

novel technologies in this field. Disclosed herein are various compositions and processes for producing peptide-MHC multimers that address these limitations.

SUMMARY

[0006] The present disclosure provides compositions and methods for identifying neoepitopes, identifying and isolating T cell receptors, engineering of primary cells to express specific T cell receptors, expanding engineered T cells and for treatment of disorders using cell therapy. In various examples, the present disclosure provides improved cell therapy methods and compositions for identifying neoepitopes, identifying and isolating T cell receptors, engineering of primary cells to express specific T cell receptors, expanding engineered T cells and for treatment of proliferative diseases, disorders, and conditions.

[0006a] In one aspect, the present disclosure refers to a method for identifying the antigen specificity of a T cell comprising:

- a) contacting a sample with a plurality of distinct particle sets,
 - i) wherein each particle comprises a polypeptide comprising, in an amino to carboxyl terminus orientation, (i) ~~a unique~~the antigen peptide, (ii) a β 2M peptide, and (iii) an MHC peptide, an operably associated barcode, and at least one identifying label,
 - ii) wherein the sample comprises T cells and is selected from a blood sample, a bone marrow sample, a tissue sample, a tumor sample, and a peripheral blood mononuclear cell (PBMC) sample, and
 - iii) wherein contacting comprises providing conditions suitable for a single T cell to bind to an antigen peptide of at least one particle set;
- b) isolating ~~a one or more~~ T cells bound to ~~the~~a particle set;
- c) identifying the barcodes of the particles bound to the isolated T cell; and
- d) determining a ratio of a most represented barcode and a second most represented~~each~~ barcode identified in (c);
- e) determining the antigen specificity of the T cell based on the ratio of the most represented barcode and the second most represented barcode;

wherein the antigen specificity of the T cell comprises the sequence of the antigen peptide and the TCR sequences of the bound T cell.

~~[0006b] In another aspect, the present disclosure refers to a method of modifying a cell, comprising:~~

- ~~a) —introducing into the cell a homologous recombination (HR) template nucleic acid sequence comprising:~~

- ~~i) first and second homology arms homologous to first and second endogenous sequences of the cell;~~
 - ~~ii) a T cell receptor (TCR) gene sequence obtained by the method as described herein, wherein the TCR gene sequence is positioned between the first and second HR arms; and~~
 - ~~iii) a first 2A coding sequence positioned upstream of the TCR gene sequence and a second 2A coding sequence positioned downstream of the TCR gene sequence, wherein the first and second 2A coding sequences code for the same amino acid sequence that are codon-diverged relative to each other;~~
- ~~b) —recombining the HR template nucleic acid into an endogenous locus of the cell comprising the first and second endogenous sequences homologous to the first and second homology arms of the HR template nucleic acid.~~

~~[0006c] In another aspect, the present disclosure refers to a composition comprising a modified cell obtained by the method as described herein.~~

~~[0006d] In yet another aspect, the present disclosure refers to a use of the composition as described herein in the manufacture of a medicament for the treatment of cancer.~~

~~[0006e] In yet another aspect, the present disclosure refers to the composition as described herein for use as a therapy.~~

[0007] In one example, provided herein is a method for identifying antigen specificity of a T cell, comprising providing two or more distinct particle sets, each distinct particle set comprising a unique antigen peptide and at least one defined barcode operably associated with the identity of the antigen peptide, and each set comprises a first particle comprising a first identifying label and a second particle comprising a second identifying label distinct from the first identifying label; providing a sample known or suspected to comprise one or more T cells; contacting the sample with the two or more particle sets, wherein the contacting comprises providing conditions sufficient for a single T cell to bind to the unique antigen of at least one particle set; isolating one or more T cells that are bound to the particle set(s) by their associated first and second identifying label; performing an assay to identify one or more barcodes bound to the particle set that is bound to the isolated T cell; determining a ratio of the barcodes bound to the isolated T cell wherein the ratio is calculated by identifying a first copy number of a predominant barcode and a second copy number of a distinct barcode and dividing the first copy number by the second copy number; and identifying the antigen specificity of the T cell based on the ratio.

[0008] In one example, provided herein is a method for identifying antigen specificity of a T cell, comprising: obtaining or having obtained at least one antigen-specific T cell bound to two or more distinct particle sets, each distinct particle set comprising a unique antigen peptide and at least one defined barcode operably associated with the identity of the antigen peptide, wherein each set comprises a first particle comprising a first identifying label and a second particle comprising a second identifying label distinct from the first identifying label; performing or having performed at least one assay to identify one or more barcodes detectably bound to the particle set that is bound to the T cell; and determining or having determined a ratio of the barcodes bound to the T cell that identifies the antigen specificity of

CLAIMS

1. A method for identifying the antigen specificity of a T cell comprising:
 - a) contacting a sample with a plurality of distinct particle sets,
 - i) wherein each particle comprises a polypeptide comprising, in an amino to carboxyl terminus orientation, (i) ~~a unique~~the antigen peptide, (ii) a β 2M peptide, and (iii) an MHC peptide, an operably associated barcode, and at least one identifying label,
 - ii) wherein the sample comprises T cells and is selected from a blood sample, a bone marrow sample, a tissue sample, a tumor sample, and a peripheral blood mononuclear cell (PBMC) sample, and
 - iii) wherein contacting comprises providing conditions suitable for a single T cell to bind to an antigen peptide of at least one particle set;
 - b) isolating ~~a one or more~~ T cells bound to ~~the~~a particle set;
 - c) identifying the barcodess of the particless bound to the isolated T cell; and
 - d) determining a ratio of a most represented barcode and a second most represented~~each~~ barcode identified in (c);
 - e) determining the antigen specificity of the T cell based on the ratio of the most represented barcode and the second most represented barcode;

wherein the antigen specificity of the T cell comprises the sequence of the antigen peptide and the TCR sequences of the bound T cell.
2. The method of claim 1, wherein the ratio is calculated by identifying a copy number of ~~the most represented~~a first barcode and a copy number of ~~the~~a second most represented barcode and dividing the copy number of the most represented~~first~~ barcode by the copy number of the second most represented barcode, optionally wherein the ratio of each barcode corresponds to the antigen specificity of the isolated T cell.
3. The method of claim 1 or 2, wherein each distinct particle set comprises at least one or more barcodes, wherein each barcode is associated with the identity of the antigen peptide.

4. The method of any one of claims 1-3, wherein the isolated T cell is identified as an antigen-specific T cell if the ratio of the first barcode is above a threshold, optionally wherein the threshold is greater than 2.
5. The method of any of the claims 1-4, wherein the identifying the barcode determines a sequence of each barcode and/or a copy number of each barcode and comprises a nucleotide-based assay comprising PCR, RT-PCR, sequencing, hybridization assay, or a combination thereof.
6. The method of any one of claims 1-5, wherein the at least one identifying label is a fluorophore, optionally wherein the fluorophore is allophycocyanin (APC) or phycoerythrin (PE).
7. The method of any one of claims 1-6, wherein the antigen peptide is selected from the group consisting of a tumor antigen, a neoantigen, a tumor neoantigen, a viral antigen, a bacterial antigen, a phospho-antigen, and a microbial antigen, optionally wherein the antigen peptide is a neoantigen identified from tumor sequencing data from a subject.
8. The method of claim 7, wherein the neoantigen is determined using an *in silico* predictive algorithm, optionally wherein the predictive algorithm comprises an MHC binding algorithm capable to predict binding between the neoantigen and an MHC peptide.
9. The method of any one of claims 1-8, wherein the isolated T cell is a CD8+ T cell.
10. The method of any one of claims 1-9, wherein the plurality of distinct particle sets comprises from 2 to 500 distinct particle sets.
11. The method of any one of claims 1-10, wherein:
 - a) the MHC peptide is a human MHC peptide, optionally wherein the MHC peptide is an HLA-A, HLA-B, or HLA-C peptide, optionally wherein the MHC peptide comprises HLA-A*01:01, HLA-A*02:01, HLA-A*03:01, HLA-A*24:02, HLA-A*30:02, HLA-A*31:01, HLA-A*32:01, HLA-A*33:01, HLA-A*68:01, HLA-A*11:01, HLA-A*23:01, HLA-A*30:01, HLA-A*33:03, HLA-A*25:01, HLA-A*26:01, HLA-A*29:02, HLA-A*68:02, HLA-B*07:02, HLA-B*14:02, HLA-B*18:01, HLA-B*27:02, HLA-B*39:01, HLA-B*40:01, HLA-B*44:02, HLA-B*46:01, HLA-B*50:01, HLA-B*57:01, HLA-B*58:01, HLA-B*08:01, HLA-B*15:01, HLA-B*15:03, HLA-B*35:01, HLA-B*40:02, HLA-B*42:01, HLA-B*44:03, HLA-B*51:01, HLA-B*53:01, HLA-B*13:02, HLA-B*15:07, HLA-

B*27:05, HLA-B*35:03, HLA-B*37:01, HLA-B*38:01, HLA-B*41:02, HLA-B*44:05, HLA-B*49:01, HLA-B*52:01, HLA-B*55:01, HLA-C*02:02, HLA-C*03:04, HLA-C*05:01, HLA-C*07:01, HLA-C*01:02, HLA-C*04:01, HLA-C*06:02, HLA-C*07:02, HLA-C*16:01, HLA-C*03:03, HLA-C*07:04, HLA-C*08:01, HLA-C*08:02, HLA-C*12:02, HLA-C*12:03, HLA-C*14:02, HLA-C*15:02, or HLA-C*17:01;

- b) the β 2M peptide is a human β 2M peptide, optionally wherein the β 2M peptide comprises a mutation, optionally wherein the mutation is S88C;
- c) the polypeptide is biotinylated; and/or
- d) the particle is selected from the group consisting of a magnetic bead, an agarose bead, a styrene polymer particle, and a dextran polymer particle, optionally the particle is streptavidin coated.

12. The method of any one of claims 1-11, further comprising:

- ~~a) — monitoring changes in the antigen-specific T cells of a subject receiving immunotherapy, optionally wherein the immunotherapy is an adoptive cell transfer or a checkpoint inhibitor;~~
- ~~b)a) identifying at least one T cell receptor (TCR) sequence, optionally wherein the at least one TCR sequence comprises a TCR alpha sequence, a TCR beta sequence, or a combination thereof; and/or~~
- ~~c) — manufacturing a soluble TCR polypeptide.~~

~~13. A method of modifying a cell, comprising:~~

- ~~a) — introducing into the cell a homologous recombination (HR) template nucleic acid sequence comprising:~~
 - ~~i) — first and second homology arms homologous to first and second endogenous sequences of the cell;~~
 - ~~ii) — a T cell receptor (TCR) gene sequence obtained by the method of claim 12, wherein the TCR gene sequence is positioned between the first and second HR arms; and~~
 - ~~iii) — a first 2A coding sequence positioned upstream of the TCR gene sequence and a second 2A coding sequence positioned downstream of the TCR~~

~~gene sequence, wherein the first and second 2A-coding sequences code for the same amino acid sequence that are codon-diverged relative to each other;~~

~~b) —recombining the HR-template nucleic acid into an endogenous locus of the cell comprising the first and second endogenous sequences homologous to the first and second homology arms of the HR-template nucleic acid.~~

~~14. A composition comprising a modified cell obtained by the method of claim 13.~~

~~15. Use of the composition of claim 14 in the manufacture of a medicament for the treatment of cancer.~~

~~16. The composition of claim 14 for use as a therapy.~~