every possible pMHC combination is extremely high, and since there are few if any 'holes' characterized in the TCR repertoire, it has been posited that a large degree of TCR cross-reactivity is a requirement of functional antigen recognition. How the T cell repertoire can simultaneously be MHC restricted, cross- reactive enough to ensure all potential antigenic challenges can be met, yet still specific enough to avoid aberrant autoimmunity, has remained an open and pressing question in immunology.

[0006] There have been a number of strategies used to determine the specificity of orphan TCRs (Birnbaum et al., (2012) Immunol Rev *250*, 82-101). Mass spectrometry can provide an unbiased method of antigen isolation, but is restricted to experiments requiring large cell numbers, typically 10^7 to 10^9, and the targets must still be presented by the correct HLA. Traditionally, most studies of T cell antigen specificities have involved testing candidate antigens empirically. For example, studies of anti-tumor T cell specificities have correctly postulated that there are productive T cell responses towards neo-antigens. Such studies involve sequencing of tumors to identify mutations, using epitope prediction algorithms to predict immunogenic mutant peptides, and testing for T cell responses directed at these mutant peptides (Kreiter et al., (2015) Nature 520, 692-696; Rajasagi et al., (2014) Blood 124, 453-462; Tran et al., (2014) Science 344, 641-645). Other strategies query established T cell specificities in patients by using pHLA multimers (Bentzen et al., (2016) Nat Biotechnol *34*, 1037-1045; Newell et al., (2013) Nat Biotechnol 31, 623-629).

[0007] High-throughput and sensitive approaches to determining the specificity of 'orphan' TCRs (i.e. TCRs of unknown antigen specificity) that could help uncover potential targets for cancer immunotherapy, autoimmunity, and infection and provide mechanistic insight into disease pathogenesis are of great interest.

SUMMARY

[0001] In one aspect, the present disclosure refers to a T cell receptor (TCR) or antibody comprising the amino acid sequence of SEQ ID NO: 258 and the amino acid sequences of SEQ ID NO: 259, SEQ ID NO: 260, or a combination thereof, wherein the T cell receptor or the antibody binds to and/or is stimulated by a peptide comprising a sequence of MMDFFNAQM (SEQ ID NO: 279).

[0001a] In one aspect, the present disclosure refers to a T cell receptor (TCR) or antibody comprising the amino acid sequence of SEQ ID NO: 261 and the amino acid sequence of SEQ ID NO: 262, wherein the T cell receptor or the antibody binds to and/or is stimulated by a peptide comprising a sequence of MMDFFNAQM (SEQ ID NO: 279) or VLDFQGQL (SEQ ID NO:280).

[0001b] In one aspect, the present disclosure refers to a T cell receptor (TCR) or antibody comprising the amino acid sequence of SEQ ID NO: 258 and the amino acid sequence of SEQ ID NO: 259, wherein the T cell receptor or the antibody binds to and/or is stimulated by a peptide comprising a sequence of MMDFFNAQM (SEQ ID NO: 279).

[0001c] In one aspect, the present disclosure refers to a T cell receptor (TCR) or antibody comprising the amino acid sequence of SEQ ID NO: 261 and the amino acid sequence of SEQ ID NO: 263, wherein the T cell receptor or the antibody binds to and/or is stimulated by a peptide comprising a sequence of MMDFFNAQM (SEQ ID NO: 279).

[0001d] In one aspect, the present disclosure refers to a T cell receptor (TCR) or antibody comprising the amino acid sequence of SEQ ID NO: 258 and the amino acid sequence of SEQ ID NO: 260, wherein the T cell receptor or the antibody binds to and/or is stimulated by a peptide comprising a sequence of MMDFFNAQM (SEQ ID NO: 279).

[0001e] In one aspect, the present disclosure refers to a T cell receptor (TCR) or antibody comprising the amino acid sequence of SEQ ID NO: 495 and the amino acid sequence of SEQ ID NO: 484, wherein the T cell receptor or the antibody binds to and/or is stimulated by a peptide comprising a sequence selected from the group consisting of: LLEDLDWDV (SEQ ID NO: 272), NMEYMTWDV (SEQ ID NO: 273), TMETIDWKV (SEQ ID NO: 274), VLEEVDWLI (SEQ ID NO: 275), KLEQLDWTV (SEQ ID NO: 276), TLEELDWCL (SEQ ID NO: 277), and NVEYYDIKL (SEQ ID NO: 278).

[0001f] In one aspect, the present disclosure refers to an immune cell engineered to comprise the T cell receptor or the antibody as disclosed herein.

[0001g] In one example, the present disclosure refers to a peptide ligand comprising an amino acid sequence of any of SEQ ID NO:1-SEQ ID NO:257.

[0001h] In one example, the present disclosure refers to a peptide ligand consisting of an amino acid sequence of any of SEQ ID NO:1-SEQ ID NO:257.

[0001i] In one example, the present disclosure refers to a polynucleotide encoding a peptide as disclosed herein.

[0001j] In one example, the present disclosure refers to a pharmaceutical composition comprising polynucleotide as disclosed herein, a peptide or combination of peptides as disclosed herein; and a pharmaceutically acceptable excipient.

[0001k] In one example, the present disclosure refers to an antigen presenting cell comprising the peptide or the combination of peptides as disclosed herein.

[0001l] Compositions are provided for ligands for a T cell receptor (TCR) of interest in a defined MHC context. The composition may comprise or consist of a defined peptide, or may comprise or consist of a polynucleotide encoding such a peptide. Such peptides may be fragments of naturally occurring antigenic proteins; may be fragments of neoantigenic proteins that are the subject of somatic mutation during tumorigenesis, or may be a synthetically generated mimic of an antigenic protein. The synthetic peptides can act as highly potent agonists of T cell receptors. In some examples a peptide, or encoding sequence, is selected from sequences provided herein, including without limitation any one or a combination of the peptide sequences set forth in SEQ ID NO:1-257. A peptide may be provided as short antigenic sequence active in stimulating T cells; or may be provided in the form of the larger protein, e.g. an intact domain, a soluble protein portion, a complete protein, etc. In some examples, peptide antigens are identified that are shared between patients

and provide a means for broadly applicable therapy. In other examples identification of antigens provides for a personalized medicine approach.

[0002] Identification of T cell receptors and cognate antigens provides targets for immunotherapy, including screening of patient T cells for responsiveness, vaccination with peptides or nucleic acids encoding such peptides, cell-based therapies, protein-based therapies, etc. The peptides and methods disclosed herein are useful in classifying TCRs based on peptide antigen specificities, which allows the identification of clinical candidate TCRs that recognize shared antigens across patients.

[0002a] In one example, the present disclosure refers to method of screening for a set of peptide antigens of a TCR of interest, the method comprising:

(a) contacting the TCR of interest with a population of host cells which express a multiplexed library of at least 10^8 different polynucleotides encoding single chain polypeptides on the cell surface, the single chain polypeptides comprising–

binding domains of the MHC protein; and

a peptide ligand;

(b) selecting for host cells expressing a single chain polypeptide that binds to the TCR of interest;

(c) iterating step (b) for at least three rounds;

(d) sequencing a nucleic acid encoding the single chain polynucleotides of the final selected population to determine a dataset of possible amino acids for each position of the peptide ligand;

(e) inputting the dataset to computer readable medium to generate a search algorithm; and

(f) searching a sequence database with the search algorithm to identify the set of peptide antigens that bind to the T cell receptor.

[0002b] In one example, the present disclosure refers to a set of peptide antigens for a TCR of interest identified by a method comprising:

(a) contacting the TCR of interest with a population of host cells which express a multiplexed library of at least 10^6 different polynucleotides encoding single chain polypeptides on the cell surface, the single chain polypeptides comprising–

binding domains of an MHC protein; and

a peptide ligand;

(b) selecting for host cells expressing a single chain polypeptide that binds to the TCR of interest;

(c) iterating step (b) for at least three rounds to generate a final selected population of host cells;

(d) sequencing a nucleic acid encoding the single chain polynucleotides of the final selected population to determine a dataset of possible amino acids for each position of the peptide ligand;

(e) inputting the dataset to computer readable medium to generate a search algorithm;

(f) searching a sequence database with the search algorithm to identify the set of peptide antigens for the TCR of interest.

[0003] In some examples, methods are provided for vaccination against cancer, for example colorectal cancer, the method comprising administering an effective dose of a vaccine composition, which composition may comprise a peptide identified herein; a combination of peptides, e.g. 2, 3, 4, 5, 6, 7, 8, 9, 10 or more distinct peptides; a complex of a peptide and at least a portion of an MHC protein; an autologous or allogeneic T cell that has been stimulated to respond to an antigenic peptide identified herein; a nucleic acid encoding an antigenic peptide identified herein; and optionally a pharmaceutically acceptable excipient, which may comprise a vaccine adjuvant. The peptide vaccination strategy may be used to initially prime an immune response, e.g. with a synthetic peptide provided herein, followed by a boost with the corresponding known wildtype antigen or wildtype whole protein.

[0003a] In one example, the present disclosure refers to use of an effective dose of the pharmaceutical composition as disclosed herein, or the antigen presenting cell as disclosed herein in the manufacture of a medicament for inducing an immune response to a cancer cell antigen in a subject in need thereof.

[0003b] In one example, the present disclosure refers to a method of determining a subject's response to an antigen, the method comprising: analyzing a sample comprising T cells from the subject for T cell stimulation in response to a peptide according to any SEQ ID NO: 1-257, wherein T cell stimulation is indicative that an immune response is inducible in the subject according to the use as disclosed herein.

[0004] The defined peptides are identified by screening peptide-MHC libraries by yeast-display was used to identify the recognition landscape of individual T cell receptors. The screening method may be utilized in a multiplex method to screen a plurality of peptide libraries simultaneously, e.g. screening 2, 3, 4 or more libraries simultaneously. Multiplexing allows improved efficiency of antigen discovery. Each library may comprise a unique epitope tag, e.g. an epitope targetable by an antibody, to allow identification; may comprise DNA barcodes; protein barcodes; etc. Each library utilizing the epitope tags were generated separately and diversities calculated, e.g. based on colony counts from limiting dilution of the initial libraries on growth plates. Pooling T cell receptors for library selection can further multiplex the selection, e.g. multiplexing of peptide sequence, peptide lengths, collections of different MHC or HLA alleles, *etc*. For selections, each barcode, epitope tag, etc. may be monitored via anti-epitope tag staining to detect the level of peptide-specific enrichment, statistical algorithms and machine-learning algorithms may be used for identification.

[0005] In some examples sequences of T cell receptors responsive to cancer antigens are provided. T cell receptor sequences may include, without limitation, the proteins having an alpha chain with sequence set forth in SEQ ID NO:258, optionally combined with a beta chain sequence of SEQ ID NO:259 or SEQ ID NO:260. The binding regions (CDR) sequences of these T cell receptors may be grafted onto an antibody framework to provide a TCR-like antibody. Because T cell receptors are adaptable and often unique from patient-to-

WHAT IS CLAIMED IS:

1. A T cell receptor (TCR) or antibody comprising the amino acid sequence of SEQ ID NO: 258 and the amino acid sequences of SEQ ID NO: 259, SEQ ID NO: 260, or a combination thereof, wherein the T cell receptor or the antibody binds to and/or is stimulated by a peptide comprising a sequence of MMDFFNAQM (SEQ ID NO: 279).

2. The T cell receptor or the antibody of claim 1, comprising the amino acid sequence of SEQ ID NO: 258 and the sequence of SEQ ID NO: 259 or the sequence of SEQ ID NO: 260.

3. A T cell receptor (TCR) or antibody comprising the amino acid sequence of SEQ ID NO: 261 and the amino acid sequence of SEQ ID NO: 262, wherein the T cell receptor or the antibody binds to and/or is stimulated by a peptide comprising a sequence of MMDFFNAQM (SEQ ID NO: 279) or VLDFQGQL (SEQ ID NO:280).

4. A T cell receptor (TCR) or antibody comprising the amino acid sequence of SEQ ID NO: 258 and the amino acid sequence of SEQ ID NO: 259, wherein the T cell receptor or the antibody binds to and/or is stimulated by a peptide comprising a sequence of MMDFFNAQM (SEQ ID NO: 279).

5. A T cell receptor (TCR) or antibody comprising the amino acid sequence of SEQ ID NO: 261 and the amino acid sequence of SEQ ID NO: 263, wherein the T cell receptor or the antibody binds to and/or is stimulated by a peptide comprising a sequence of MMDFFNAQM (SEQ ID NO: 279).

6. A T cell receptor (TCR) or antibody comprising the amino acid sequence of SEQ ID NO: 258 and the amino acid sequence of SEQ ID NO: 260, wherein the T cell receptor or the antibody binds to and/or is stimulated by a peptide comprising a sequence of MMDFFNAQM (SEQ ID NO: 279).

7. A T cell receptor (TCR) or antibody comprising the amino acid sequence of SEQ ID NO: 495 and the amino acid sequence of SEQ ID NO: 484, wherein the T cell receptor or the antibody binds to and/or is stimulated by a peptide comprising a sequence selected from the group consisting of: LLEDLDWDV (SEQ ID NO: 272), NMEYMTWDV (SEQ ID NO: 273), TMETIDWKV (SEQ ID NO: 274), VLEEVDWLI (SEQ ID NO: 275), KLEQLDWTV (SEQ ID NO: 276), TLEELDWCL (SEQ ID NO: 277), and NVEYYDIKL (SEQ ID NO: 278).

8. An immune cell engineered to comprise the T cell receptor or the antibody of any one of claims 1-7.

9. The T cell receptor (TCR) of any of claims 1-6, wherein an alpha chain of the TCR comprises SEQ ID NO: 258, and wherein SEQ ID NO: 258 is a sequence of a T cell receptor alpha chain of TCR 2A and TCR 3B.

10. The T cell receptor (TCR) of any of claims 1-6, wherein a beta chain of the TCR comprises SEQ ID NO: 259, and wherein SEQ ID NO: 259 is a sequence of a T cell receptor beta chain of TCR 2A.

11. The T cell receptor (TCR) of any of claims 1-6, wherein a beta chain of the TCR comprises SEQ ID NO: 259, and wherein SEQ ID NO: 260 is a sequence of a T cell receptor beta chain of TCR 3B.

12. The T cell receptor (TCR) of any one of claims 1-6, wherein a complementarity determining region of an alpha chain of the TCR comprises SEQ ID NO: 261, and wherein SEQ ID NO: 261 is a sequence of a complementarity determining region of a TCR alpha chain of TCR 2A and TCR 3B.

13. The T cell receptor (TCR) of any one of claims 1-6, wherein a complementarity determining region of a beta chain of the TCR comprises SEQ ID NO: 262, and wherein SEQ ID NO: 262 is a sequence of a complementarity determining region of a TCR beta chain of TCR 2A.

14. The T cell receptor (TCR) of any one of claims 1-6, wherein a complementarity determining region of a beta chain of the TCR comprises SEQ ID NO: 263, and wherein SEQ ID NO: 263 is a sequence of a complementarity determining region of a TCR beta chain of TCR 3B.

15. The T cell receptor (TCR) of claim 7, wherein a complementarity determining region of an alpha chain of the TCR comprises SEQ ID NO: 495, and wherein SEQ ID NO: 495 is a sequence of a complementarity determining region of a TCR alpha chain of TCR 4B.

16. The T cell receptor (TCR) of claim 7, wherein a complementarity determining region of a beta chain of the TCR comprises SEQ ID NO: 484, and wherein SEQ ID NO: 484 is a sequence of a complementarity determining region (CDR) of a T cell receptor beta chain of TCR 4B.