

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) 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 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 one or more T cells bound to a particle;
- c) identifying the barcode of the particle bound to the isolated T cell; and
- d) determining a ratio of each 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

the 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.

[0009] In one example, provided herein is a method for identifying antigen specificity of a T cell, comprising: obtaining or having obtained a dataset comprising data associated with one or more barcodes detectably bound to the particle set that is bound, directly or indirectly, to the T cell, wherein the one or more barcodes are each operably associated with a unique antigen peptide; and determining or having determined a ratio of the barcodes bound to the T cell that identifies the antigen specificity of the 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.

[0010] In some examples, the dataset comprises the one or more barcodes and the one or more barcode copy numbers.

[0011] In some examples, the unique antigen peptide is the same for each distinct particle set.

[0012] In some examples, the first particle comprises a first barcode and the second particle comprises a second barcode distinct from the first barcode, wherein the first and second barcodes are associated with the identity of the antigen.

[0013] In some examples, the ratio of the barcodes corresponds to the antigen specificity of the isolated T cell.

[0014] In some examples, the isolated T cell is identified as an antigen-specific T cell if the ratio of the barcodes is above a threshold.

[0015] In some examples, the threshold is at least or greater than 2.

[0016] In some examples, the threshold is at least or greater than 5.

[0017] In some examples, the threshold is at least or greater than 10.

[0018] In some examples, the threshold is between 2 and 5.

[0019] In some examples, the threshold is between 5 and 10.

[0020] In some examples, the threshold is at least or greater than 2, 3, 4, 5, 6, 7, 8, 9, 10, 2-5, 3-6, 4-7, 5-8, 5-10, 7-10, or greater than 10.

[0021] In some examples, the assay is a nucleotide-based assay.

[0022] In some examples, the nucleotide-based assay is a PCR assay, an RT-PCR assay, a sequencing assay, or a hybridization assay.

[0023] In some examples, the assay determines the sequence(s) of the one or more barcodes.

[0024] In some examples, the assay determines the sequence(s) and copy number(s) of the one or more barcodes.

[0025] In some examples, the method further comprises obtaining the T cell receptor (TCR) CDR sequences.

[0026] In some examples, the method further comprises obtaining the TCR alpha and beta chain sequences.

[0027] In some examples, the antigen specificity of the T cell comprises each of (a) the sequence of the antigen peptide and (b) the TCR sequences of the bound T cell.

[0028] In some examples, the first identifying label of each first particle is the same in each set.

[0029] In some examples, the second identifying label of each second particle is the same in each set.

[0030] In some examples, the first identifying label of each first particle is the same in each set, and wherein the second identifying label of each second particle is the same in each set.

[0031] In some examples, the first and second identifying labels are fluorophores.

[0032] In some examples, the first fluorophore is allophycocyanin (APC).

[0033] In some examples, the second fluorophore is phycoerythrin (PE).

[0034] In some examples, the particle set comprises a third particle comprising a third barcode distinct from the first and second barcode, wherein the first, second, and third barcodes are associated with the identity of the antigen.

[0035] In some examples, the unique antigen peptide is selected from the group consisting of: a tumor antigen peptide, a neoantigen peptide, a tumor neoantigen peptide, a viral antigen peptide, a bacterial antigen peptide, a phospho-antigen peptide, and a microbial antigen peptide.

[0036] In some examples, the unique antigen peptide is a neoantigen peptide.

[0037] In some examples, wherein the neoantigen is derived from tumor sequencing data from a subject used to identify one or more somatic mutations present in the data relative to wild-type.

[0038] In some examples, an in silico predictive algorithm is used to determine the neoantigen.

[0039] In some examples, the predictive algorithm further comprises an MHC binding algorithm to predict binding between the neoantigen and an MHC peptide.

[0040] In some examples, the sample is selected from a blood sample, a bone marrow sample, a tissue sample, a tumor sample, or a peripheral blood mononuclear cell (PBMC) sample.

[0041] In some examples, the sample is a PBMC sample.

[0042] In some examples, the sample is a tumor sample.

[0043] In some examples, the sample is a bone marrow sample.

[0044] In some examples, the T cell is a human T cell.

[0045] In some examples, the T cell is a CD8⁺ T cell.

[0046] In some examples, the CD8⁺ T cell is a human CD8⁺ T cell.

[0047] In some examples, the method comprises a library of distinct particle sets.

[0048] In some examples, the library comprises 2 to 500 distinct particle sets.

[0049] In some examples, the library comprises at least 60 distinct particle sets.

[0050] In some examples, each particle comprises an MHC peptide.

[0051] In some examples, the MHC peptide is a mammalian MHC peptide.

[0052] In some examples, the MHC peptide is a human MHC peptide.

[0053] In some examples, the MHC peptide is a class I HLA peptide.

[0054] In some examples, the HLA peptide comprises an HLA-A, HLA-B, or HLA-C peptide.

[0055] In some examples, the HLA 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.

[0056] In some examples, the HLA peptide comprises a Y84A or a Y84C mutation.

[0057] In some examples, each particle comprises an HLA I peptide and a β 2M peptide.

[0058] In some examples, the β 2M peptide is a mammalian β 2M peptide.

[0059] In some examples, the β 2M peptide is a human β 2M peptide.

[0060] In some examples, the β 2M peptide comprises a mutation to allow or increase binding to thiol dyes.

[0061] In some examples, the mutation is S88C.

[0062] In some examples, each particle comprises a polypeptide comprising, in an amino to carboxyl terminus orientation, (i) the antigen peptide, (ii) a β 2M peptide, and (iii) an MHC peptide.

[0063] In some examples, the polypeptide further comprises a first universal target peptide before the antigen peptide, and a second universal target peptide that is distinct from the first universal target peptide between the antigen peptide and the β 2M peptide.

[0064] In some examples, each particle comprises a polypeptide comprising, in an amino to carboxyl terminus orientation, (i) a first universal target peptide, (ii) the antigen peptide, (iii) a second universal target peptide that is distinct from the first universal target peptide, (iv) a β 2M peptide, and (v) an MHC peptide.

[0065] In some examples, the antigen peptide is 7-15 amino acids, 7-10, 8-9, 7, 8, 9, 10, 11, 12, 13, 14, or 15 amino acids in length.

[0066] In some examples, the polypeptide comprising the unique antigen peptide is biotinylated.

[0067] In some examples, each particle in a distinct particle set comprises a streptavidin core and at least one copy of the unique antigen peptide.

[0068] In some examples, the particle comprises one, two, three, or four copies of the unique antigen peptide.

[0069] In one example, provided herein is a library comprising two or more distinct particle sets each comprising a unique antigen peptide and a 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.

[0070] In some examples, the identifying label is a fluorophore.

[0071] In some examples, the fluorophore is APC or PE.

[0072] In one example, provided herein is a particle comprising a tetrameric solid support bound to a unique barcode and three or fewer attached polypeptide molecules, the polypeptide molecules comprising in an amino to carboxyl terminus orientation, (i) an

antigenic peptide, (ii) a β 2M peptide, and (iii) an MHC peptide, wherein the barcode is operably associated with the identity of the antigen peptide.

[0073] In some examples, the polypeptide further comprises a first universal target peptide before the antigen peptide, and a second universal target peptide that is distinct from the first universal target peptide between the antigen peptide and the β 2M peptide.

[0074] In some examples, the solid support is a streptavidin core.

[0075] In some examples, the polypeptide molecules are biotinylated.

[0076] In some examples, the polypeptide molecules are bound to the streptavidin core via a biotin-streptavidin interaction.

[0077] In some examples, the particle further comprises an identifying label.

[0078] In some examples, the identifying label is a fluorophore.

[0079] In some examples, the fluorophore is APC or PE.

[0080] In one example, provided herein is a library comprising the particles, wherein the library comprises two or more distinct particles, wherein each distinct particle comprises a unique antigen peptide.

[0081] In one example, provided herein is a method of monitoring an immune repertoire in a subject, 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, wherein the sample is obtained from a subject over time; 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 associated with the first and second identifying label; performing an assay to identify one or more barcodes 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 from step (e) and dividing the first copy number by the second copy number; identifying the antigen specificity of the T cell based on the ratio; and monitoring changes in the antigen specific T cells identified by the method in the subject.

[0082] In some examples, the first and second identifying labels are fluorophores.

[0083] In some examples, the fluorophore is APC or PE.

[0084] In one example, provided herein is a method of monitoring an immune repertoire in a subject, comprising obtaining or having obtained a dataset comprising data associated with one or more barcodes detectably bound, directly or indirectly, to the T cell, wherein the one or more barcodes are each operably associated with a unique antigen peptide; determining or having determined a ratio of the barcodes bound to the T cell that identifies the antigen specificity of the 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 from step (a) and dividing the first copy number by the second copy number; identifying a sequence of the unique antigen peptide bound to an antigen specific T cell; and monitoring changes in the antigen specific T cells identified by the method in the subject.

[0085] In some examples, the dataset comprises the one or more barcodes and the one or more barcode copy numbers.

[0086] In some examples, the unique antigen peptide is the same for each distinct particle set.

[0087] In some examples, monitoring the changes in the T cells comprises administering to the subject a soluble, labeled antigen-specific TCR.

[0088] In some examples, the subject is administered an immunotherapy based upon the changes in the identified antigen-specific T cells.

[0089] In some examples, the immunotherapy is a checkpoint inhibitor.

[0090] In some examples, the checkpoint inhibitor is an anti-PD-1 antibody, an anti-PD-L1 antibody, or an anti-CTLA-4 antibody. In some examples the anti-PD-1 antibody is selected from the group comprising pembrolizumab, nivolumab, and cemiplimab. In some examples the anti-PD-L1 antibody is selected from the group comprising atezolizumab, avelumab, and durvalumab. In some examples, the anti-CTLA4 antibody is ipilimumab. In some examples the checkpoint inhibitor is an anti-TIGIT antibody. In some examples, the anti-TIGIT antibody is selected from the group comprising AB154 (Arcus), tiragolumab (Genentech), BMS-986297 (BMS), MK-7684 (Merck), and etigilimab (OncoMed).

[0091] In one example, provided herein is a method of identifying an antigen, 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 associated with the first and second identifying labels; performing an assay to identify one or more barcodes 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 from step (e) and dividing the first copy number by the second copy number; and identifying a sequence of the unique antigen peptide bound to an antigen specific T cell.

[0092] In some examples, the first and second identifying labels are fluorophores.

[0093] In some examples, the fluorophore is APC or PE.

[0094] In one example, provided herein is a method of identifying an antigen, comprising

[0095] obtaining or having obtained a dataset comprising data associated with one or more barcodes detectably bound, directly or indirectly, to the T cell, wherein the one or more barcodes are each operably associated with a unique antigen peptide; determining or having determined a ratio of the barcodes bound to the 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 from step (a) and dividing the first copy number by the second copy number; and identifying a sequence of the unique antigen peptide bound to an antigen specific T cell.

[0096] In some examples, step (a) comprises obtaining a T cell-based sample and assaying it to obtain the dataset.

[0097] In some examples, the dataset comprises the one or more barcodes and the one or more barcode copy numbers.

[0098] In some examples, the unique antigen peptide is the same for each distinct particle set.

[0099] In one example, provided herein is a method of identifying an HLA and peptide complex, 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 associated with the first and second identifying labels; performing an assay to identify one or more barcodes 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 from step (e) and dividing the first copy number by the second copy number; and identifying the HLA and peptide complex bound to an antigen specific T cell.

[00100] In some examples, the first and second identifying labels are fluorophores.

[00101] In some examples, the fluorophore is APC or PE.

[00102] In one example, provided herein is a method of identifying an HLA and peptide complex, comprising obtaining or having obtained a dataset comprising data associated with one or more barcodes detectably bound, directly or indirectly, to the T cell, wherein the one or more barcodes are each operably associated with a unique antigen peptide;; determining or having determined a ratio of the barcodes bound to the 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 from step (a) and dividing the first copy number by the second copy number; and identifying the HLA and peptide complex bound to an antigen specific T cell.

[00103] In some examples, step (a) comprises obtaining a T cell-based sample and assaying it to obtain the dataset.

[00104] In some examples, the dataset comprises the one or more barcodes and the one or more barcode copy numbers.

[00105] In some examples, the unique antigen peptide is the same for each distinct particle set.

[00106] In one example, provided herein is a method of identifying a subject for treatment with an immunotherapy, 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 associated with the first and second identifying labels; performing an assay to identify one or more barcodes 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 from step (e) and dividing the first copy number by the second copy number.

[00107] In some examples, the first and second identifying labels are fluorophores.

[00108] In some examples, the fluorophore is APC or PE.

[00109] In one example, provided herein is a method of identifying a subject for treatment with an immunotherapy, comprising obtaining or having obtained a dataset comprising data associated with one or more barcodes detectably bound, directly or indirectly, to the T cell, wherein the one or more barcodes are each operably associated with a unique antigen peptide; and determining or having determined a ratio of the barcodes bound to the 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 from step (a) and dividing the first copy number by the second copy number.

[00110] In some examples, step (a) comprises obtaining a T cell-based sample and assaying it to obtain the dataset.

[00111] In some examples, the dataset comprises the one or more barcodes and the one or more barcode copy numbers.

[00112] In some examples, the unique antigen peptide is the same for each distinct particle set.

[00113] In some examples, the immunotherapy comprises a T cell vaccine, a dendritic cell vaccine, a nucleic acid vaccine, a peptide vaccine, a viral vaccine, a soluble TCR, a TCR-drug conjugate, an antibody, or an antibody-drug conjugate.

[00114] In some examples, the antibody comprises a monoclonal antibody.

[00115] In one example, provided herein is a method of identifying a unique TCR sequence, 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 associated with the first and second identifying labels; performing an assay to identify one or more barcodes 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 from step (e) and dividing the first copy number by the second copy number; and identifying the unique TCR sequence.

[00116] In some examples, the first and second identifying labels are fluorophores.

[00117] In some examples, the fluorophore is APC or PE.

[00118] In one example, provided herein is a method of identifying a unique TCR sequence, comprising obtaining or having obtained a dataset comprising data associated with one or more barcodes detectably bound, directly or indirectly, to the T cell, wherein the one or more barcodes are each operably associated with a unique antigen peptide; determining or having determined a ratio of the barcodes bound to the 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 from step (a) and dividing the first copy number by the second copy number; and identifying the unique TCR sequence.

[00119] In some examples, step (a) comprises obtaining a T cell-based sample and assaying it to obtain the dataset.

[00120] In some examples, the dataset comprises the one or more barcode sequences and the one or more barcode copy numbers.

[00121] In some examples, the unique antigen peptide is the same for each distinct particle set.

[00122] In some examples, the method further comprises manufacturing a soluble TCR polypeptide comprising the identified unique TCR sequence.

[00123] In some examples, the soluble TCR polypeptide is linked to a label or a drug.

[00124] In some examples, the method is repeated to identify at least two unique TCR sequences.

[00125] In some examples, the method further comprises manufacturing a library comprising the at least two unique TCR sequences.

[00126] In one example, provided herein is a method of treating cancer in a subject, comprising obtaining or having obtained a dataset comprising data associated with one or more barcodes detectably bound, directly or indirectly, to a T cell, wherein the one or more barcodes are each operably associated with a unique antigen peptide; determining or having determined a ratio of the barcodes bound to the T cell that identifies the antigen specificity of the 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 from step (a) and dividing the first copy number by the second copy number; identifying at least one or both of the T cell's TCR

sequences and creating an engineered T cell comprising at least one or both of the TCR sequences; and administering the engineered T cell to the subject.

[00127] In some examples, the method further comprises administering an immunotherapy.

[00128] In some examples, the immunotherapy is a checkpoint inhibitor.

[00129] In some examples, the checkpoint inhibitor is an anti-PD-1 antibody, an anti-PD-L1 antibody, or an anti-CTLA-4 antibody.

[00130] In some examples, the T cell is autologous.

[00131] In some examples, the engineered T cell is autologous.

[00132] In some examples, the unique antigen peptide is presented by HLA class I on the cell surface of the subject's cancer.

[00133] In certain examples, the presently disclosed subject matter provides methods for processing T cells. In certain examples, the methods comprise: (a) contacting a sample with a plurality of distinct particle sets; (b) isolating one or more T cells bound to a particle; (c) identifying the barcode of the particle bound to the isolated T cell; and (d) determining a ratio of each barcode. In certain examples, each particle comprises a unique antigen peptide, an operably associated barcode, and at least one identifying label. In certain examples, the sample comprises T cells. In certain examples, contacting comprises providing conditions suitable for a single T cell to bind to a unique antigen peptide of at least one particle set.

[00134] In certain examples, the ratio is calculated by identifying a copy number of a first barcode and a copy number of a second barcode and dividing the copy number of the first barcode by the copy number of the second barcode. In certain examples, the unique antigen peptide is the same for each distinct particle set. In certain examples, each distinct particle set comprises at least one or more barcodes, wherein each barcode is associated with the identity of the antigen peptide. In certain examples, the ratio of each barcode corresponds to the antigen specificity of the isolated T cell. In certain examples, the isolated T cell is identified as an antigen-specific T cell if the ratio of the first barcode is above a threshold. In certain examples, the threshold is at least or greater than 2, 3, 4, 5, 6, 7, 8, 9, 10, 2-5, 3-6, 4-7, 5-8, 5-10, 7-10, or greater than 10.

[00135] In certain examples, the identifying the barcode comprises a nucleotide-based assay. In certain examples, nucleotide-based assay is a PCR, an RT-PCR, a sequencing, or a hybridization assay. In certain examples, the nucleotide-based assay determines a sequence of each barcode. In certain examples, the nucleotide-based assay determines a

copy number of each barcode. In certain examples, the nucleotide-based assay determines (a) a sequence of each barcode and/or (b) a copy number of each barcode.

[00136] In certain examples, the methods further comprise obtaining a T cell receptor (TCR) CDR sequence. In certain examples, the methods further comprise obtaining a TCR gene sequence. In certain examples, the TCR gene sequence is a TCR alpha or a TCR beta chain sequence.

[00137] In certain examples, the methods comprise identifying the antigen specificity of a T cell. In certain examples, the antigen specificity of the T cell comprises the sequence of the antigen peptide and the TCR sequences of the bound T cell.

[00138] In certain examples, the at least one identifying label is the same in each distinct particle set. In certain examples, the methods comprise at least two different identifying labels. In certain examples, the at least one identifying label is a fluorophore. In certain examples, the fluorophore is selected from the group comprising allophycocyanin (APC) and phycoerythrin (PE). In certain examples, the at least two different identifying labels are fluorophores, wherein the fluorophores are selected from the group comprising allophycocyanin (APC) and phycoerythrin (PE).

[00139] In certain examples, 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. In certain examples, the neoantigen is identified from tumor sequencing data from a subject. In certain examples, an in silico predictive algorithm is used to determine the neoantigen. In certain examples, the predictive algorithm further comprises an MHC binding algorithm to predict binding between the neoantigen and an MHC peptide.

[00140] In certain examples, the sample is selected from a blood sample, a bone marrow sample, a tissue sample, a tumor sample, or a peripheral blood mononuclear cell (PBMC) sample. In certain examples, wherein the T cell is a human T cell. In certain examples, the T cell is a CD8⁺ T cell.

[00141] In certain examples, the methods comprise a library of distinct particle sets. In certain examples, the library comprises 2 to 500 distinct particle sets. In certain examples, each particle comprises an MHC peptide. In certain examples, the MHC peptide is a human MHC peptide. In certain examples, the MHC peptide is a class I HLA peptide. In certain examples, the HLA peptide comprises an HLA-A, HLA-B, or HLA-C peptide. In certain examples, the HLA 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.

[00142] In certain examples, each particle comprises an HLA peptide and a β 2M peptide. In certain examples, the β 2M peptide is a human β 2M peptide. In certain examples, the β 2M peptide comprises a mutation. In certain examples, the mutation is S88C.

[00143] In certain examples, each particle comprises a polypeptide comprising, in an amino to carboxyl terminus orientation, (i) the antigen peptide, (ii) a β 2M peptide, and (iii) an MHC peptide. In certain examples, the antigen peptide is 7-15 amino acids, 7-10, 8-9, 7, 8, 9, 10, 11, 12, 13, 14, or 15 amino acids in length. In certain examples, the polypeptide is biotinylated. In certain examples, the particles are selected from the group consisting of magnetic beads, agarose beads, styrene polymer particles, and dextran polymer particles. In certain examples, wherein the particles are streptavidin coated.

[00144] In certain examples, the presently disclosed subject matter provides methods for monitoring an immune repertoire in a subject. In certain examples, the methods comprise monitoring changes in the antigen-specific T cells in the subject. In certain examples, the methods comprise administering an immunotherapy to the subject. In certain examples, the immunotherapy is an adoptive cell transfer or a checkpoint inhibitor. In certain examples, any of the methods disclosed herein are used for monitoring an immune repertoire in a subject.

[00145] In certain examples, the presently disclosed subject matter provides methods for identifying at least one TCR sequence. In certain examples, the at least one TCR sequence is a TCR alpha sequence, a TCR beta sequence, or a combination thereof. In certain examples, the methods further comprise manufacturing a soluble TCR

polypeptide. In certain examples, any of the methods disclosed herein are used for identifying at least one TCR sequence.

[00146] In certain examples, the presently disclosed subject matter provides libraries of particles. In certain examples, the library comprises at least two particle sets. In certain examples, each particle set comprises an antigen peptide, a barcode operably associated with the identity of the antigen peptide, and at least one identifying label. In certain examples, the at least one identifying label is the same in each particle set. In certain examples, at least two different identifying labels in each distinct particle set. In certain examples, the at least one identifying label is a fluorophore. In certain examples, the fluorophore is selected from the group comprising allophycocyanin (APC) and phycoerythrin (PE). In certain examples, the at least two different identifying labels are fluorophores, wherein the fluorophores are selected from the group comprising allophycocyanin (APC) and phycoerythrin (PE).

[00147] In certain examples, the presently disclosed subject matter further provides particles. In certain examples, a particle comprises at least one polypeptide, a barcode, and an identifying label. In certain examples, the polypeptide comprises an antigen peptide, a β 2M peptide, and an MHC peptide. In certain examples, the barcode is operably associated with the identity of the antigen peptide. In certain examples, the particle is selected from the group consisting of magnetic beads, agarose beads, styrene polymer particles, and dextran polymer particles. In certain examples, the identifying label is a fluorophore. In certain examples, the particle is streptavidin coated. In certain examples, the polypeptide is labeled.

[00148] In certain examples, the presently disclosed subject matter further discloses methods of treating cancer in a subject. In certain examples, the methods comprise: (a) preparing a plurality of particles each comprising a plurality of labeled polypeptides; (b) contacting the plurality of particles with a plurality of T cells from the subject under conditions suitable for antigen-specific binding of a T cell to the particle; (c) isolating the T cells bound to the particle and identifying the TCR gene sequence of the isolated T cell; (d) preparing a polynucleotide comprising homology arms and at least one TCR gene sequence; (e) recombining the polynucleotide into an endogenous locus of the T cell of the subject; (f) culturing the modified T cell to produce a population of T cells; and (g) administering a therapeutically effective number of the modified T cells to the subject to thereby treat the cancer. In certain examples, the polypeptides comprise an antigen peptide, a β 2M sequence, an HLA sequence and a detectable label. In certain examples, the TCR gene

sequence is patient specific. In certain examples, the TCR gene sequence is positioned between the homology arms.

[00149] In certain examples, the presently disclosed subject matter further discloses methods of modifying a cell. In certain examples, the methods comprise: (a) introducing into the cell a homologous recombination (HR) template nucleic acid sequence; and (b) recombining the HR template nucleic acid into an endogenous locus of the cell. In certain examples, the HR template nucleic acid comprises: (a) first and second homology arms homologous to first and second endogenous sequences of the cell; (b) a T cell receptor (TCR) gene sequence obtained by any of the methods disclosed herein; and (c) 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. In certain examples, the first and second endogenous sequences are homologous to the first and second homology arms of the HR template nucleic acid. In certain examples, the TCR gene sequence is positioned between the first and second HR arms. In certain examples, the 2A-coding sequence is a P2A-coding sequence. In certain examples, the HR template further comprises a sequence coding for a flexible linker. In certain examples, the sequence coding for the flexible linker is positioned immediately upstream of the 2A-coding sequences. In certain examples, the flexible linker has a Gly Ser Gly amino acid sequence. In certain examples, the HR template further comprises a sequence coding for a protease cleavage sequence. In certain examples, the protease cleavage sequence is a Furin sequence. In certain examples, the protease cleavage sequence is a TEV sequence. In certain examples, the protease cleavage sequence is upstream of the second 2A-coding sequence.

[00150] In certain examples, the presently disclosed subject matter further discloses compositions comprising modified cells. In certain examples, the modified cell comprises an exogenous nucleic acid sequence integrated into an endogenous locus. In certain examples, the exogenous nucleic acid sequence comprises: (a) a TCR gene sequence; and (b) 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. In certain examples, the TCR gene sequence is identified by any of the methods disclosed herein. In certain examples, the first and second 2A-coding sequences code for the same amino acid sequence that are codon-diverged relative to each other. In certain examples, the 2A-coding sequence is a P2A-coding sequence. In certain examples, the exogenous

nucleic acid sequence further comprises a sequence coding for a flexible linker. In certain examples, the sequence coding for the flexible linker is positioned immediately upstream of the 2A-coding sequences. In certain examples, the flexible linker has a Gly Ser Gly amino acid sequence. In certain examples, the exogenous nucleic acid further comprises a sequence coding for a protease cleavage sequence. In certain examples, the protease cleavage sequence is a Furin sequence. In certain examples, the protease cleavage sequence is a TEV sequence. In certain examples, the protease cleavage sequence is upstream of the second 2A-coding sequence.

BRIEF SUMMARY OF DRAWINGS

[00151] These and other features, aspects, and advantages of disclosed compositions and methods will become better understood with regard to the following description, and accompanying drawings, where:

[00152] Figure 1 shows the design of an exemplary comPACT mini-gene. SS refers to the optional signal sequence; US1 refers to the first universal target site; NeoE refers to Neoepitope, the antigenic peptide sequence site; US2 refers to the second universal target site; L1 refers to the optional first linker sequence; Beta2m refers to the β -2-microglobulin domain sequence; L2 refers to the optional second linker sequence; MHC heavy chain refers to the MHC heavy chain allele; L3 refers to the optional third linker sequence; and purification cluster refers to the optional purification cluster with a biotinylation sequence, a protease cleavage site, and an affinity tag sequence. While Figure 1 shows a His6 (SEQ ID NO: 34) (6-His tag (SEQ ID NO: 34)) as the affinity tag, any other appropriate affinity tag could be used including but not limited to histidine tags of different lengths (poly-His tags), HAT tags, FLAG tags (or FLAG epitopes), epitopes that are specific to any antibody used for purification, galactose-binding protein tags, fluorescent tags, GST tags, HA tags, HaloTags, MBP tags, Myc tags, poly-Asp tag, poly-Phe tag, protein C, Streptavidin/Biotin tags, Strep-tags, protein G, or any other protein purification tag that is capable of purifying a comPACT polypeptide.

[00153] Furthermore, while Figure 1 shows that a nickel resin (see, “Ni” in figure) was used to purify the His6 tagged (SEQ ID NO: 34) comPACT polypeptide, other His6 (SEQ ID NO: 34) affinity resins have been used. Specifically, Zinc resin has been used to successfully purify a His6 tagged (SEQ ID NO: 34) comPACT polypeptide from a solution. Cobalt and Calcium resins are two other exemplary His6 (SEQ ID NO: 34) affinity resins that could be used.

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) 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 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 one or more T cells bound to a particle;
 - c) identifying the barcode of the particle bound to the isolated T cell; and
 - d) determining a ratio of each barcodewherein 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 a first barcode and a copy number of a second barcode and dividing the copy number of the first barcode by the copy number of the second 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) 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.