

CLAIMS

What is claimed is:

1. A method for identifying an antigen capable of activating a T cell, and optionally a T-cell receptor (TCR) α chain sequence and/or a TCR β chain sequence of a TCR that specifically binds the antigen, the method comprising:

(I) sorting an activated T cell, based on the expression of an activation-induced marker (AIM) from a composition comprising a unique biological sample, which unique biological sample comprises:

(a) a T cell and a surface bound Major Histocompatibility Complex (MHC), wherein the T cell is capable of recognizing a peptide presented in the context of the surface bound MHC,

(b) a unique antigen,

(c) a unique hashtag oligonucleotide (HTO) that is used to specifically identify the unique antigen, wherein the unique HTO is conjugated to a molecule that labels the T cell with the unique HTO, and optionally,

(d) medium that supports activation of the T cell, and

(II) performing single cell sequencing analysis on the activated T cell sorted in (I) to identify the unique HTO conjugated to the molecule that labeled the activated T cell with the unique HTO, wherein identifying the unique HTO identifies the antigen capable of activating the activated T cell, and optionally wherein the single cell sequencing analysis also identifies one or more of the following:

(i) one or more genes expressed by the activated T cell, and/or

(ii) TCR α and/or β chain sequences of a TCR expressed by the activated T cell.

2. The method of claim 1, comprising, before sorting, one or both of the following step(s): creating a plurality of biological samples by equally distributing a collection of cells comprising T cells and antigen presenting cells (APCs) isolated from a subject into individual samples, wherein each biological sample optionally comprises media and cytokines that support T cell and/or APC viability, activation and/or activity, and

creating a plurality unique biological samples by delivering to each of a plurality of biological samples a unique antigen and/or a unique HTO that is used to specifically identify the unique antigen, wherein the unique HTO is conjugated to a molecule that labels a T cell with the unique HTO,

wherein each of the plurality of biological samples comprises a collection of cells comprising T cells and APCs isolated from a subject and optionally medium that supports viability, activity, and/or activation of the T cells and APCs, and

wherein after delivery of the unique antigen and/or the unique HTO conjugated to a molecule that labels a T cell with the unique HTO, each of the plurality of biological samples becomes a unique biological sample that comprises

(a) a collection of cells comprising T cells and APCs isolated from a subject,

(b) a unique antigen,

(c) a unique HTO that specifically identifies the unique antigen and is conjugated to a molecule that labels the T cell with the HTO, and optionally

(d) medium that supports viability, activity, and/or activation of the T cells and APCs,

such the composition sorted in (I) comprises a plurality of unique biological samples.

3. The method of claim 2, wherein the APCs comprise monocyte-derived dendritic cells, dendritic cells, monocytes, macrophages, B cells, or a combination thereof.

4. The method of any one of claims 1-3, wherein sorting comprises fluorescence activated cell sorting of activated T cells based on the expression of the activation-induced marker (AIM).

5. The method of claim 4, wherein the AIM is selected from the group consisting of CD137/4-1BB, CD107, IFN γ , PD-1, CD40L, OX40, CD25, CD69, CD28, HLA-DR, CX3CR1, TIM3, LAG3, TIGIT, and any combination thereof.

6. The method of claim 4 or claim 5, wherein fluorescence activated cell sorting is based on detection with fluorescently labeled antibody to the AIM.
7. The method of any one of the preceding claims, comprising performing further functional and/or phenotypic analysis on the activated T cell analyzed in II, optionally wherein the further functional and/or phenotypic analysis is selected from the group consisting of flow cytometric analysis, CITE-seq analysis, multimer analysis, and a combination thereof.
8. The method of claim 7, wherein the further functional and/or phenotypic analysis measures the protein and/or RNA expression levels of one or more of CD3, CD4, CD8, CD25, CD27, CD28, CD45RA, CD62L, HLA-DR, CD137/4-1BB, CD69, CD278, CD274, CD279, CD127, CD197, IFN γ , GZMH, GNLY, CD38, CCL3, and LAG3.
9. The method of any one of the preceding claims, wherein peripheral blood mononuclear cells provide the T cell and surface bound MHC.
10. The method of any one of the preceding claims, wherein the molecule that labels the T cell with the unique HTO comprises an antibody that binds a cell surface molecule.
11. The method of any one of the preceding claims, wherein the AIM is or comprises CD137/4-1BB.
12. The method of any one of the preceding claims, wherein the method comprises identifying a TCR α chain sequence and/or a TCR β chain sequence of a TCR that specifically binds the antigen and the TCR α chain sequence and/or a TCR β chain sequence are a TCR α chain variable region sequence and/or a TCR β chain variable region sequence, respectively.
13. The method of any one of the preceding claims, wherein the method comprises identifying a TCR α chain sequence and/or a TCR β chain sequence of a TCR that specifically binds the antigen and the method further comprises utilizing the TCR α chain sequence and/or a TCR β chain sequence in making a therapeutic.

14. A composition comprising a biological sample that comprises:
- (a) a T cell and a surface bound Major Histocompatibility Complex (MHC), wherein the T cell is capable of recognizing a peptide presented in the context of the surface bound MHC,
 - (b) an antigen,
 - (c) a hashtag oligonucleotide (HTO) that specifically identifies the antigen, wherein the HTO is conjugated to a molecule that labels the T cell with the HTO, and optionally
 - (d) medium that supports activation of the T cell.
15. The composition of claim 14, wherein
- (a) the MHC is expressed on the surface of an antigen presenting cell (APC), optionally wherein:
 - the T cell and APC are autologous,
 - the T cell and the APC are each isolated from a human donor, and/or
 - the APC is selected from the group consisting of a monocyte-derived dendritic cell, a dendritic cell, a monocyte, a macrophage, a B cell and a combination thereof,
 - (b) the antigen
 - (I) is selected from the group consisting of
 - (i) a bacterial antigen or portion thereof,
 - (ii) a viral antigen or portion thereof,
 - (iii) an allergen or portion thereof,
 - (iv) a tumor associated antigen or a portion thereof, and
 - (v) a combination thereof, and/or
 - (II) comprises
 - (i) an amino acid sequence,
 - (ii) a nucleotide sequence,
 - (iii) lysate, and
 - (iv) a combination thereof,
 - (c) the HTO conjugated molecule comprises an antibody that binds a cell surface molecule or a lipid, and/or

(d) the medium comprises a cytokine that supports the viability of the T cell and/or an APC.

16. The composition of claim 15, wherein
the antibody binds a cell surface marker selected from the group consisting of $\beta 2$ microglobulin, CD298, CD2, CD3, CD4, CD8, and any combination thereof, or
the lipid that incorporates into a cell membrane.

17. The composition of claim 15 or claim 16, wherein the cytokine that supports the viability of the T cell and/or the APC is selected from the group consisting of IL-2, IL-7, IL-15, GM-CSF, IL-4, and any combination thereof.

18. The composition of any one of claims 15-17, further comprising a second biological sample, wherein the second biological sample comprises:

- (a) a second T cell and a second surface bound MHC, wherein the second T cell is capable of recognizing a peptide presented in the context of the second surface bound MHC,
- (b) a second antigen,
- (c) a second HTO that specifically identifies the second antigen, wherein the HTO is conjugated to a second molecule that labels the second T cell with the second HTO, and optionally
 - (d) a medium that supports activation of the second T cell, wherein
 - (i) the T cell and second T cell are isolated from the same subject,
 - (ii) the antigen and the second antigen are not identical,
 - (iii) the molecule that labels the T cell with the HTO and the second molecule that labels the second T cell with the second HTO are identical, and the HTO and the second HTO are not identical.

19. The composition of any one of claims 14-18, wherein the composition further comprises an agent that allows sorting an activated T cell based on expression of an activation-induced marker (AIM).

20. The composition of claim 19, wherein the agent that allows sorting activated T cell based on expression of an AIM is a fluorescently labeled antibody that specifically binds the AIM.
21. The composition of claim 19 or claim 20, wherein the AIM is selected from the group consisting of CD137/4-1BB, CD107, IFN γ , PD-1, CD40L, OX40, CD25, CD69, CD28, HLA-DR, CX3CR1, TIM3, LAG3, and/or TIGIT.
22. The composition of any one of claims 14-21, wherein the composition comprises an antibody and/or MHC multimers useful for flow cytometric analysis or CITE-seq analysis of the composition.
23. A kit comprising
a plurality of unique antigens, and
a plurality of unique hashtag oligonucleotides (HTOs), each of which specifically identifies only one of the plurality of unique antigens.
24. The kit of claim 23, wherein the kit further comprises an agent that allows sorting of activated T cells based on their expression of an activation-induced marker (AIM), optionally wherein the agent that allows sorting activated T cell based on expression of an AIM is a fluorescently labeled antibody that specifically binds the AIM.
25. The kit of claim 23 or claim 24, wherein each of the plurality of unique HTOs is conjugated to an identical molecule such that the kit comprises a plurality of unique HTO-conjugated molecules.
26. The kit of any one of claims 23-25, wherein each of the plurality of unique antigens comprises unique and overlapping peptide sequences from a single protein.
27. The kit of claim 26, wherein the single protein is selected from the group consisting of a pathogenic antigen, a tumor associated antigen, or a transplantation antigen.

28. Use of the method of any one of claims 1-13, the composition of any one of claims 14-22, or the kit of any one of claims 23-27 for analyzing a T cell mediated immune response of a patient to a vaccine.
29. Use of the method of any one of claims 1-13, the composition of any one of claims 14-22, or the kit of any one of claims 23-27 for analyzing a T cell mediated immune response of a patient to an immunotherapy.
30. Use of the method of any one of claims 1-13, the composition of any one of claims 14-22, or the kit of any one of claims 23-27 for analyzing a T cell mediated immune response in a patient during immunotherapy of the patient.
31. Use of the method of any one of claims 1-13, the composition of any one of claims 14-22, or the kit of any one of claims 23-27 for analyzing T cell responses of a patient to an autoantigen.
32. Use of the method of any one of claims 1-13, the composition of any one of claims 14-22, or the kit of any one of claims 23-27 for analyzing T cell responses of a patient to a transplant antigen.
33. Use of the method of any one of claims 1-13, the composition of any one of claims 14-22, or the kit of any one of claims 23-27 to identify one or more TCR variable region sequences of an activated T cell.
34. The use of claim 33, wherein the one or more TCR variable region sequences comprises a CDR3 sequence of a TCR α chain and/or a CDR3 sequence of a TCR β chain.
35. Use of the one or more TCR variable region sequences identified in claim 33 or claim 34 in making a human therapeutic.

36. The use of claim 35, wherein the human therapeutic comprises a T cell comprising the one or more TCR variable region sequences identified using the method of any one of claims 1-13, the composition of any one of claims 14-22, or the kit of any one of claims 23-27.