

CLAIMS

What is claimed is:

1. A method for identifying a unique antigen capable of activating a T cell, and optionally a T-cell receptor (TCR) α chain nucleotide sequence and/or a TCR β chain nucleotide 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,

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,

wherein the unique biological sample comprises:

(a) a surface bound Major Histocompatibility Complex (MHC),

(b) a unique antigen, wherein the unique antigen is capable of binding the surface bound MHC,

(c) a unique hashtag oligonucleotide (HTO) that (i) is used to specifically identify the unique antigen and (ii) is conjugated to a molecule that incorporates into the cell membrane of T cells and/or binds a cell surface marker expressed by T cells, and

(d) a T cell comprising the unique HTO, wherein the T cell is capable of recognizing the unique antigen when the unique antigen is bound to the surface bound MHC, and

(II) performing single cell sequencing analysis on the activated T cell sorted in (I) to identify the unique HTO, wherein identifying the unique HTO identifies the unique antigen, 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 nucleotide sequences of a TCR that specifically binds the unique antigen and is expressed by the activated T cell.

2. The method of claim 1, comprising, before sorting:
creating a plurality of unique biological samples such that the composition sorted in (I) comprises the plurality of unique biological samples,
wherein each of the plurality of unique biological samples comprises
 - (a) antigen presenting cells (APCs) expressing a cell surface MHC,
 - (b) a unique antigen that binds the cell surface MHC expressed by the APCs,
 - (c) a unique HTO that (i) specifically identifies the unique antigen and (ii) is conjugated to a molecule that incorporates into the cell membrane of T cells and/or binds a cell surface marker expressed by T cells, and
 - (d) T cells comprising the unique HTO,
wherein the APCs and the T cells are isolated from a subject, and optionally
 - (e) wherein each unique biological sample comprises medium that supports viability, activity, and/or activation of the T cells and APCs.
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 fluorescence activated cell sorting is based on detection with fluorescently labeled antibody to the AIM.
6. 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.

7. The method of claim 6, 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.
8. The method of any one of the preceding claims, wherein peripheral blood mononuclear cells provide the T cell and the surface bound MHC.
9. The method of any one of the preceding claims, wherein the molecule that binds a cell surface marker expressed by the T cells comprises an antibody that binds a cell surface molecule.
10. The method of any one of the preceding claims, wherein the AIM is or comprises CD137/4-1BB.
11. The method of any one of the preceding claims, wherein the method comprises identifying a TCR α chain nucleotide sequence and/or a TCR β chain nucleotide sequence of a TCR that specifically binds the antigen and the TCR α chain nucleotide sequence and/or the TCR β chain nucleotide sequence are a TCR α chain variable region nucleotide sequence and/or a TCR β chain variable region nucleotide sequence, respectively.
12. The method of any one of the preceding claims, wherein the method comprises identifying a TCR α chain nucleotide sequence and/or a TCR β chain nucleotide sequence of a TCR that specifically binds the antigen, and the method further comprises utilizing the TCR α chain nucleotide sequence and/or the TCR β chain nucleotide sequence in making a therapeutic.
13. A composition comprising a biological sample that comprises:
 - (a) a surface bound MHC expressed on the surface of an APC,
 - (b) a unique antigen, wherein the unique antigen is capable of binding the surface bound MHC,

(c) a unique HTO that (i) specifically identifies the unique antigen, and (ii) is conjugated to a molecule that incorporates into the cell membrane of T cells and/or binds a cell surface marker expressed by T cells,

(d) a T cell comprising the unique HTO, wherein the T cell is capable of recognizing the unique antigen when the unique antigen is bound to the surface bound MHC, and

(e) an agent that allows sorting an activated T cell based on expression of an AIM.
and optionally

(f) medium that supports activation of the T cell.

14. The composition of claim 13, wherein

(a) (i) the T cell and the APC are autologous,

(ii) the T cell and the APC are each isolated from a human donor, and/or

(iii) 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 unique antigen

(I) is selected from the group consisting of

(i) a bacterial antigen,

(ii) a viral antigen,

(iii) an allergen,

(iv) a tumor associated antigen, 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 molecule that binds a cell surface marker expressed by T cells 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 the APC.

15. The composition of claim 14, wherein
- (i) the cell surface marker expressed by T cells is selected from the group consisting of $\beta 2$ microglobulin, CD298, CD2, CD3, CD4, CD8, and any combination thereof, or
 - (ii) the molecule that incorporates into the cell membrane of T cells comprises a lipid.
16. The composition of claim 14 or claim 15, 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.
17. The composition of any one of claims 14-16, further comprising a second unique biological sample, wherein the second unique biological sample comprises:
- (a) a second surface bound MHC expressed on the surface of an APC,
 - (b) a second unique antigen, wherein the second unique antigen is capable of binding the second surface bound MHC,
 - (c) a second unique HTO that (i) specifically identifies the second unique antigen, and (ii) is conjugated to the molecule that incorporates into the cell membrane of T cells and/or binds a cell surface marker expressed by T cells, and
 - (d) a second T cell comprising the second unique HTO, wherein the second T cell is capable of recognizing the second unique antigen when the second unique antigen is bound to the second surface bound MHC,
- 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, and
 - (iii) the molecule that incorporates into the cell membrane of T cells and/or binds a cell surface marker expressed by T cells is identical for the unique biological sample and the second unique biological sample, and the unique HTO and the second unique HTO are not identical.
18. The composition of claim 17, wherein the composition further comprises a medium that supports activation of the second T cell.

19. The composition of claim of any one of claims 13-18, wherein the agent that allows sorting the activated T cell based on expression of an AIM is a fluorescently labeled antibody that specifically binds the AIM.
20. The composition of any one of claims 13-19, 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.
21. The composition of any one of claims 13-20, wherein the composition comprises an antibody and/or MHC multimers useful for flow cytometric analysis or CITE-seq analysis of the composition.
22. Use of the method of any one of claims 1-12, or the composition of any one of claims 13-21, for analyzing a T cell mediated immune response of a patient:
- (i) to a vaccine,
 - (ii) to an immunotherapy,
 - (iii) during immunotherapy of the patient,
 - (iv) to an autoantigen, or
 - (v) to a transplant antigen.
23. Use of the method of any one of claims 1-12, or the composition of any one of claims 13-21 to identify one or more TCR variable region nucleotide sequences of an activated T cell.
24. The use of claim 23, wherein the one or more TCR variable region nucleotide sequences comprises a CDR3 nucleotide sequence of a TCR α chain nucleotide sequence and/or a CDR3 nucleotide sequence of a TCR β chain nucleotide sequence.