

CLAIMS

1. An isolated cell derived from a T cell, the cell comprising one or more reporter systems, wherein the endogenous T cell receptor (TCR) alpha gene locus and/or beta gene locus of the cell is modified such that surface expression of a TCR-CD3 complex is disrupted.
2. The cell according to claim 1, wherein the isolated cell is derived from a human or murine T cell.
3. The cell according to any one of claims 1 or 2, wherein the isolated cell is derived from a Jurkat T cell.
4. The cell according to any one of claims 1 to 3, wherein the cell further comprises a co-receptor.
5. The cell according to claim 4, wherein the co-receptor is encoded by genes that are operably linked to their corresponding promoter or to a recombinant promoter.
6. The cell according to claim 5 wherein the recombinant promoter is a constitutive promoter.
7. The cell according to any one of claims 4 to 6, wherein the co-receptor is CD4 or CD8.
8. The cell according to any one of claims 1 to 7, wherein the one or more reporter system comprises a polynucleotide comprising a promoter region operably linked to a polynucleotide sequence encoding a detectable marker.
9. The cell according to claim 8, wherein the promoter region comprises one or more transcriptional response elements.

10. The cell according to claim 9, wherein at least one of the one or more transcriptional response elements is the nuclear factor of activated T cell (NFAT) response element.
11. The cell according to any one of claims 8 to 10, wherein the promoter region comprises 3 NFAT response elements.
12. The cell according to any one of claims 8 to 11, wherein the detectable marker is a fluorescent protein.
13. The cell according to claim 12, wherein the fluorescent protein is a green fluorescent protein (GFP).
14. The cell according to any one of claims 1 to 13, wherein expression and/or activity of at least one receptor involved in programmed cell death or T cell exhaustion is reduced or disrupted.
15. The cell according to claim 14, wherein the receptor involved in programmed cell death or T cell exhaustion is a Fas receptor, TNFR2 and/or PD-1, in particular, wherein the receptor is a Fas receptor.
16. The cell according to any one of claims 1 to 15, wherein expression and/or activity of at least one protein involved in antigen presentation is reduced or disrupted, in particular wherein the protein is beta-2 microglobulin.
17. The cell according to any one of claims 1 to 16, wherein expression and/or activity of at least one protein promoting non-homologous end joining is reduced or disrupted, in particular wherein the protein is p53 or p53-binding protein 1.
18. The cell according to any one of claims 1 to 17, wherein expression and/or activity of at least one protein promoting homology directed repair is increased, in particular, wherein the protein is BRCA1.
19. The cell according to any one of claims 1 to 18, wherein the cell comprises a gene

encoding a recombinant endonuclease.

20. The cell according to claim 19, wherein the recombinant endonuclease is *Streptococcus pyogenes* Cas9.

21. A method for producing a cell expressing a TCR-CD3 complex on the cell surface, the method comprising the steps of:

- a) providing a cell according to any one of claims 1 to 20;
- b) introducing a DNA fragment comprising coding sequences encoding a TCR alpha variable domain, a TCR beta variable domain and at least one of a TCR alpha constant domain and/or a TCR beta constant domain into the genome of the cell of step (a);
- c) expressing the coding sequences encoded on the DNA fragment introduced in step (b);
- and
- d) obtaining a cell expressing a TCR-CD3 complex on the cell surface.

22. The method according to claim 21, wherein the DNA fragment of step (b) further comprises homology arms at its 5' and 3' ends that are homologous to the endogenous TCR alpha locus or the endogenous TCR beta gene locus of the cell.

23. The method according to claim 22, wherein the DNA fragment further comprises a splice donor site that is located downstream of the most 3' coding sequence of the DNA fragment and upstream of the 3' homology arm.

24. The method according to any one of claims 21 to 23, wherein the DNA fragment is introduced into the genome of the cell such that the coding sequences located on the DNA fragment replace the endogenous TCR alpha VJ exon or the TCR beta VDJ exon and are transcriptionally linked to the endogenous gene encoding the TCR alpha constant domain or the TCR beta constant domain, respectively.

25. The method according to any one of claims 21 to 24, wherein the DNA fragment is introduced into the genome of the cell through homologous recombination, homology-directed repair, homology-independent targeted insertion or microhomology-mediated end joining.

26. The method according to claim 25, wherein the DNA fragment is introduced into the

genome of the cell through CRISPR-Cas9-mediated homology-directed repair.

27. A library of DNA fragments, wherein the library comprises DNA fragments comprising a coding sequence encoding a TCR alpha variable domain, a coding sequence encoding a TCR beta variable domain and at least one of a coding sequence encoding a TCR alpha constant domain and/or a coding sequence encoding a TCR beta constant domain.

28. The library of DNA fragments according to claim 27, wherein the coding sequences encoding the TCR alpha variable domain, the TCR beta variable domain and at least one of the TCR alpha constant domain and/or the TCR beta constant domain have been obtained by sequencing TCR genes in a population of T cells, in particular, wherein the population of T cells comprises tumor-infiltrating lymphocytes.

29. The library of DNA fragments according to claim 28, wherein the coding sequences encoding the TCR alpha and beta variable domains comprised in at least one DNA fragment of the library have been obtained from the same T cell.

30. The library of DNA fragments according to claim 27, wherein at least one DNA fragment in the library comprises one or more mutations in at least one coding sequence encoding a TCR alpha variable domain, a TCR beta variable domain and at least one of a TCR alpha constant domain and/or a TCR beta constant domain compared to a DNA fragment with a known sequence.

31. The library of DNA fragments according to claim 30, wherein the one or more mutations have been introduced into the coding sequences encoding the complementarity determining regions (CDRs) of the variable domains of the T cell receptor, in particular wherein the one or more mutations have been introduced into the coding sequences encoding the CDR3 of the variable alpha and/or variable beta domains of the T cell receptor.

32. The library of DNA fragments according to any one of claims 30 or 31, wherein the one or more mutations have been introduced into the DNA fragments by deep mutational scanning.

33. A method for determining the potential of a cell expressing a T cell receptor to bind to and/or to be activated by an antigen, the method comprising the steps of:

- a) producing a cell expressing a T cell receptor using the method according to any one of claims 21 to 26;
- b) contacting the cell of step (a) with an antigen; and
- c) determining the potential of the cell to bind to the antigen and/or to be activated by the antigen.

34. The method according to claim 33, wherein the cell is determined to bind to the antigen, if the binding between the cell and the antigen is stronger compared to the binding between the cell and an irrelevant antigen, or, if the binding between the cell and the antigen is stronger compared to the binding between the antigen and a cell that expresses an irrelevant or no T cell receptor;

and/or

wherein the cell is determined to be activated by the antigen, if the activation of the cell by the antigen is higher compared to the activation of a cell that has been contacted with an irrelevant or no antigen, or, if the activation of the cell by the antigen is higher compared to the activation of a cell that expresses an irrelevant or no T cell receptor and has been contacted with the antigen.

35. The method according to any one of claims 33 or 34, wherein the antigen is part of an antigen library.

36. A method for identifying at least one T cell receptor with optimized antigen-binding or antigen-induced signaling properties, the method comprising the steps of:

- a) producing a plurality of cells using the method according to any one of claims 21 to 26, wherein the plurality of cells is produced by introducing the library of DNA fragments according to any one of claims 30 to 32 into said cells;
- b) contacting the plurality of cells of step (a) with a target antigen or with one or more non-target antigens;
- c) isolating at least one cell from the plurality of cells, in which the binding between the cell and the target antigen and/or in which the activation of the cell by the target antigen is increased compared to a cell that expresses a T cell receptor that has been used as starting point for the optimized T cell receptor;

and/or,

in which the binding between the cell and the non-target antigen and/or in which the

activation of the cell by the non-target antigen is decreased compared to a cell that expresses a T cell receptor that has been used as a starting point for the optimized T cell receptor; and

d) identifying at least one T cell receptor with optimized antigen-binding or antigen-induced signaling properties by sequencing the DNA fragment comprised in the at least one cell isolated in step (c).

37. The method according to claim 36, wherein the at least one identified T cell receptor is included in a training set for a machine learning algorithm to predict T cell receptors with improved antigen-binding or antigen-induced signaling properties.

38. A method for identifying at least one antigen-specific T cell receptor, the method comprising the steps of:

a) producing a plurality of cells using the method according to any one of claims 21 to 26, wherein the plurality of cells is produced by introducing the library of DNA fragments according to any one of claims 28 to 29 into said cells;

b) contacting the plurality of cells of step (a) with an antigen;

c) isolating at least one cell from the plurality of cells, in which the binding between the cell and the antigen of step (b) is stronger compared to the binding between the cell and an irrelevant antigen, or, in which the binding between the cell and the antigen is stronger compared to the binding between the antigen and a cell that expresses an irrelevant or no T cell receptor;

and/or

in which the activation of the cell by the antigen of step (b) is higher compared to the activation of a cell that has been contacted with an irrelevant or no antigen, or, in which the activation of the cell by the antigen is higher compared to the activation of a cell that expresses an irrelevant or no T cell receptor and has been contacted with the antigen; and

d) identifying at least one antigen-specific T cell receptor by sequencing the DNA fragment comprised in the at least one cell isolated in step (c).

39. The method according to claim 38, wherein the antigen is presented on the surface of a tumor cell.

40. The method according to claim 39, wherein the tumor cell and the polynucleotide

sequences of the TCR coding sequences in the library of DNA fragments have been obtained from the same subject.

41. The method according to any one of claims 33 to 38, wherein the antigen or the target antigen is a peptide bound to a major histocompatibility complex (MHC), in particular wherein the peptide is presented by an MHC on the surface of an antigen-presenting cell (APC) or by an MHC multimer.

42. The method according to any one of claims 36 or 37, wherein the non-target antigen is a peptide that is presented by an APC, by an MHC multimer, or wherein the non-target antigen is a cell that does not present the target antigen on its cell surface.

43. The method according to any one of claims 41 or 42, wherein the peptide is pulsed onto the APC or wherein the peptide is genetically encoded by the APC.

44. The method according to any one of claims 33 to 43, wherein the binding between an antigen and a cell expressing a T cell receptor is determined by measuring the avidity of the T cell receptor for the antigen, in particular wherein the antigen is a peptide bound to a fluorescently-labeled MHC multimer.

45. The method according to claim 44, wherein the avidity is measured by flow cytometry.

46. The method according to any one of claims 33 to 43, wherein the activation of a cell expressing a T cell receptor is determined by measuring the expression of the reporter system or by measuring the expression of an activation marker after contact with an antigen, in particular wherein the antigen is a peptide presented on the surface of an APC.

47. The method according to claim 46, wherein the activation marker is CD69.

48. A T cell receptor as identified by the method according to any one of claims 36 to 47 or a functional portion thereof.

49. The T cell receptor or the functional portion thereof according to claim 48, wherein the functional portion thereof is comprised in a soluble T cell receptor.

50. The T cell receptor or the functional portion thereof according to claim 49, wherein the soluble T cell receptor is coupled to a biologically active molecule, in particular wherein the biologically active molecule is an agonistic antibody, a cytokine, or a cytotoxic agent.

51. A T cell receptor or a functional portion thereof, binding specifically to the MAGE-A3 antigen EVDPIGHLY (SEQ ID NO:11), wherein the T cell receptor, or the functional portion thereof, comprises a CDR3 β region comprising an amino acid sequence selected from the group consisting of sequences set forth in SEQ ID NO: 59, 54, 52, 57, 53, 50, 51, 55, 56, 58, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245 and 246.

52. The T cell receptor or the functional portion thereof according to claim 51, wherein the T cell receptor comprises a CDR1 α region comprising the sequence DSAIYN (SEQ ID NO:247), and/or a CDR1 β region comprising the sequence SGHRS (SEQ ID NO:248) , and/or a CDR2 α region comprising the sequence IQSSQRE (SEQ ID NO:249) , and/or a CDR2 β region comprising the sequence YFSETQ (SEQ ID NO:250) , and/or a CDR3 α region comprising the sequence AVRPGGAGSYQLT (SEQ ID NO:251).

53. The T cell receptor or the functional portion thereof according to claim 51 or 52, wherein the T cell receptor or the functional portion thereof comprises

a) a TCR α variable domain comprising an amino acid sequence as set forth in SEQ ID NO:9 or a TCR α variable domain comprising an amino acid sequence having at least 90% sequence identity to the amino acid sequence set forth in SEQ ID NO:9; and

b) a TCR β variable domain comprising an amino acid sequence selected from the group consisting of sequences set forth in SEQ ID NO: 261, 256, 254, 259, 255, 252, 253, 257, 258,

260, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 409, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448 and 449; or a TCR β variable domain comprising an amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of sequences set forth in SEQ ID NO: 261, 256, 254, 259, 255, 252, 253, 257, 258, 260, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 409, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448 and 449.

54. The T cell receptor or the functional portion thereof according to any one of claims 51 to 53, wherein the functional portion thereof is comprised in a soluble T cell receptor.

55. The T cell receptor or the functional portion thereof according to claim 54, wherein the soluble T cell receptor is coupled to a biologically active molecule, in particular wherein the biologically active molecule is an agonistic antibody, a cytokine, or a cytotoxic agent.

56. A polynucleotide encoding a T cell receptor or a functional portion thereof according to any one of claims 48 to 55.

57. A viral vector comprising the polynucleotide according to claim 56, in particular wherein the viral vector is derived from a lentivirus, a retrovirus or an adenovirus.
58. A cell comprising the polynucleotide according to claim 51 or the viral vector according to claim 57, in particular wherein the cell is a T cell.
59. A pharmaceutical composition comprising the T cell receptor or the functional fragment thereof according to any one of claims 48 to 55, a polynucleotide according to claim 56, a viral vector according to claim 57 or a cell according to claim 58 and a pharmaceutically acceptable carrier.
60. The pharmaceutical composition according to claim 59 comprising an additional active ingredient.
61. The T cell receptor or the functional portion thereof according to any one of claims 48 to 55, the polynucleotide according to claim 56, the viral vector according to claim 57, the cell according to claim 58 or the pharmaceutical composition according to claim 59 or 60 for use in therapy, in particular for use in cancer therapy.
62. The T cell receptor or the functional portion thereof, the polynucleotide, the viral vector the cell or the pharmaceutical composition for use according to claim 61, wherein the cancer is a MAGE-A3-positive cancer.
63. The T cell receptor or the functional portion thereof, the polynucleotide, the viral vector, the cell or the pharmaceutical composition for use according to claim 61 or 62, wherein the T cell receptor or the functional portion thereof, the polynucleotide, the viral vector, the cell or the pharmaceutical composition are administered to a subject.
64. The cell according to claim 58 or the pharmaceutical composition comprising a cell according to claim 58 for use in T cell therapy.
65. The cell or the pharmaceutical composition for use according to claim 64, wherein the T cell therapy is an autologous T cell therapy.

66. The cell or the pharmaceutical composition for use according to claim 64 or 65, wherein the T cell therapy is to treat cancer.

67. The cell or the pharmaceutical composition for use according to any one of claims 64 to 66, wherein the cancer is a MAGE-A3-positive cancer.