

(WO2019/070755) have been described. Recently, activation markers from tumor infiltrating T lymphocytes were described (WO 2021/188954 A1, Lowery et al. (2022), Science 10.1126/science.abl5447).

5 T-cell activation has long been acknowledged to involve presentation of an antigen, e.g. an epitope of a polypeptide, in the context of major histocompatibility complexes (MHCs). MHC class I, interacting with TCR complexes comprising the CD8 protein on CD8⁺ T-cells, is expressed by all nucleate cells, while MHC class II, interacting with TCR complexes comprising the CD4 protein on CD4⁺ T-cells, is only expressed by professional antigen presenting cells, mostly B- cells and dendritic cells. However,
10 other surface molecules of cells have been found to be involved in T-cell interaction and activation as well (cf. e.g. Iwabuchi & van Kaer (2019), Front Immunol 10:1837 (doi: 10.3389/fimmu.2019.01837).

Nonetheless, there is still a need for improved methods for providing T-cells reactive to specific antigens, e.g. cancer antigens, and corresponding TCRs. This problem is solved by the embodiments characterized in the claims and described herein below.
15

Summary

In one aspect, the present disclosure relates to a method of identifying a T-cell reactive to cells of a subject presenting a T-cell activating antigen (reactive T-cell), comprising (a) determining expression
20 of at least one of CCL3L1, CCL4, CCL4L2, CCL3, and CXCL13 in T-cells from a sample of said subject; and (b) identifying a reactive T-cell based on the determination of step (a) preferably wherein said T-cell activating antigen is a cancer antigen or an autoimmune T-cell activating antigen, more preferably is a cancer antigen.

25 In another aspect, the present disclosure relates to a method of identifying a TCR binding to a T-cell activating antigen presented on a cell, preferably a cancer cell, of a subject, said method comprising (A) identifying a reactive T-cell according to the method as disclosed herein, (B) providing the amino acid sequences of at least the complementarity determining regions (CDRs) of the TCR of the reactive T-cell identified in step (A); and, hereby, (C) identifying a TCR binding to an activating antigen presented on
30 a cell.

In another aspect, the present disclosure relates to a method of providing a T-cell recognizing a cell presenting a T-cell activating anti-gen, preferably a cancer cell, said method comprising (i) identifying
35 a TCR binding to a cell presenting a T-cell activating antigen according to the method as disclosed herein, (ii) expressing a TCR comprising at least the complementarity determining regions (CDRs) of the TCR of step (I) in a T-cell, and, thereby, (iii) providing a T-cell recognizing a cell presenting a T-cell activating antigen, preferably a cancer cell.

In another aspect, the present disclosure relates to a reactive T-cell identified by the method as disclosed
40 herein and/or obtained or obtainable by the method as disclosed herein, preferably comprising a T-cell receptor comprising an amino acid sequence of SEQ ID NO:1 and/or SEQ ID NO:2, for use in therapy.

In another aspect, the present disclosure relates to use of a reactive T-cell identified by the method as disclosed herein and/or obtained or obtainable by the method as disclosed herein preferably comprising a T-cell receptor comprising an amino acid sequence of SEQ ID NO:1 and/or SEQ ID NO:2 in the manufacture of a medicament for treating and/or preventing cancer in a subject.

5

In another aspect, the present disclosure relates to a method of identifying at least one biomarker of reactive T-cells, comprising (I) providing expression data of a plurality of biomarkers of T-cells in a sample of a subject, (II) providing a clustering said plurality of T-cells based on the expression of the biomarkers of step (I); (III) providing amino acid sequences of at least the complementarity determining regions (CDRs) of TCRs of T-cells of step (II); (IV) determining reactivity of T-cells expressing a TCR comprising the CDRs of step (III) to cells presenting a T-cell activating antigen; (V) repeating steps (III) and (IV) at least once for further T-cells clustering with T-cells whose TCRs are determined to be reactive to cells presenting a T-cell activating antigen in step (IV), wherein the TCRs of said further T-cells are non-identical to the TCRs of step (IV); (VI) determining at least one cluster of step (II) comprising the highest fraction of T-cells comprising T-cell receptors recognizing cells presenting a T-cell activating antigen; and (VII) determining at least one biomarker expressed by the highest fraction of T-cells in the cluster determined in step (VI), thereby identifying at least one biomarkers of cancer -reactive T-cells.

20 **Detailed description**

In accordance, the present invention relates to a method of identifying a T-cell reactive to cells of a subject presenting a T-cell activating antigen (reactive T-cell), comprising

(a) determining expression of at least one of CCL4, CCL4L2, CCL3, CCL3L1, and CXCL13 in T-cells from a sample of said subject; and

25 (b) identifying a reactive T-cell based on the determination of step (a).

Preferably, the present invention relates to a method of identifying a T-cell reactive to cancer cells of a subject (cancer-reactive T-cell), comprising

(a) determining expression of at least one of CCL4, CCL4L2, CCL3, CCL3L1, and CXCL13 in T-cells from a sample of said subject; and

30 (b) identifying a cancer-reactive T-cell based on the determination of step (a).

In general, terms used herein are to be given their ordinary and customary meaning to a person of ordinary skill in the art and, unless indicated otherwise, are not to be limited to a special or customized meaning. As used in the following, the terms “have”, “comprise” or “include” or any arbitrary grammatical variations thereof are used in a non-exclusive way. Thus, these terms

35

Claims

1. A method of identifying a T-cell reactive to cells of a subject presenting a T-cell activating antigen (reactive T-cell), comprising
 - (a) determining expression of at least one of CCL3L1, CCL4, CCL4L2, CCL3, and CXCL13 in T-cells from a sample of said subject; and
 - (b) identifying a reactive T-cell based on the determination of step (a), preferably wherein said T-cell activating antigen is a cancer antigen or an autoimmune T-cell activating antigen, more preferably is a cancer antigen.
2. The method of claim 1, wherein step (a) comprises determining expression of at least two, preferably at least three, more preferably at least four, of CCL3L1, CCL4, CCL4L2, CCL3, and CXCL13.
3. The method of claim 1 or 2, wherein step (a) comprises further determining expression of at least one biomarker selected from the list consisting of IFNG, HAVCR2, FNBP1, CSRN1P1, SPRY1, RHOH, FOXN2, HIF1A, TOB1, RILPL2, CD8B, GABARAPL1, TNFSF14, EGR1, EGR2, TAGAP, TNFSF9, ANXA1, MAP3K8, PIK3R1, DUSP2, DUSP4, DUSP6, CLIC3, RASGEF1B, LAG3, XCL2, NR4A2, DNAJB6, NFKBID, MCL1, EVI2A, SLC7A5, H3F3B, NR4A3, REL, IRF4, CST7, ATF3, TNF, GPR171, BCL2A1, ITGA1, TNFAIP3, NR4A1, RUNX3, HERPUD2, FASLG, CBLB, PTGER4, SLA, XCL1, BHLHE40, LYST, KLRD1, ZNF682, CTSW, SLC2A3, NLRP3, SCML4, VSIR, LINC01871, and ZFP36L1.
4. The method of any one of claims 1 to 3, wherein step (a) comprises further determining expression of at least one biomarker selected from the list consisting of LAG3, GABARAPL1, CBLB, SLA, KLRD1, and CLEC2B, preferably comprises determining all biomarkers of claim 1 and/or of claim 4.
5. The method of any one of claims 1 to 4, wherein step (a) comprises further determining expression of at least one biomarker selected from the list consisting of CTSD, CD7, CD3D, LSP1, SNAP47, GAPDH, KLRK1, TNS3, VCAM1, KLRC2, PMAIP1, FYN, CTLA4, GSTP1, AREG, FAM3C, SH3BGRL3, CD3E, SRGAP3, SRGN, SIRPG, SCPEP1, RHOB, ANKRD28, LINC02446, RABAC1, IKZF3, BCAS4, CD2,

BLOC1S1, RHOA, EID1, MYL6, CLIC1, IQGAP1, ARPC2, PHYKPL, PRDM1, EVL, TPI1, ADGRE5, PAXX, RGS2, HERPUD1, IFI27L2, SEPTIN7, UBB, JUN, CFLAR, LITAF, ANXA5, STAT3, RSRP1, PRDX5, SEM1, SERPINB1, RNF19A, IL2RG, ENSA, SRP14, ATP6V0C, LY6E, BIN1, AKAP13, PDE4D, PELI1, PARK7, MSN, SERTAD1, RAC2, SELENOH, PSMB8, CKLF, KLRC1, RNASEK, MT2A, TXNIP, and FOXP3.

6. The method of any one of claims 1 to 5, wherein step (a) comprises determining expression of at least CCL3L1 + CCL3; CCL3L1 + CCL3 + LAG3 + KLRD1; CCL3L1 + CCL3 + CXCL13; CCL3L1 + CCL3 + CXCL13 + KLRD1; CCL3L1 + CCL3 + CXCL13 + LAG3; CCL3L1 + CCL3 + CXCL13 + LAG3 + KLRD1; CCL3L1 + CCL3 + CCL4 + CCL3; CCL3L1 + CCL4 + CCL3 + CXCL13; CCL3L1 + CCL4 + CCL3 + CXCL13 + KLRD1; CCL3L1 + CCL4 + CCL3 + CXCL13 + LAG3; CCL3L1 + CCL4 + CCL3 + CXCL13 + LAG3 + KLRD1; CCL3L1 + CCL4 + CCL3 + LAG3; CCL3L1 + CCL4 + CCL3 + LAG3 + KLRD1; CCL3L1 + CCL4 + CCL4L2 + CCL3 + CXCL13 + KLRD1; CCL3L1 + CCL4 + CCL4L2 + CCL3 + CXCL13 + LAG3 ; CCL3L1 + CCL4 + CCL4L2 + CCL3 + CXCL13 + LAG3 + KLRD1; CCL3L1 + CCL4 + CCL4L2 + CCL3 + KLRD1; CCL3L1 + CCL4 + CCL4L2 + CXCL13; CCL3L1 + CCL4 + CCL4L2 + CXCL13 + KLRD1; CCL3L1 + CCL4 + CCL4L2 + CXCL13 + LAG3; CCL3L1 + CCL4 + CCL4L2 + CXCL13 + LAG3 + KLRD1; CCL3L1 + CCL4 + CCL4L2 + LAG3 + KLRD1; CCL3L1 + CCL4 + CXCL13; CCL3L1 + CCL4 + CXCL13 + KLRD1; CCL3L1 + CCL4 + CXCL13 + LAG3; CCL3L1 + CCL4 + CXCL13 + LAG3 + KLRD1; CCL3L1 + CCL4 + LAG3 + KLRD1; CCL3L1 + CCL4L2 + CCL3; CCL3L1 + CCL4L2 + CCL3 + CXCL13; CCL3L1 + CCL4L2 + CCL3 + CXCL13 + KLRD1; CCL3L1 + CCL4L2 + CCL3 + CXCL13 + LAG3; CCL3L1 + CCL4L2 + CCL3 + CXCL13 + LAG3 + KLRD1; CCL3L1 + CCL4L2 + CCL3 + KLRD1; CCL3L1 + CCL4L2 + CCL3 + LAG3 + KLRD1; CCL3L1 + CCL4L2 + CXCL13; CCL3L1 + CCL4L2 + CXCL13 + KLRD1; CCL3L1 + CCL4L2 + CXCL13 + LAG3; CCL3L1 + CCL4L2 + CXCL13 + LAG3 + KLRD1; CCL3L1 + CCL4L2 + KLRD1; CCL3L1 + CCL4L2 + LAG3; CCL3L1 + CCL4L2 + LAG3 + KLRD1; CCL3L1 + CXCL13; CCL3L1 + CXCL13 + KLRD1; CCL3L1 + CXCL13 + LAG3; CCL3L1 + CXCL13 + LAG3 + KLRD1; CCL3L1 + LAG3 + KLRD1; CCL3 + CXCL13; CCL3 + CXCL13 + KLRD1; CCL3 + CXCL13

+ LAG3; CCL3 + CXCL13 + LAG3 + KLRD1; CCL3 + LAG3 + KLRD1; CCL4 + CCL3; CCL4 + CCL3 + CXCL13; CCL4 + CCL3 + CXCL13 + KLRD1; CCL4 + CCL3 + CXCL13 + LAG3; CCL4 + CCL3 + CXCL13 + LAG3 + KLRD1; CCL4 + CCL3 + KLRD1; CCL4 + CCL3 + LAG3 + KLRD1; CCL4 + CCL4L2 + CCL3 + CXCL13; CCL4 + CCL4L2 + CCL3 + CXCL13 + KLRD1; CCL4 + CCL4L2 + CCL3 + CXCL13 + LAG3; CCL4 + CCL4L2 + CCL3 + CXCL13 + LAG3 + KLRD1; CCL4 + CCL4L2 + CCL3 + KLRD1; CCL4 + CCL4L2 + CCL3 + LAG3; CCL4 + CCL4L2 + CXCL13; CCL4 + CCL4L2 + CXCL13 + KLRD1; CCL4 + CCL4L2 + CXCL13 + LAG3; CCL4 + CCL4L2 + CXCL13 + LAG3 + KLRD1; CCL4 + CCL4L2 + KLRD1; CCL4 + CCL4L2 + LAG3 + KLRD1; CCL4 + CXCL13; CCL4 + CXCL13 + KLRD1; CCL4 + CXCL13 + LAG3; CCL4 + CXCL13 + LAG3 + KLRD1; CCL4 + LAG3 + KLRD1; CCL4L2 + CCL3; CCL4L2 + CCL3 + CXCL13; CCL4L2 + CCL3 + CXCL13 + KLRD1; CCL4L2 + CCL3 + CXCL13 + LAG3; CCL4L2 + CCL3 + CXCL13 + LAG3 + KLRD1; CCL4L2 + CCL3 + KLRD1; CCL4L2 + CCL3 + LAG3 + KLRD1; CCL4L2 + CXCL13; CCL4L2 + CXCL13 + KLRD1; CCL4L2 + CXCL13 + LAG3; CCL4L2 + CXCL13 + LAG3 + KLRD1; CCL4L2 + LAG3 + KLRD1; CXCL13 + LAG3; CXCL13 + LAG3 + KLRD1; KLRD1; KLRD1 + CCL3; KLRD1 + CCL3L1; KLRD1 + CCL4L2; KLRD1 + CXCL13; KLRD1 + LAG3; all biomarkers of Table 1; all biomarkers of Table 5; or all biomarkers of Table 6.

7. The method of any one of claims 1 to 6, wherein said T-cell activating antigen is a cancer antigen, and wherein preferably said sample is a tumor sample.
8. The method of claim 7, wherein said cancer is a brain metastasis of a non-brain primary tumor, is lung cancer, or is glioblastoma, preferably is a brain metastasis of a non-brain primary tumor or is lung cancer.
9. A method of identifying a TCR binding to a T-cell activating antigen presented on a cell, preferably a cancer cell, of a subject, said method comprising
 - (A) identifying a reactive T-cell according to the method of any one of claims 1 to 4,
 - (B) providing the amino acid sequences of at least the complementarity determining regions (CDRs) of the TCR of the reactive T-cell identified in step (A); and, hereby,
 - (C) identifying a TCR binding to an activating antigen presented on a cell.

10. The method of any one of claims 1 to 9, wherein expression of at least one biomarker of step a) and/or the nucleic acid sequences encoding the amino acid sequences of step (B) is/are determined by single-cell sequencing, preferably by single-cell RNA sequencing.
11. The method of claim 9 or 10, wherein said method comprises further step B1) expressing a TCR comprising at least the CDRs determined in step B) in a host cell, preferably a T-cell.
12. The method of claim 11, wherein said method further comprises further step B2) determining binding of the TCR expressed in step B1) to a T-cell activating antigen, preferably complexed in a major histocompatibility complex (MHC), preferably MHC class I, molecule, preferably in a tetramer assay.
13. The method of claim 11 or 12, wherein said method further comprises step B3) determining recognition of cells presenting a T-cell activating antigen by the TCR expressed in step B1).
14. The method of any one of claims 9 to 13, wherein said method further comprises step B4) producing a soluble TCR comprising at least the CDRs determined in step B) and determining binding of said soluble TCR to a cancer cell and/or to a cancer antigen complexed in a major histocompatibility complex (MHC), preferably MHC class I, molecule.
15. A method of providing a T-cell recognizing a cell presenting a T-cell activating antigen, preferably a cancer cell, said method comprising
 - (i) identifying a TCR binding to a cell presenting a T-cell activating antigen according to the method according to any one of claims 9 to 12,
 - (ii) expressing a TCR comprising at least the complementarity determining regions (CDRs) of the TCR of step (I) in a T-cell, and, thereby,
 - (iii) providing a T-cell recognizing a cell presenting a T-cell activating antigen, preferably a cancer cell.

16. A reactive T-cell identified by the method according to any one of claims 1 to 8 and/or obtained or obtainable by the method according to any one of claims 9 to 14, preferably comprising a T-cell receptor comprising an amino acid sequence of SEQ ID NO:1 and/or SEQ ID NO:2, for use in therapy.
17. Use of a reactive T-cell identified by the method according to any one of claims 1 to 8 and/or obtained or obtainable by the method according to any one of claims 9 to 14, preferably comprising a T-cell receptor comprising an amino acid sequence of SEQ ID NO:1 and/or SEQ ID NO:2 in the manufacture of a medicament for treating and/or preventing cancer in a subject.
18. A method of identifying at least one biomarker of reactive T-cells, comprising
 - (I) providing expression data of a plurality of biomarkers of T-cells in a sample of a subject,
 - (II) providing a clustering said plurality of T-cells based on the expression of the biomarkers of step (I);
 - (III) providing amino acid sequences of at least the complementarity determining regions (CDRs) of TCRs of T-cells of step (II);
 - (IV) determining reactivity of T-cells expressing a TCR comprising the CDRs of step (III) to cells presenting a T-cell activating antigen;
 - (V) repeating steps (III) and (IV) at least once for further T-cells clustering with T-cells whose TCRs are determined to be reactive to cells presenting a T-cell activating antigen in step (IV), wherein the TCRs of said further T-cells are non-identical to the TCRs of step (IV);
 - (VI) determining at least one cluster of step (II) comprising the highest fraction of T-cells comprising T-cell receptors recognizing cells presenting a T-cell activating antigen; and
 - (VII) determining at least one biomarker expressed by the highest fraction of T-cells in the cluster determined in step (VI), thereby identifying at least one biomarkers of cancer-reactive T-cells.
19. The subject matter of any one of claims 1 to 18, wherein said T-cell(s) is/are CD8⁺ T-cell(s) or CD4⁺ T-cells, preferably are CD8⁺ T-cell(s).

20. The subject matter of any one of claims 9 to 19, wherein said TCR comprises, preferably consists of, a TCR alpha chain and a TCR beta chain.