the TCR-b variable region comprises a CDR1 comprising the amino acid sequence of SEQ ID NO:14 and/or a CDR2 comprising the amino acid sequence of SEQ ID NO:15.
31. The polypeptide of any one of claims 25-30, wherein the TCR-b variable region  
5 comprises an amino acid sequence with at least 70% sequence identity to SEQ ID NO:28.
32. The polypeptide of claim 31, wherein the TCR-a variable region comprises the amino acid sequence of SEQ ID NO:28.
33. A nucleic acid encoding for the polypeptide of any one of claims 1-32.
34. An engineered T-cell Receptor (TCR) comprising a TCR-a polypeptide and a TCR-b  
10 polypeptide, wherein the TCR-a polypeptide comprises a CDR3 comprising an amino acid sequence with at least 80% sequence identity to LVVEGNRDDKII (SEQ ID NO:9) and the TCR-b polypeptide comprises a CDR3 comprising an amino acid sequence with at least 80% sequence identity to ASPPTPSSYNEQF (SEQ ID NO:16).
35. The TCR of claim 34, wherein the TCR-a polypeptide comprises a CDR3 comprising  
15 the amino acid sequence of SEQ ID NO:9 and the TCR-b polypeptide comprises a CDR3 comprising the amino acid sequence of SEQ ID NO:16.
36. The TCR of claim 34 or 35, wherein the TCR comprises a TCR-a polypeptide comprising a variable region comprising CDR1, CDR2, and CDR3 and a TCR-b polypeptide comprising a variable region comprising CDR1, CDR2, and CDR3.
- 20 37. The TCR of claim 36, wherein the TCR-a polypeptide comprises a CDR1 with at least 80% sequence identity to SEQ ID NO:7 and/or the TCR-b polypeptide comprises a CDR1 with at least 80% sequence identity to SEQ ID NO:14.
38. The TCR of claim 37, wherein the TCR-a polypeptide comprises a CDR1 comprising the amino acid sequence of SEQ ID NO:7 and the TCR-b polypeptide comprises a CDR1  
25 comprising the amino acid sequence of SEQ ID NO:14.
39. The TCR of any one of claims 36-38, wherein the TCR-a polypeptide comprises a CDR2 with at least 80% sequence identity to SEQ ID NO:8 and the TCR-b polypeptide comprises a CDR2 with at least 80% sequence identity to SEQ ID NO:15.
40. The TCR of claim 39, wherein the TCR-a polypeptide comprises a CDR2 comprising  
30 the amino acid sequence of SEQ ID NO:8 and the TCR-b polypeptide comprises a CDR2 comprising the amino acid sequence of SEQ ID NO:15.
41. The TCR of any one of claims 36-40, wherein the CDR1, CDR2, and CDR3 of the TCR-a polypeptide comprise the amino acid sequence of SEQ ID NO: 7, 8, and 9, respectively

and wherein the CDR1, CDR2, and CDR3 of the TCR-b polypeptide comprise the amino acid sequence of SEQ ID NO:14, 15, and 16, respectively

42. The TCR of any one of claims 36-41, wherein the TCR-a variable region comprises an amino acid sequence with at least 70% sequence identity to SEQ ID NO:5 and the TCR-b variable region comprises an amino acid sequence with at least 70% sequence identity to SEQ ID NO:12.
43. The TCR of claim 42, wherein the TCR-a polypeptide comprises an amino acid sequence with at least 70% sequence identity to SEQ ID NO:3 and the TCR-b polypeptide comprises an amino acid sequence with at least 70% sequence identity to SEQ ID NO:10.
44. An engineered T-cell Receptor (TCR) comprising a TCR-a polypeptide and a TCR-b polypeptide, wherein the TCR-a polypeptide comprises a CDR3 comprising an amino acid sequence with at least 80% sequence identity to AVGSMDSNYQLI (SEQ ID NO:25) and the TCR-b polypeptide comprises a CDR3 comprising an amino acid sequence with at least 80% sequence identity to ASSQERTWPYNEQF (SEQ ID NO:30).
45. The TCR of claim 44, wherein the TCR-a polypeptide comprises a CDR3 comprising the amino acid sequence of SEQ ID NO:25 and the TCR-b polypeptide comprises a CDR3 comprising the amino acid sequence of SEQ ID NO:30.
46. The TCR of claim 44 or 45, wherein the TCR comprises a TCR-a polypeptide comprising a variable region comprising CDR1, CDR2, and CDR3 and a TCR-b polypeptide comprising a variable region comprising CDR1, CDR2, and CDR3.
47. The TCR of claim 46, wherein the TCR-a polypeptide comprises a CDR1 with at least 80% sequence identity to SEQ ID NO:23 and/or the TCR-b polypeptide comprises a CDR1 with at least 80% sequence identity to SEQ ID NO:14.
48. The TCR of claim 47, wherein the TCR-a polypeptide comprises a CDR1 comprising the amino acid sequence of SEQ ID NO:23 and the TCR-b polypeptide comprises a CDR1 comprising the amino acid sequence of SEQ ID NO:14.
49. The TCR of any one of claims 46-48, wherein the TCR-a polypeptide comprises a CDR2 with at least 80% sequence identity to SEQ ID NO:24 and the TCR-b polypeptide comprises a CDR2 with at least 80% sequence identity to SEQ ID NO:15.
50. The TCR of claim 49, wherein the TCR-a polypeptide comprises a CDR2 comprising the amino acid sequence of SEQ ID NO:24 and the TCR-b polypeptide comprises a CDR2 comprising the amino acid sequence of SEQ ID NO:15.
51. The TCR of any one of claims 46-50, wherein the CDR1, CDR2, and CDR3 of the TCR-a polypeptide comprise the amino acid sequence of SEQ ID NO: 23, 24, and 25,

respectively and wherein the CDR1, CDR3, and CDR3 of the TCR-b polypeptide comprise the amino acid sequence of SEQ ID NO:14, 15, and 30, respectively.

52. The TCR of any one of claims 46-51, wherein the TCR-a variable region comprises an amino acid sequence with at least 70% sequence identity to SEQ ID NO:21 and the TCR-b variable region comprises an amino acid sequence with at least 70% sequence identity to SEQ ID NO:28.
53. The TCR of claim 52, wherein the TCR-a polypeptide comprises an amino acid sequence with at least 70% sequence identity to SEQ ID NO:19 and the TCR-b polypeptide comprises an amino acid sequence with at least 70% sequence identity to SEQ ID NO:26.
54. The TCR of any one of claims 34-53, wherein the TCR comprises a modification or is chimeric.
55. The TCR of any one of claims 34-54, wherein the TCR-a polypeptide and TCR-b polypeptide are operably linked.
56. The TCR of claim 55, wherein the TCR-a polypeptide and TCR-b polypeptide are operably linked through a peptide bond.
57. The TCR of claim 56, wherein 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.
58. The TCR of claim 56, wherein 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.
59. The TCR of any one of claims 34-58, wherein the TCR is a single chain TCR.
60. A fusion protein comprising the TCR of any one of claims 34-57 and a CD3 binding region.
61. The fusion protein of claim 60, 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.
62. The TCR of any one of claims 34-59 or the fusion protein of claim 60 or 61, wherein the TCR or fusion protein is conjugated to a detection or therapeutic agent.
63. The TCR or fusion protein of claim 62, wherein the agent comprises a fluorescent molecule, radiative molecule, or toxin.
64. A nucleic acid encoding a TCR-a polypeptide comprising a CDR3 comprising an amino acid sequence with at least 80% sequence identity to LVVEGNRDDKII (SEQ ID NO:9) and the TCR-b polypeptide comprises a CDR3 comprising an amino acid sequence with at least 80% sequence identity to ASPPTPSSYNEQF (SEQ ID NO:16).

65. The nucleic acid of claim 64, 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.
66. The nucleic acid of claim 65, wherein the nucleic acid encodes for a TCR-a comprising a CDR1 with at least 80% sequence identity to SEQ ID NO:7 and/or a TCR-b comprising a CDR1 with at least 80% sequence identity to SEQ ID NO:14.
67. The nucleic acid of claim 65 or 66, wherein the nucleic acid encodes for a TCR-a comprising a CDR2 with at least 80% sequence identity to SEQ ID NO:8 and a TCR-b comprising a CDR2 with at least 80% sequence identity to SEQ ID NO:15.
68. The nucleic acid of any one of claims 64-67, wherein the nucleic acid encodes a TCR-a variable region comprising an amino acid sequence with at least 70% sequence identity to SEQ ID NO:5 and/or a TCR-b variable region comprising an amino acid sequence with at least 70% sequence identity to SEQ ID NO:12.
69. The nucleic acid of claim 68, wherein the nucleic acid encodes a TCR-a polypeptide chain comprising an amino acid sequence with at least 70% sequence identity to SEQ ID NO:3 and/or a TCR-b chain comprising an amino acid sequence with at least 70% sequence identity to SEQ ID NO:10.
70. The nucleic acid of claim 69, wherein the nucleic acid comprises SEQ ID NO:1 and/or SEQ ID NO:2.
71. A nucleic acid encoding a TCR-a polypeptide comprising a CDR3 comprising an amino acid sequence with at least 80% sequence identity to AVGSMDSNYQLI (SEQ ID NO:25) and the TCR-b polypeptide comprises a CDR3 comprising an amino acid sequence with at least 80% sequence identity to ASSQERTWPYNEQF (SEQ ID NO:30).
72. The nucleic acid of claim 71, 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.
73. The nucleic acid of claim 72, wherein the nucleic acid encodes for a TCR-a comprising a CDR1 with at least 80% sequence identity to SEQ ID NO:23 and/or a TCR-b comprising a CDR1 with at least 80% sequence identity to SEQ ID NO:14.
74. The nucleic acid of claim 72 or 73, wherein the nucleic acid encodes for a TCR-a comprising a CDR2 with at least 80% sequence identity to SEQ ID NO:24 and a TCR-b comprising a CDR2 with at least 80% sequence identity to SEQ ID NO:15.
75. The nucleic acid of any one of claims 71-74, wherein the nucleic acid encodes a TCR-a variable region comprising an amino acid sequence with at least 70% sequence identity to

SEQ ID NO:21 and/or a TCR-b variable region comprising an amino acid sequence with at least 70% sequence identity to SEQ ID NO:28.

76. The nucleic acid of claim 75, wherein the nucleic acid encodes a TCR-a polypeptide chain comprising an amino acid sequence with at least 70% sequence identity to SEQ ID NO:19 and/or a TCR-b chain comprising an amino acid sequence with at least 70% sequence identity to SEQ ID NO:26.
77. The nucleic acid of claim 76, wherein the nucleic acid comprises SEQ ID NO:17 and/or SEQ ID NO:18.
78. The nucleic acid of any one of claims 64-77, wherein the nucleic acid comprises a TCR-a (TRA) and TCR-b (TRB) gene.
79. The nucleic acid of any one of claims 64-78, wherein the nucleic acid is polycistronic.
80. The nucleic acid of claim 79, wherein the nucleic acid comprises an internal ribosome entry site (IRES) or a P2A linker.
81. The nucleic acid(s) of any one of claims 64-80, wherein the nucleic acid comprises a cDNA encoding the TCR-a and/or TCR-b genes.
82. The nucleic acid(s) of any one of claims 64-81, wherein the nucleic acid further encodes for a polypeptide comprising a CD3 binding region.
83. The nucleic acid of claim 82, 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.
84. A nucleic acid vector comprising the nucleic acid(s) of any one of claims 33 or 64-83.
85. The vector of claim 84, wherein the vector comprises a promoter that directs the expression of the nucleic acid.
86. The vector of claim 85, wherein the promoter comprises a murine stem cell virus (MSCV) promoter.
87. The vector of any one of claims 84-86, wherein the vector comprises the TCR-a and TCR-b genes.
88. A cell comprising the polypeptide of any one of claims 1-32, TCR of any one of claims 34-59, 62, or 63, the fusion protein of any one of claims 60-63, the nucleic acid(s) of any one of claims 33 or 64-83, or the vector of any one of claims 84-87.
89. The cell of claim 88, wherein the cell comprises a stem cell, a progenitor cell, an immune cell, or a natural killer (NK) cell.

90. The cell of claim 89, 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).
91. The cell of claim 89 or 90, wherein the cell is isolated or derived from peripheral blood mononuclear cell (PBMCs).
92. The cell of claim 90 or 91, wherein the T cell comprises a cytotoxic T lymphocyte (CTL), a CD8<sup>+</sup> T cell, a CD4<sup>+</sup> T cell, an invariant NK T (iNKT) cell, a gamma-delta T cell, a NKT cell, or a regulatory T cell.
93. The cell of any one of claims 88-92, wherein the cell is isolated from a cancer patient.
94. A composition comprising the polypeptide of any one of claims 1-32, TCR of any one of claims 34-59, 62, or 63, the fusion protein of any one of claims 60-63, the nucleic acid(s) of any one of claims 33 or 64-83, the vector of any one of claims 84-87, or the cell of any one of claims 88-93.
95. The composition of claim 94, wherein the composition has been determined to be serum-free, mycoplasma-free, endotoxin-free, and/or sterile.
96. A method of making an engineered cell comprising transferring the nucleic acid of any one of claims 33 or 64-83 or the vector of any one of claims 84-87 into a cell.
97. The method of claim 96, 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.
98. A method for treating cancer in a subject comprising administering the composition of claim 94 or 95 or the cells of any one of claims 88-93 to a subject in need thereof.
99. The method of claim 98, wherein the subject has previously been treated for the cancer.
100. The method of claim 99, wherein the subject has been determined to be resistant to the previous treatment.
101. The method of any one of claims 98-100, wherein the method further comprises the administration of an additional therapy.
102. The method of any one of claims 98-101, wherein the cancer comprises stage I, II, III, or IV cancer.
103. The method of any one of claims 98-102, wherein the cancer comprises metastatic and/or recurrent melanoma.
104. The method of any one of claims 98-103, wherein the cancer comprises lung cancer, esophagus carcinoma, head and neck cancer, ovarian cancer, or melanoma.
105. The method of any one of claims 98-104, wherein the cancer comprises lung cancer.

106. The method of any one of claims 98-105, wherein the subject is and/or has been determined to be HLA-A2 positive.

107. The method of claim 106, wherein the subject is and/or has been determined to be HLA-A0201 positive.



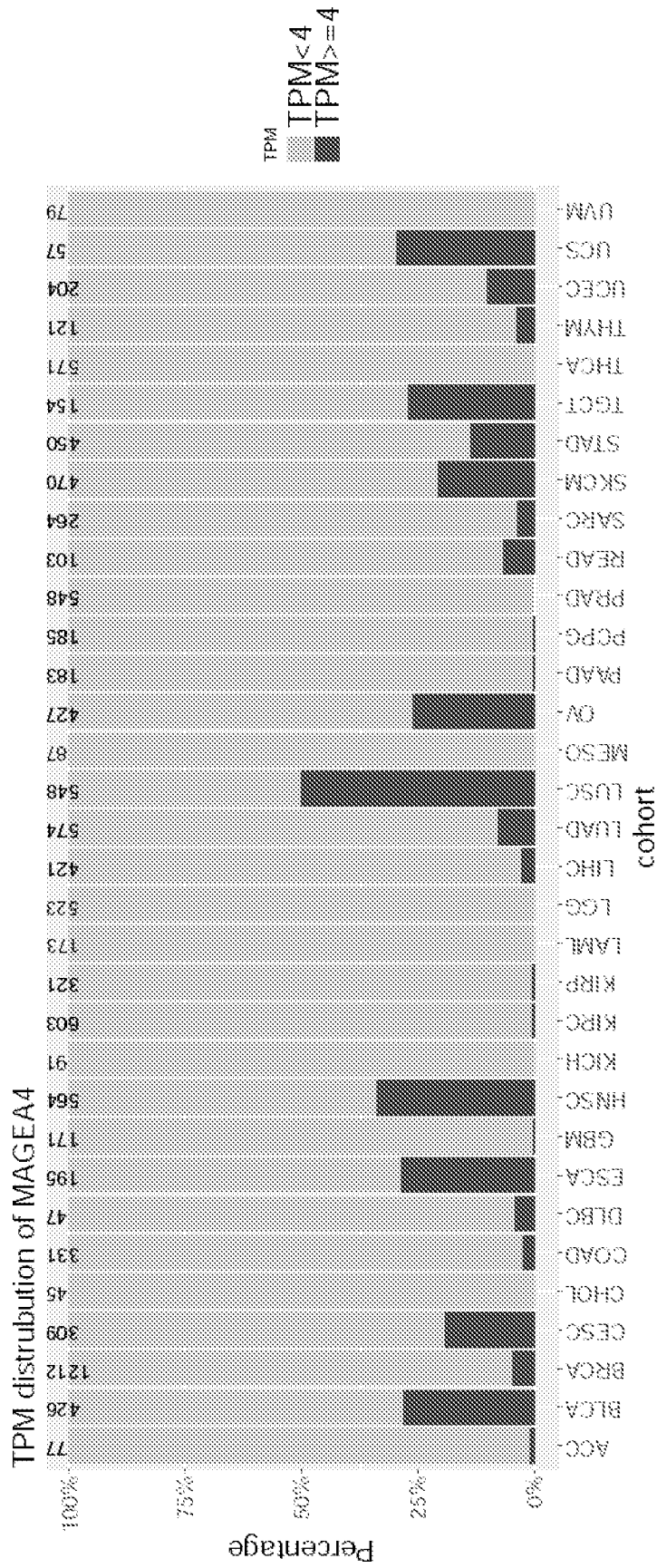


FIG. 2

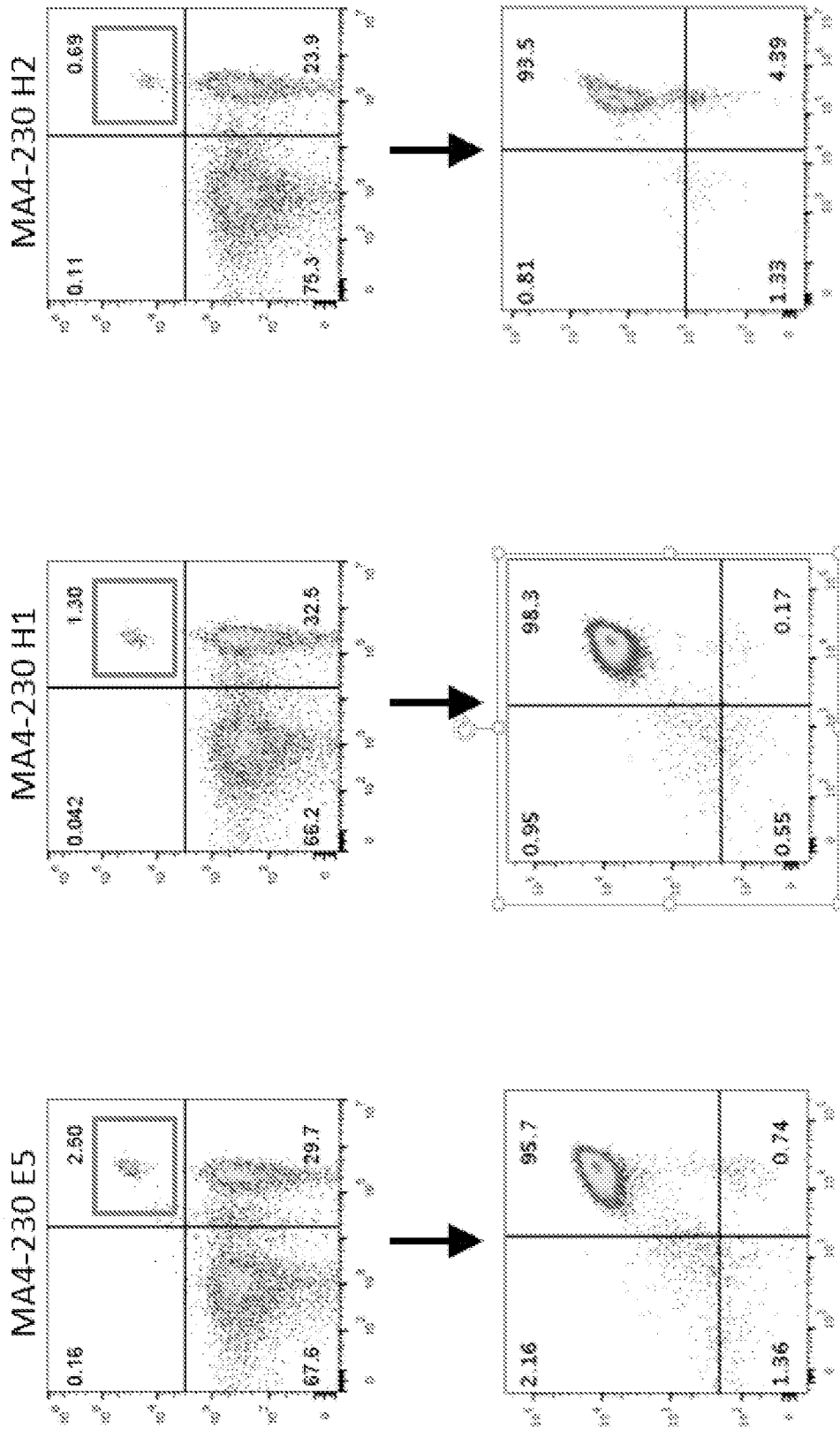


FIG. 3

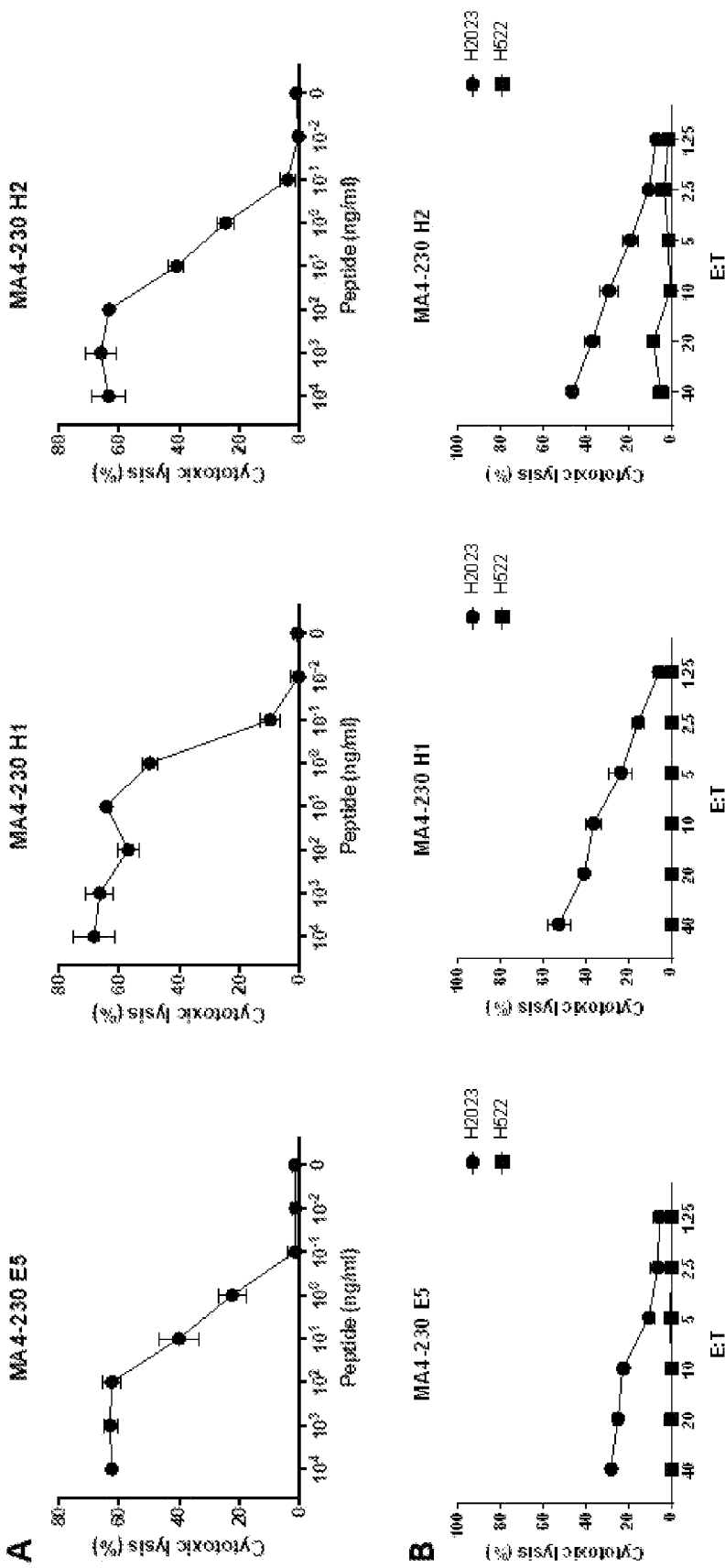


FIG. 4A-B

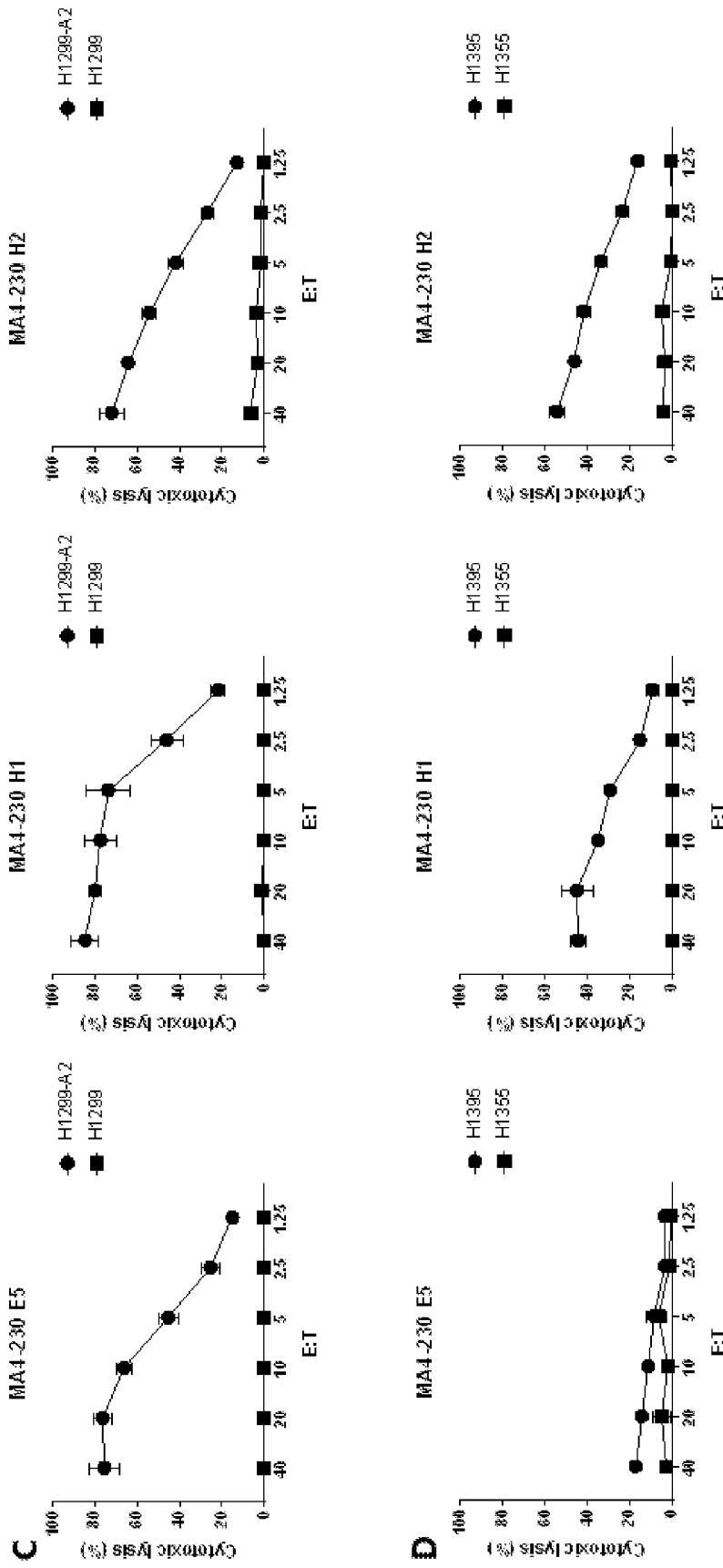


FIG. 4C-D

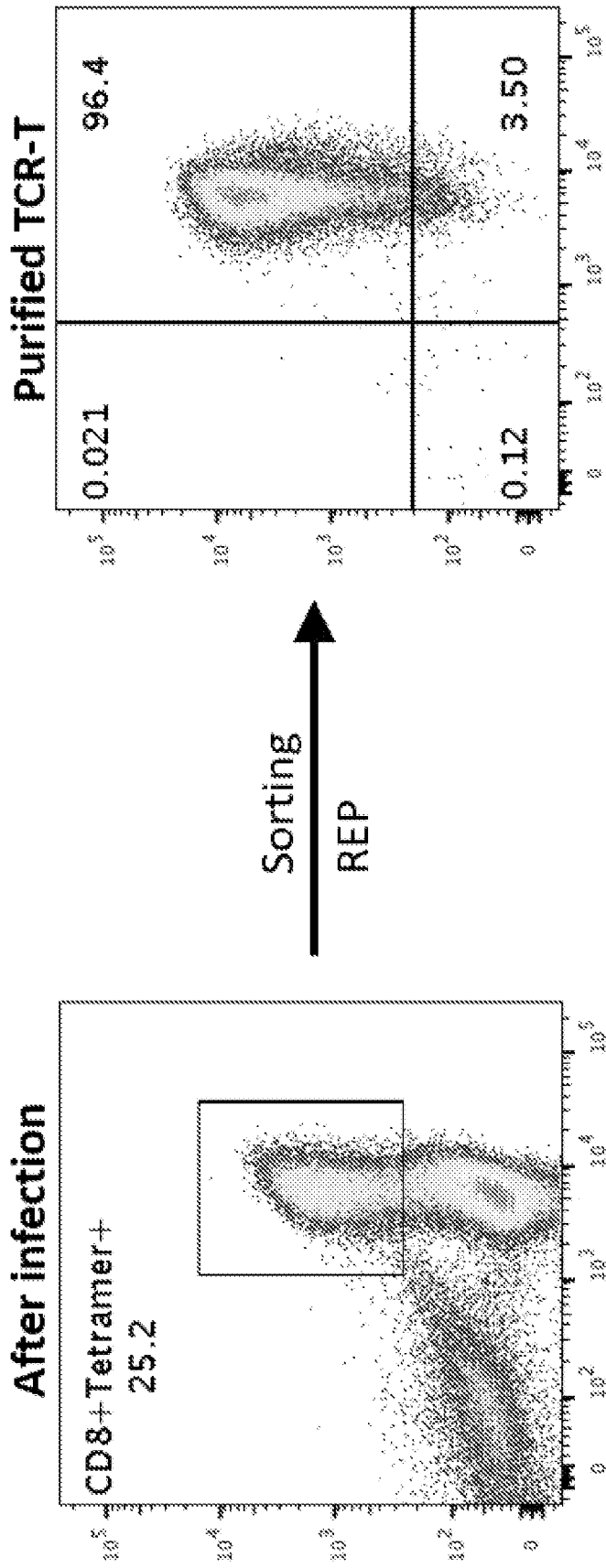


FIG. 5

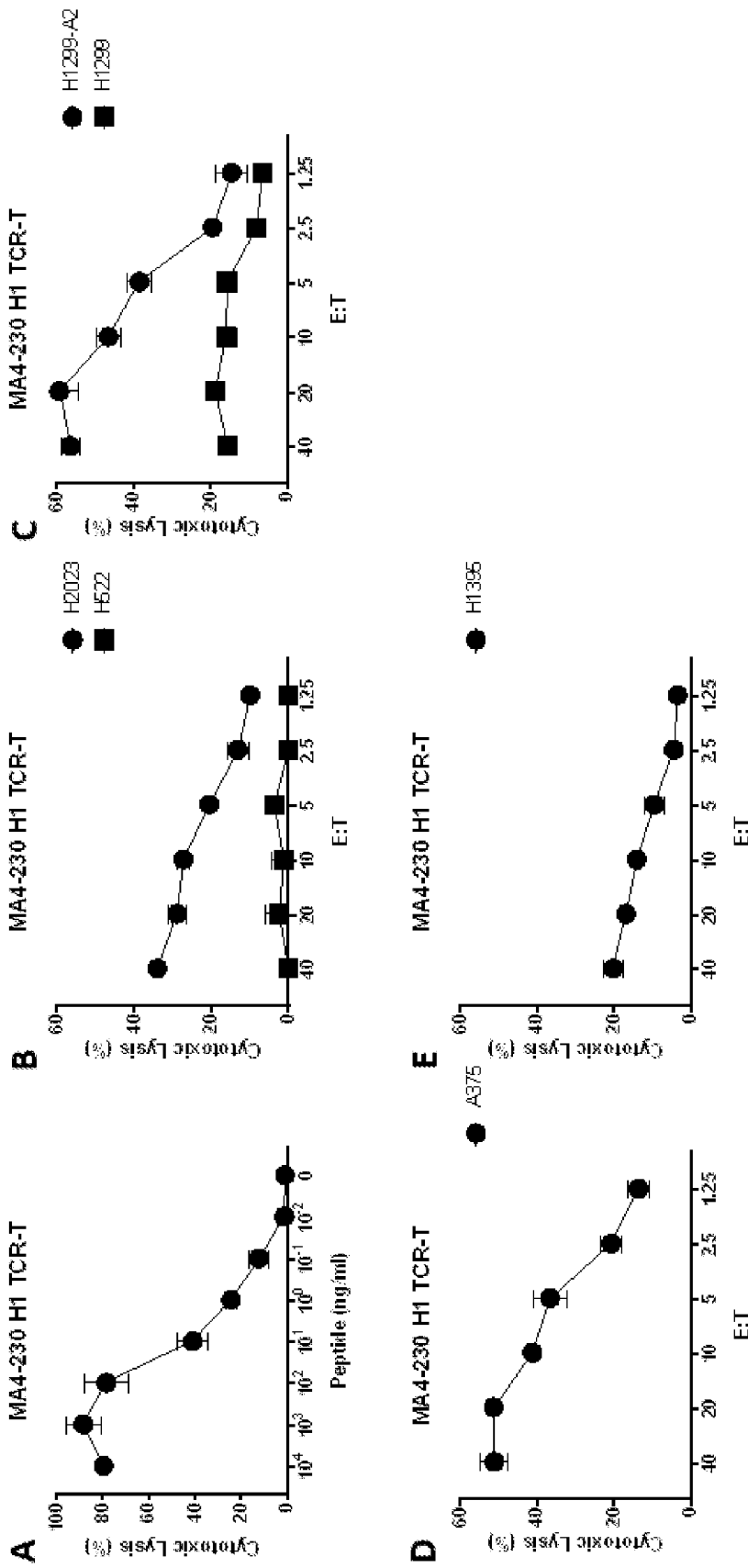


FIG. 6A-E

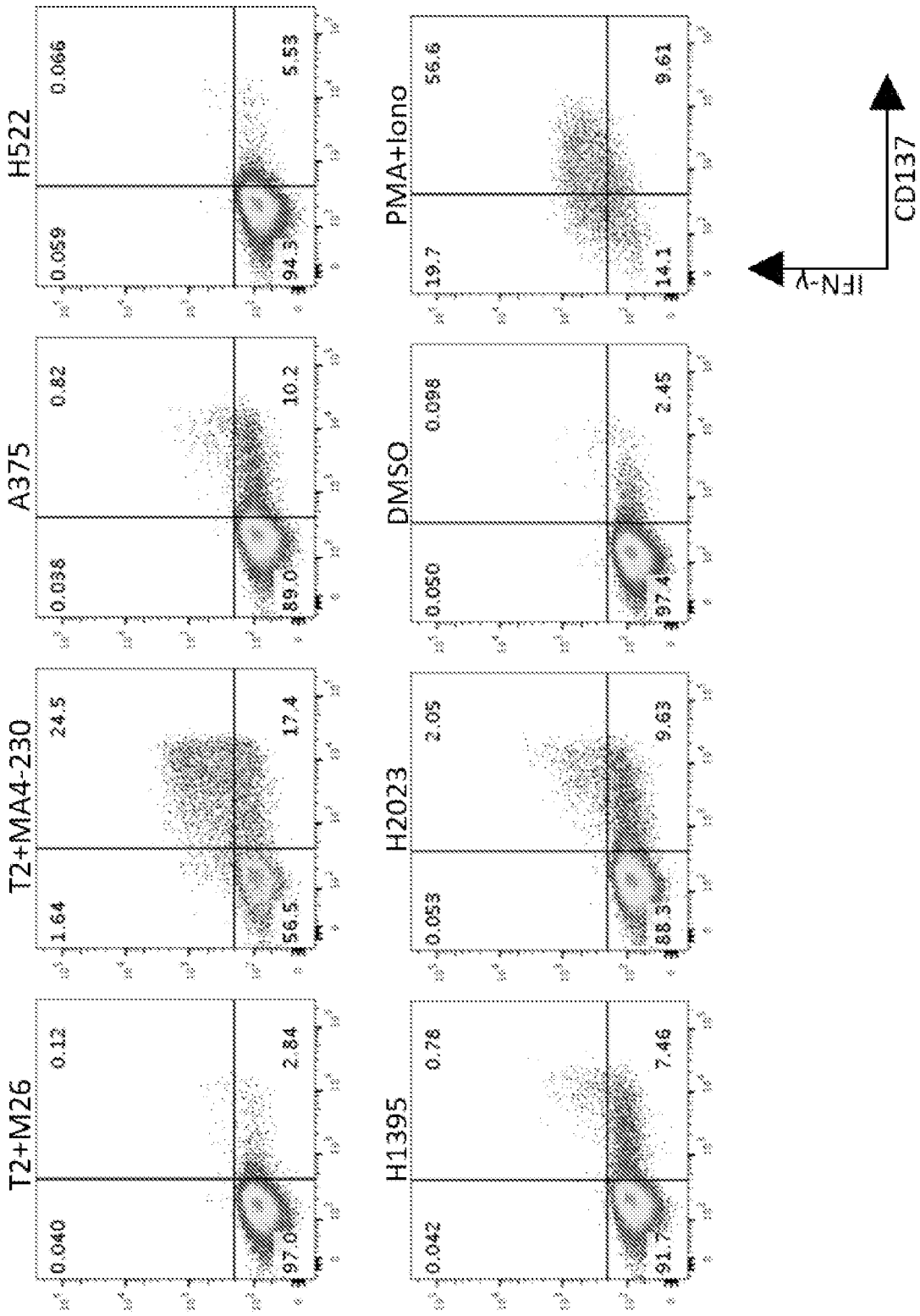


FIG. 7A

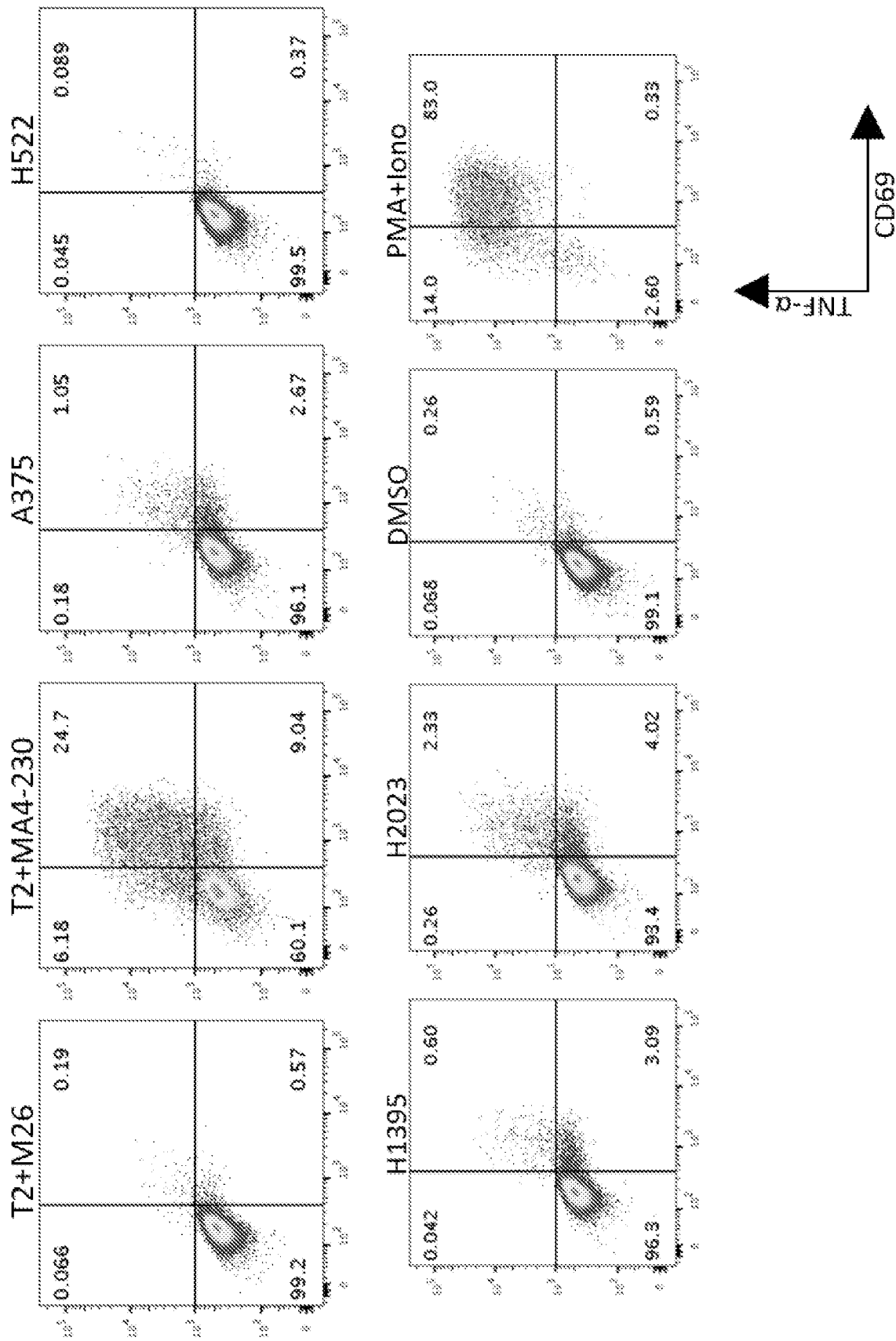


FIG. 7B

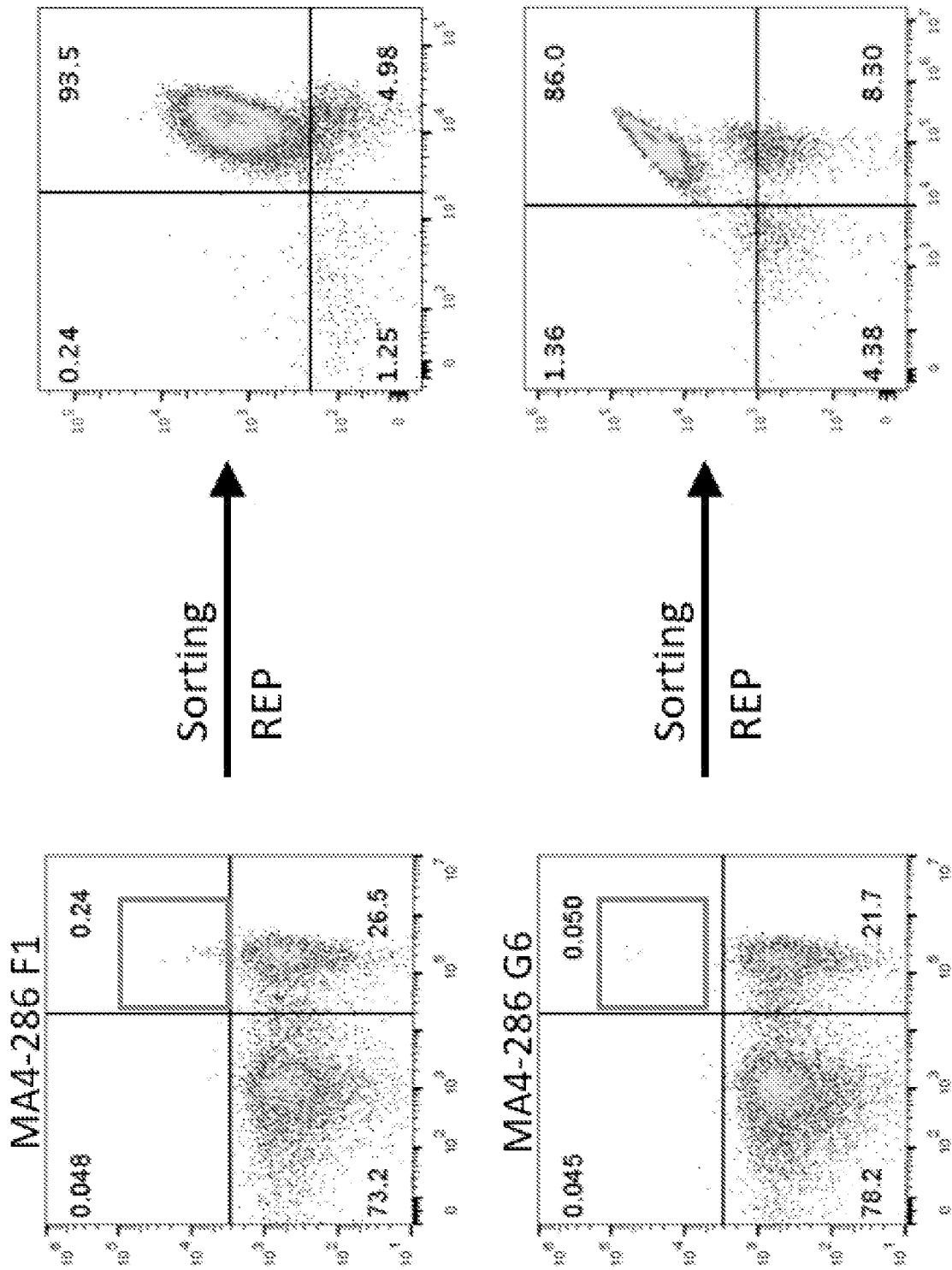


FIG. 8

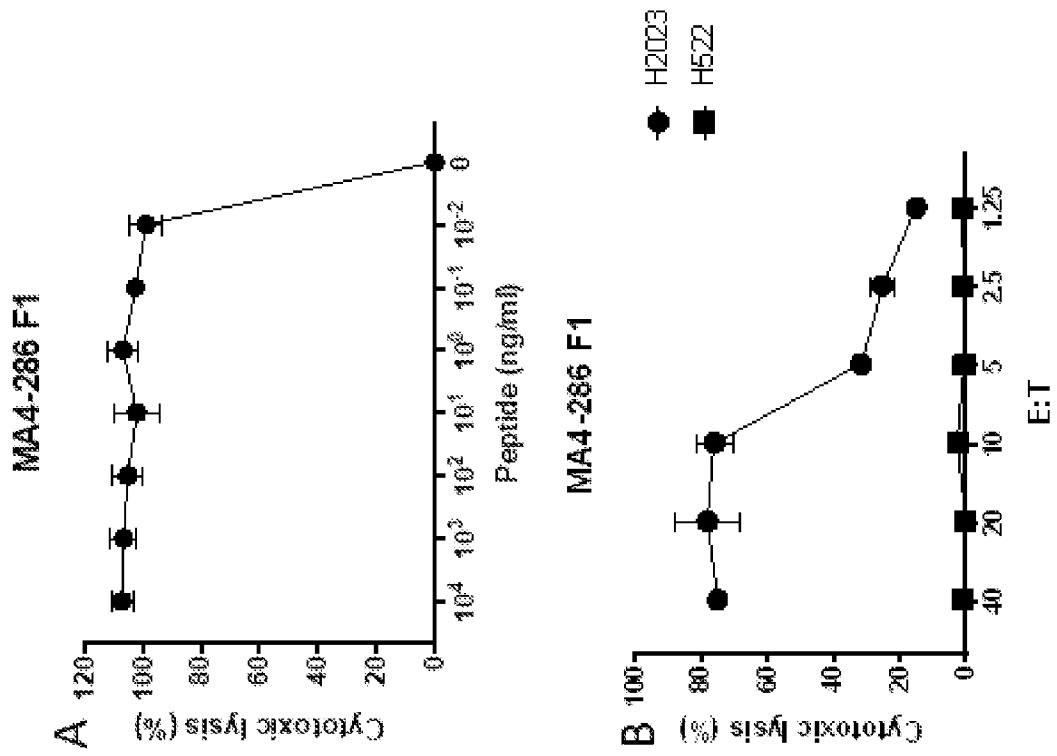
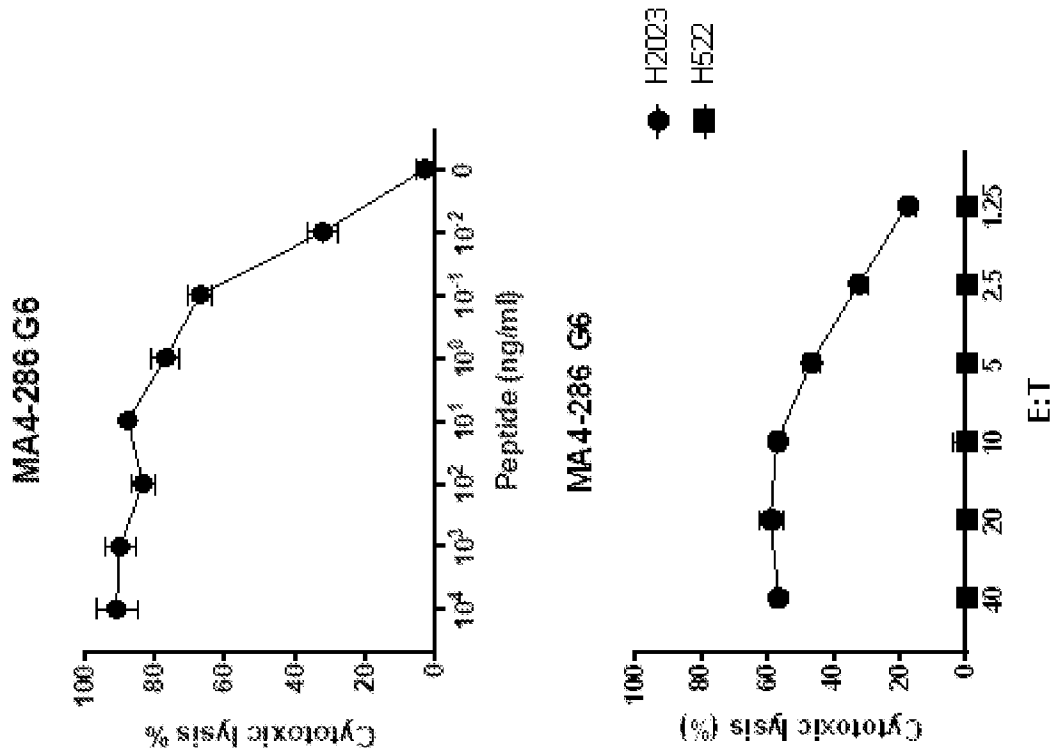


FIG. 9A-B

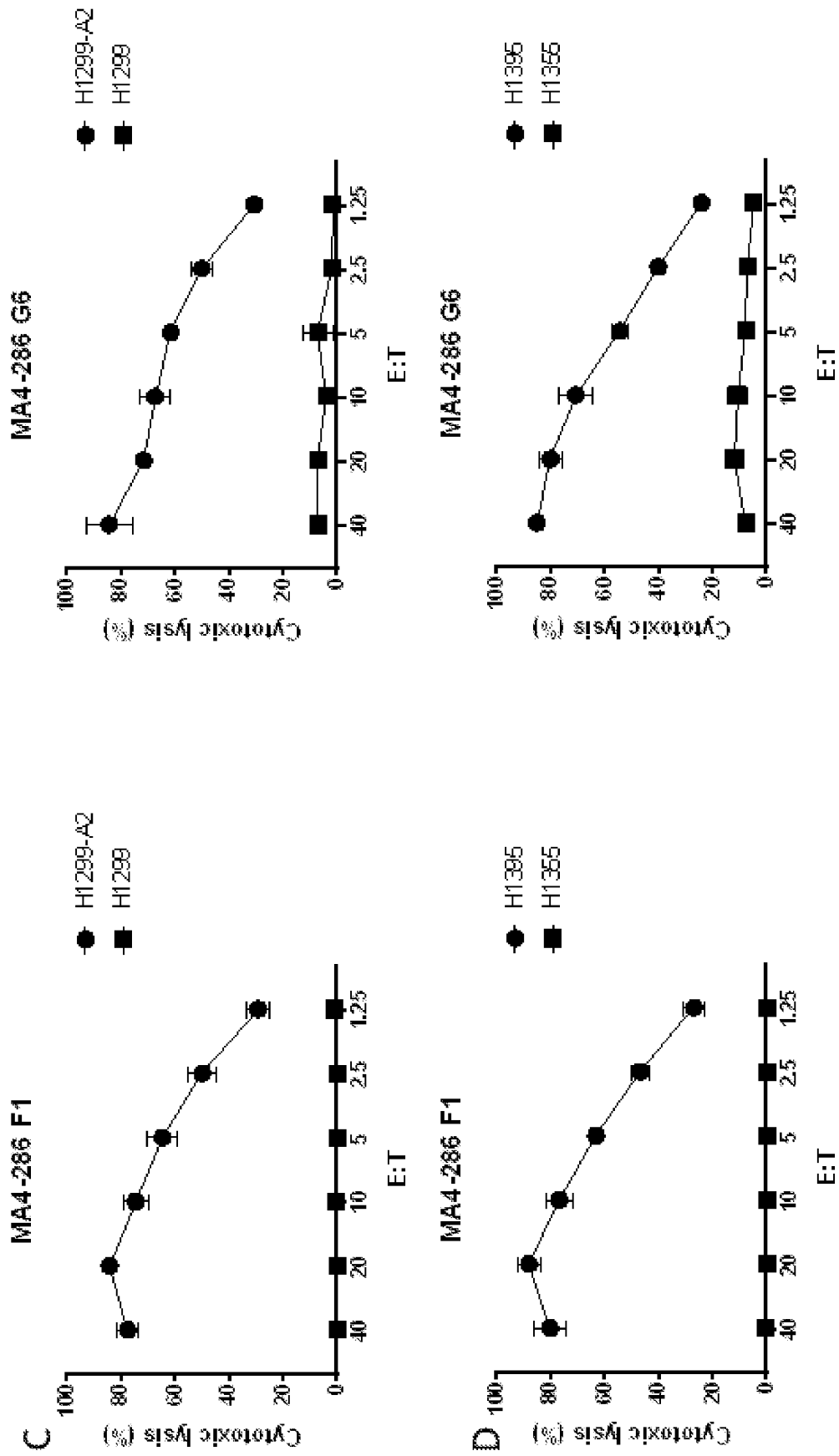


FIG. 9C-D

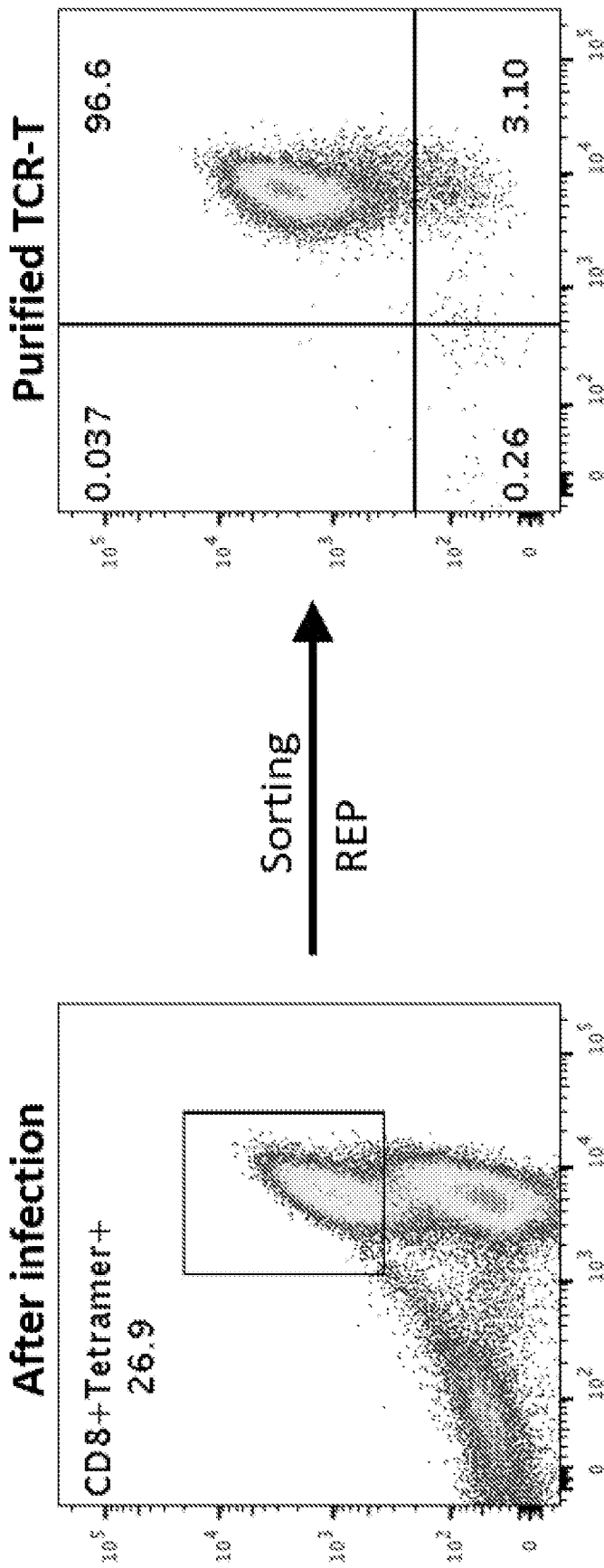


FIG. 10

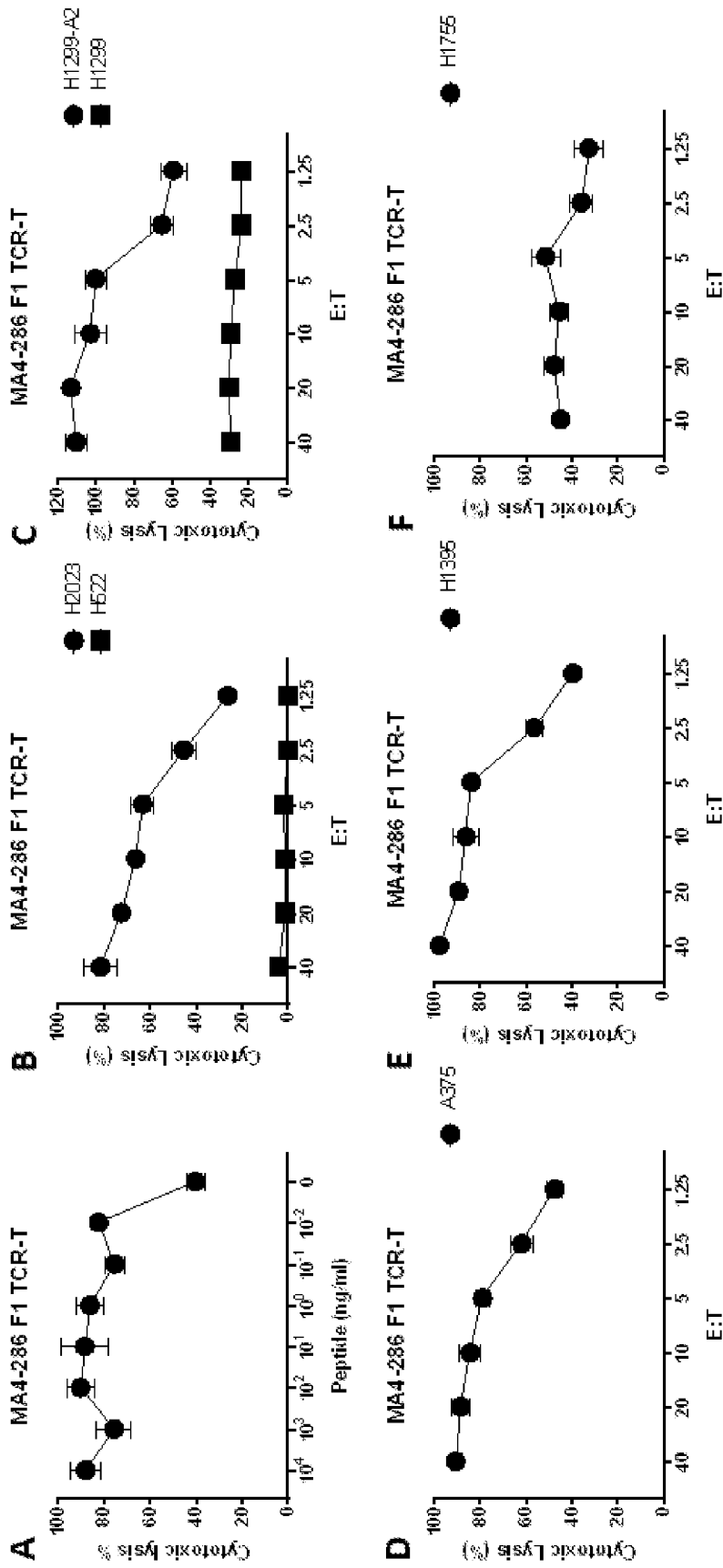


FIG. 11A-F

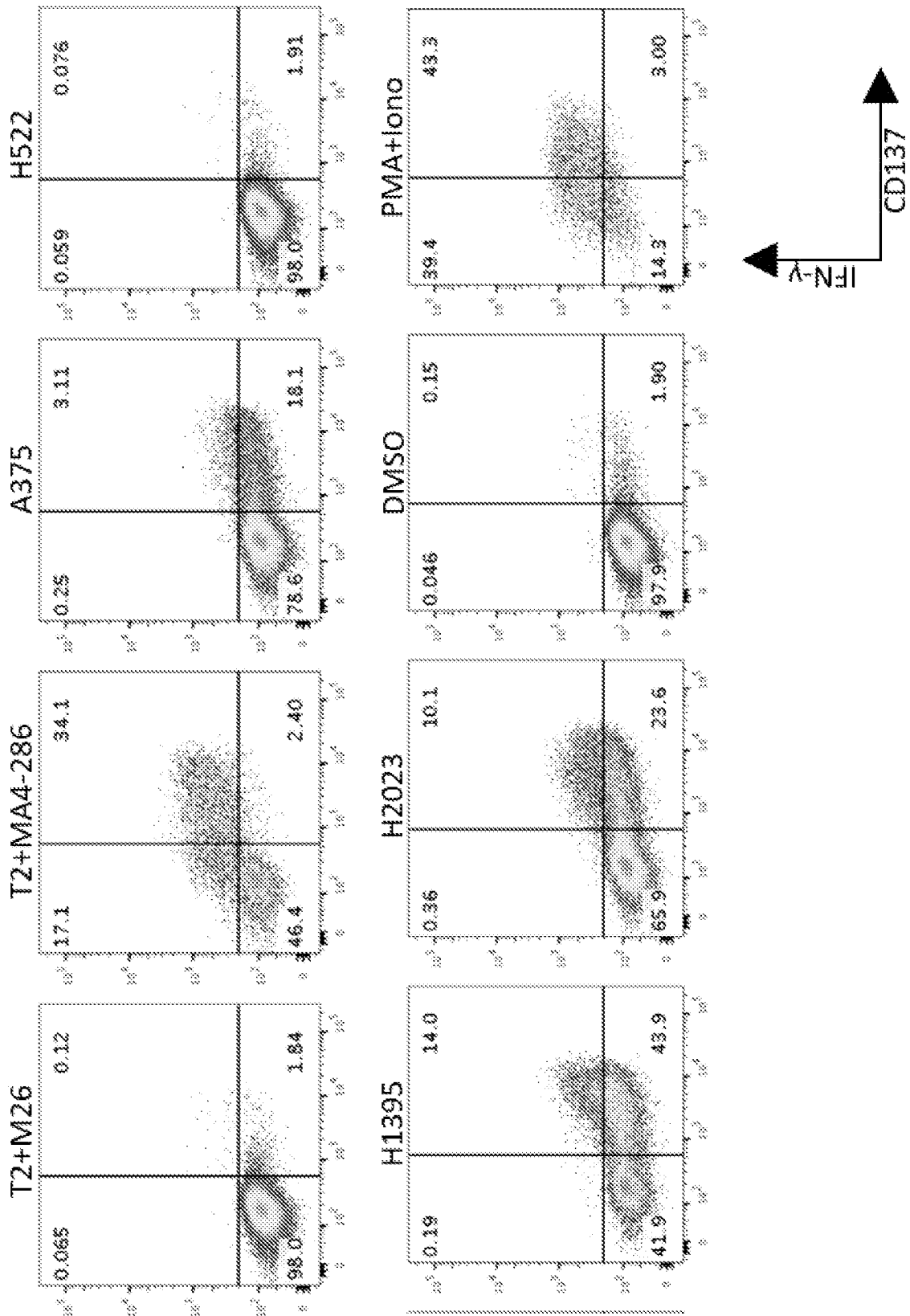


FIG. 12A

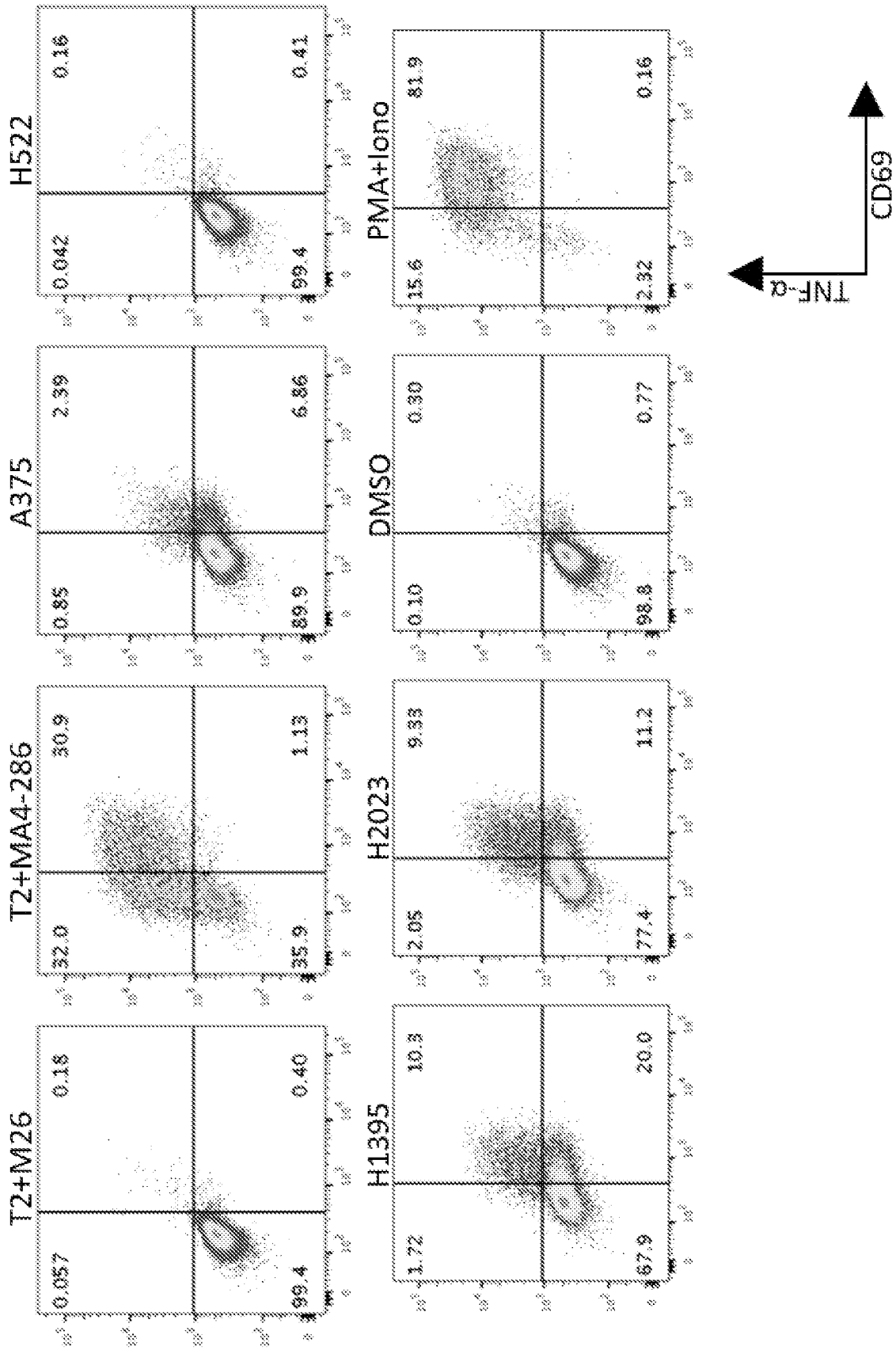


FIG. 12B

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 21/32818

A. CLASSIFICATION OF SUBJECT MATTER  
 IPC - C07K 14/725, A61K 35/17, C12N 5/0783 (2021.01)  
 CPC - C07K 14/7051, A61K 35/17, C12N 5/0636

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	US 2012/0225481 A1 (JAKOBSEN et al.) 06 September 2012 (06.09.2012) para [0007], [0066], [0168], Abstract	1-4, 9-12, 34-38
A	UniProtKB Accession No. R5L168 "R5L168_9CLOT", 24 July 2013 [online]. [Retrieved on 20 September 2021]. Retrieved from the internet: <URL: https://www.uniprot.org/uniprot/R5L168> Entire document; sequence residues 1-297	1-4, 34-38
A	WO 2019/133853 A1 (GRITSTONE ONCOLOGY, INC.) 04 July 2019 (04.07.2019) Claim 60, Claim 61, Claim 78, Page 221	1-4, 34-38
A	WO 2019/129892 A1 (MAX-DELBRLTCK-CENTRUM F R MOLEKULARE MEDIZIN IN DER HELMHOLTZGEMEINSCHAFT) 04 July 2019 (04.07.2019) pg 10 para 3, pg 13 para 2, SEQ ID No: 139	4
A	WO 2008/108257 A1 (INTERNATIONAL INSTITUTE OF CANCER IMMUNOLOGY, INC.) 12 September 2008 (12.09.2008) Claim 3, SEQ ID No: 3016, Abstract	9-12, 34-38
A	WO 2013/002086 A1 (INTERNATIONAL INSTITUTE OF CANCER IMMUNOLOGY, INC.) 03 January 2013 (03.01.2013). Claim 3, Abstract, SEQ ID No: 1492	9-12, 34-38

Further documents are listed in the continuation of Box C.

See patent family annex.

* Special categories of cited documents:	"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
"A" document defining the general state of the art which is not considered to be of particular relevance	"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
"D" document cited by the applicant in the international application	"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
"E" earlier application or patent but published on or after the international filing date	"&" document member of the same patent family
"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	

Date of the actual completion of the international search  
 04 October 2021

Date of mailing of the international search report  
**OCT 29 2021**

Name and mailing address of the ISA/US  
 Mail Stop PCT, Attn: ISA/US, Commissioner for Patents  
 P.O. Box 1450, Alexandria, Virginia 22313-1450  
 Facsimile No. 571-273-8300

Authorized officer  
 Kari Rodriguez  
 Telephone No. PCT Helpdesk: 571-272-4300

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 21/32818

**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:

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 21/32818

**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:
  
3.  Claims Nos.: 5-8, 13-16, 21-24, 29-33, 39-43, 49-63, 68-70, 75-107  
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:  
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 searched, the appropriate additional search fees must be paid.

-----Please see continuation in first extra sheet-----

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, 9-12, 34-38, limited to engineered TCR comprising TCRa CDR3 of SEQ ID NO:9, CDR1 of SEQ ID NO:7, and TCRb CDR3 of SEQ ID NO:16, and CDR1 of SEQ ID NO:14

- 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.

Continuation of Box No. III. Observations where unity of invention is lacking

Group I+, Claims 1-4, 9-12, 17-20, 25-28, 34-38, 44-48, directed to engineered T-cell Receptor (TCR) comprising a TCR-a polypeptide and a TCR-b polypeptide. The engineered TCR will be searched to the extent that the TCR encompasses a polypeptide comprising a TCR alpha (TCR-a) variable region comprising a CDR3 comprising an amino acid sequence with at least 100% sequence identity to LVVEGNRDDKII (SEQ ID NO:9), and CDR1 with at least 100% sequence identity to NIATNDY (SEQ ID NO:7) and a polypeptide comprising a TCR beta (TCR-b) variable region comprising a CDR3 comprising an amino acid sequence with at least 100% sequence identity to ASPPTPSSYNEQF (SEQ ID NO:16), and TCR-b variable region comprises a CDR1 with at least 100% sequence identity to MNHEY (SEQ ID NO:14) (note, these are the first claimed sequences for the inventive TCR polypeptides). It is believed that claims 1-4, 9-12, 34-38 encompass this first named invention, and thus these claims will be searched without fee to the extent that the TCR encompasses a polypeptide comprising a TCR alpha (TCR-a) variable region comprising a CDR3 comprising an amino acid sequence with at least 100% sequence identity to LVVEGNRDDKII (SEQ ID NO:9), and CDR1 with at least 100% sequence identity to NIATNDY (SEQ ID NO:7) and a polypeptide comprising a TCR beta (TCR-b) variable region comprising a CDR3 comprising an amino acid sequence with at least 100% sequence identity to ASPPTPSSYNEQF (SEQ ID NO:16), and TCR-b variable region comprises a CDR1 with at least 100% sequence identity to MNHEY (SEQ ID NO:14). Additional TCR polypeptide(s) will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected TCR polypeptide(s). Applicants must further indicate, if applicable, the claims which encompass 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. An exemplary election would be a TCR encompassing a polypeptide comprising a TCR alpha (TCR-a) variable region comprising a CDR3 comprising an amino acid sequence with at least 100% sequence identity to AVGSMDSNYQLI (SEQ ID NO:25), further wherein the TCR-a variable region comprises a CDR1 with at least 100% sequence identity to TLLSN (SEQ ID NO:23), and a polypeptide comprising a TCR beta (TCR-b) variable region comprising a CDR3 comprising an amino acid sequence with at least 100% sequence identity to ASSQERTWPYNEQF (SEQ ID NO:30), further wherein the TCR-b variable region comprises a CDR1 with at least 100% sequence identity to MNHEY (SEQ ID NO:14) (claims 17-20, 25-28, 44-48).

Group II+, claims 64-67, 71-74, directed to a nucleic acid encoding TCR polypeptides. Group II+ will be searched upon payment of additional fees. The nucleic acid may be searched, for example, to encompass nucleic acid encoding a TCR-a polypeptide comprising a CDR3 comprising an amino acid sequence with at least 100% sequence identity to LVVEGNRDDKII (SEQ ID NO:9) and the TCR-b polypeptide comprises a CDR3 comprising an amino acid sequence with at least 100% sequence identity to ASPPTPSSYNEQF (SEQ ID NO:16), further wherein the nucleic acid encodes for a TCR-a comprising a CDR1 with at least 100% sequence identity to SEQ ID NO:7, a TCR-a comprising a CDR2 with at least 100% sequence identity to SEQ ID NO:8 and/or a TCR-b comprising a CDR1 with at least 100% sequence identity to SEQ ID NO:14, and a TCR-b comprising a CDR2 with at least 100% sequence identity to SEQ ID NO:15, for an additional fee and election as such. It is believed that claims 64-67 read on this exemplary invention. Additional nucleic acid(s) encoding additional TCR polypeptide(s) will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected nucleic acid(s) encoding additional TCR polypeptide(s). 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. Another exemplary election would be nucleic acid encoding a TCR-a polypeptide comprising a CDR3 comprising an amino acid sequence with at least 100% sequence identity to AVGSMDSNYQLI (SEQ ID NO:25) and the TCR-b polypeptide comprises a CDR3 comprising an amino acid sequence with at least 100% sequence identity to ASSQERTWPYNEQF (SEQ ID NO:30), further wherein the nucleic acid encodes for a TCR-a comprising a CDR1 with at least 100% sequence identity to SEQ ID NO:23 and a TCR-a comprising a CDR2 with at least 100% sequence identity to SEQ ID NO:24, and/or a TCR-b comprising a CDR1 with at least 100% sequence identity to SEQ ID NO:14 and a TCR-b comprising a CDR2 with at least 100% sequence identity to SEQ ID NO:15 (claims 71-74).

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

#### Special technical features

The inventions of Groups I+ and II+ each include the special technical feature of a unique amino acid or polynucleotide sequence. Each amino acid sequence comprises a unique peptide, and is considered a distinct technical feature. Each polynucleotide sequence encodes a unique peptide, and is considered a distinct technical feature.

Additionally, Group I+ has the special technical feature of an engineered T-cell Receptor (TCR) comprising a TCR-a polypeptide and a TCR-b polypeptide, that is not required by Group II+.

Group II+ has the special technical feature of nucleic acid encoding TCR-a and TCR-b polypeptides comprising a CDR 1-3, that is not required by Group I+.

-----please see continuation on next sheet -----

Continuation of Box No. III. Observations where unity of invention is lacking

Common technical features

No technical features are shared between the TCR amino acid and/or polynucleotide sequences of Groups I+ and II+ and, accordingly, these groups lack unity a priori.

Additionally, even if Groups I+ and II+ were considered to share the technical features of including: an engineered T-cell Receptor (TCR) comprising a TCR-a polypeptide and a TCR-b polypeptide; a nucleic acid encoding TCR-a and TCR-b polypeptides comprising a CDR 1-3; these shared technical features are previously disclosed by US 2012/0225481 A1 to Jakobsen et al. (hereinafter 'Jakobsen').

Jakobsen discloses an engineered T-cell Receptor (TCR) comprising a TCR-a polypeptide and a TCR-b polypeptide (Abstract - "A T cell receptor (TCR)...comprising a TCR alpha variable domain and/or a TCR beta variable domain"; para [0066] - "According to the invention, there is provided an isolated or non-naturally occurring or engineered T cell receptor (TCR)...comprising a TCR alpha variable domain and/or a TCR beta variable domain"), wherein the TCR-a polypeptide comprises a CDR3 comprising an amino acid sequence (para [0168] - "These alpha chains are mutated only in CDR3"; para [0007] - "Each variable region comprises three CDRs (Complementarity Determining Regions) embedded in a framework sequence, one being the hypervariable region named CDR3. There are several types of alpha chain variable (Va) regions and several types of beta chain variable (Vb) regions distinguished by their framework...and by a partly defined CDR3 sequence.") and the TCR-b polypeptide comprises a CDR3 comprising an amino acid sequence (para [0168] - "the beta chains are mutated in one or more of...CDR3."; para [0007]).

Jakobsen further discloses a nucleic acid encoding a TCR-a polypeptide comprising a CDR3, CDR2 and CDR1 (Abstract - "A T cell receptor (TCR)...comprising a TCR alpha variable domain"; para [0023] - 'Another embodiment of the invention relates to DNA or RNA encoding the mutated TCRs. '; para [0168] - "These alpha chains are mutated only in CDR3"; para [0007] - "Each variable region comprises three CDRs (Complementarity Determining Regions) embedded in a framework sequence, one being the hypervariable region named CDR3. There are several types of alpha chain variable (Va) regions and several types of beta chain variable (Vb) regions distinguished by their framework...and by a partly defined CDR3 sequence.") and a TCR-b polypeptide comprising a CDR3 amino acid sequence (para [0066] - "According to the invention, there is provided an isolated or non-naturally occurring or engineered T cell receptor (TCR)...comprising...a TCR beta variable domain"; para [0168] - "the beta chains are mutated in one or more of...CDR3."; para [0007]).

As the technical features were known in the art at the time of the invention, they cannot be considered special technical features that would otherwise unify the groups.

Therefore, Group I+ and II+ inventions lack unity under PCT Rule 13 because they do not share the same or corresponding special technical feature.