pMHC tetramer combinations directly from a human blood sample with astonishing sensitivity, although this technology is presently limited to ~100 such combinations per assay [12]. Each of these approaches has contributed valuable insights into T-cell epitopes; however, these methods are slow, labor-intensive and require a high degree of user skill.

[0007] The present invention addresses this need for new and improved technologies for the efficient and systematic identification of the repertoire of T-cell epitopes.

SUMMARY

[0007a] In one aspect of the present disclosure, there is provided a (i) lentivirus-like particle or (ii) lentivirus, which lentivirus-like particle or a lentivirus is physically associated with an expression product via a mammalian cell membrane portion associated with the lentivirus-like particle or lentivirus and the mammalian transmembrane domain of the expression product, the expression product comprising in N-terminal to C-terminal order:

a 5 to 15 amino acid peptide, contiguous with

a first linker peptide sequence, contiguous with

a beta 2 microglobulin sequence, contiguous with

a second linker peptide sequence, contiguous with

a class I Major Histocompatibility Complex (MHC) heavy chain sequence, contiguous with

a third linker peptide sequence, contiguous with

a fluorescent protein or a sequence of an immunoglobulin Fc domain, contiguous with

a fourth linker peptide sequence, contiguous with

the mammalian transmembrane domain, contiguous with

a viral packaging sequence.

[0007b] In another aspect of the present disclosure, there is provided a plurality of isolated lentiviruses or isolated lentivirus-like particles described herein.

sequence encoding a beta 2 microglobulin sequence, contiguous with

[0007c] In a further aspect of the present disclosure, there is provided an isolated suspension-adapted mammalian cell expressing an expression product of a heterologous nucleic acid transduced or transfected therein, or a membrane-bound portion of such cell expressing the expression product, which expression product comprises, in N-terminal to C-terminal order:

a 5 to 15 amino acid peptide, contiguous with
a first linker peptide sequence, contiguous with
a beta 2 microglobulin sequence, contiguous with
a second linker peptide sequence, contiguous with
a class I Major Histocompatibility Complex heavy chain sequence, contiguous with
a third linker peptide sequence, contiguous with
a fluorescent protein or a sequence of an immunoglobulin Fc domain, contiguous with
a fourth linker peptide sequence, contiguous with
a mammalian transmembrane domain, contiguous with
a viral packaging sequence.

[0007d] In another aspect, the present disclosure refers to the membrane-bound portion of the cell disclosed herein, wherein the membrane bound portion comprises the expression product.

[0007e] In a further aspect of the present disclosure, there is provided a plurality of the isolated suspension-adapted cells described herein, or a plurality of membrane-bound portions of such cells expressing the expression product, wherein the plurality comprises at least two different encoded 5 to 15 amino acid peptides, wherein the encoded peptide or peptides is or are presented on an extracellular surface of the cells.

[0007f] In another aspect of the present disclosure, there is provided a method of identifying a T-cell epitope comprising:

contacting a T-cell with a plurality, comprising at least two cells, of isolated suspension-adapted cells described herein, or a membrane-bound portion of such cells expressing the expression product, or with lentivirus-like particles or lentiviruses described herein associated with a membrane portion of such cells;

recovering T-cell(s) which have formed a conjugate with a suspension-adapted cell or membrane-bound portions;

recovering DNA from the suspension-adapted cell(s);

sequencing the recovered DNA; and

identifying the 5 to 15 amino acid peptide(s) encoded for in the DNA, so as to thereby identify a T-cell epitope.

[0008] The present disclosure provides an isolated suspension-adapted cell transduced by or transfected with a heterologous nucleic acid comprising, in 5’ to 3’ order:

a leader oligonucleotide sequence, contiguous with

an oligonucleotide sequence encoding an 8, 9, 10, 11 or 12 amino acid peptide, contiguous with

an oligonucleotide sequence encoding a first linker, contiguous with

an oligonucleotide sequence encoding a beta 2 microglobulin sequence, contiguous with

an oligonucleotide sequence encoding a second linker, contiguous with

an oligonucleotide sequence encoding a Major Histocompatibility Complex heavy chain sequence, contiguous with

an oligonucleotide sequence encoding a third linker, contiguous with

an oligonucleotide sequence encoding a fluorescent protein, contiguous with

an oligonucleotide sequence encoding a fourth linker, contiguous with

an oligonucleotide sequence encoding a Major Histocompatibility Complex heavy chain transmembrane domain.

[0009] The present disclosure also provides isolated suspension-adapted cell expressing an expression product of a heterologous nucleic acid transduced or transfected therein, which expression product comprises, in N-terminal to C-terminal order:

an 8, 9, 10, 11 or 12 amino acid peptide, contiguous with

a first linker peptide sequence, contiguous with

a beta 2 microglobulin sequence, contiguous with

a second linker peptide sequence, contiguous with

a Major Histocompatibility Complex heavy chain sequence, contiguous with

a third linker peptide sequence, contiguous with

a fluorescent protein, contiguous with

What is claimed is:

1. A (i) lentivirus-like particle or (ii) lentivirus, which lentivirus-like particle or a lentivirus is physically associated with an expression product via a mammalian cell membrane portion associated with the lentivirus-like particle or lentivirus and the mammalian transmembrane domain of the expression product, the expression product comprising in N-terminal to C-terminal order:

a 5 to 15 amino acid peptide, contiguous with
a first linker peptide sequence, contiguous with
a beta 2 microglobulin sequence, contiguous with
a second linker peptide sequence, contiguous with
a class I Major Histocompatibility Complex (MHC) heavy chain sequence, contiguous with a third linker peptide sequence, contiguous with
a fluorescent protein or a sequence of an immunoglobulin Fc domain, contiguous with
a fourth linker peptide sequence, contiguous with
the mammalian transmembrane domain, contiguous with
a viral packaging sequence.

2. The lentivirus-like particle or lentivirus of claim 1, wherein the viral packaging sequence comprises the GP41 envelope protein residues 706-713.

3. The lentivirus-like particle or lentivirus of claim 1, wherein the mammalian transmembrane domain is a class I MHC heavy chain transmembrane domain.

4. A plurality of isolated lentiviruses or isolated lentivirus-like particles of claim 1 or claim 2.

5. The plurality of claim 4, wherein the plurality comprises at least 100 different encoded 5 to 15 amino acid peptides.

6. An isolated suspension-adapted mammalian cell expressing an expression product of a heterologous nucleic acid transduced or transfected therein, or a membrane-bound portion of such cell expressing the expression product, which expression product comprises, in N-terminal to C-terminal order:

a 5 to 15 amino acid peptide, contiguous with
a first linker peptide sequence, contiguous with
a beta 2 microglobulin sequence, contiguous with
a second linker peptide sequence, contiguous with
a class I Major Histocompatibility Complex heavy chain sequence, contiguous with
a third linker peptide sequence, contiguous with
a fluorescent protein or a sequence of an immunoglobulin Fc domain, contiguous with
a fourth linker peptide sequence, contiguous with
a mammalian transmembrane domain, contiguous with
a viral packaging sequence.

7. The cell of claim 6, wherein the viral packaging sequence comprises the GP41 envelope protein residues 706-713.

8. The membrane-bound portion of the cell of claim 6, wherein the membrane-bound portion comprises the expression product.

9. The membrane-bound portion of the cell of claim 6 or 7 which is a microvesicle or an exosome.

10. The isolated suspension-adapted cell, or membrane-bound portion of such cell expressing the expression product, of any one of claims 6-9, wherein the cell expresses the expression product comprising the sequence of the immunoglobulin Fc domain.

11. The isolated suspension-adapted cell, or membrane-bound portion of such cell expressing the expression product, of any one of claims 6-9, wherein the cell expresses the expression product comprising the fluorescent protein.

12. The isolated suspension-adapted cell of any of the preceding claims, or a membrane-bound portion of such cell expressing the expression product, wherein the mammalian transmembrane domain is a Major Histocompatibility Complex heavy chain transmembrane domain.

13. A plurality of the isolated suspension-adapted cells of any one of claims 6-12, or a plurality of membrane-bound portions of such cells expressing the expression product, wherein the plurality comprises at least two different encoded 5 to 15 amino acid peptides, wherein the encoded peptide or peptides is or are presented on an extracellular surface of the cells.

14. The plurality of claim 13, wherein the plurality comprises at least 100 different encoded 5 to 15 amino acid peptides.

15. A method of identifying a T-cell epitope comprising:

contacting a T-cell with a plurality, comprising at least two cells, of isolated suspension-adapted cells according to any one of claims 6-14, or a membrane-bound portion of such cells expressing the expression product, or with lentivirus-like particles or lentiviruses according to any one of claims 1-5 associated with a membrane portion of such cells;

recovering T-cell(s) which have formed a conjugate with a suspension-adapted cell or membrane-bound portions;

recovering DNA from the suspension-adapted cell(s);

sequencing the recovered DNA; and

identifying the 5 to 15 amino acid peptide(s) encoded for in the DNA, so as to thereby identify a T-cell epitope.

16. The method of claim 15, wherein the T-cells comprise peripheral T-cells obtained from a subject, such as a human subject.