

CLAIMS

1. A chimeric antigen receptor comprising a polypeptide comprising:
 - a) an extracellular domain which comprises a claudin-3 binding domain comprising a heavy chain variable region (VH) comprising a heavy chain complementarity determining region 1 (CDRH1) sequence of SEQ ID NO: 1; a heavy chain complementarity determining region 2 (CDRH2) sequence of SEQ ID NO: 2; a heavy chain complementarity determining region 3 (CDRH3) sequence of SEQ ID NO: 3;
 - b) a transmembrane domain; and
 - c) one or more intracellular signalling domains.
2. The chimeric antigen receptor according to claim 1, wherein the extracellular domain further comprises a claudin-3 binding domain comprising a light chain variable region (VL) comprising a light chain complementarity determining region 1 (CDRL1) sequence of SEQ ID NO: 4; a light chain complementarity determining region 2 (CDRL2) sequence of SEQ ID NO: 5; a light chain complementarity determining region 3 (CDRL3) sequence of SEQ ID NO: 6.
3. The chimeric antigen receptor according to claim 2, wherein the VL is located at the N-terminus of the VH, or wherein the VH is located at the N-terminus of the VL.
4. The chimeric antigen receptor according to any one of claims 2 to 3, wherein the VL and the VH are directly fused to each other via a peptide bond or linked to each other via a peptide linker.
5. The chimeric antigen receptor according to ~~any one of claims 1 to 4~~, wherein the antigen binding domain is selected from the group consisting of: a Camel Ig, Ig NAR, Fab fragments, Fab' fragments, F(ab)'2 fragments, F(ab)'3 fragments, Fv, single-chain variable fragment (scFv), bis-scFv, (scFv)₂, minibody, diabody, triabody, tetrabody, disulfide stabilized Fv protein (dsFv), and single-domain antibody (sdAb).
6. The chimeric antigen receptor according to ~~any one of claim 5~~ 1 to 5, wherein the VH comprises an amino acid sequence having at least 90% or 95% sequence identity to SEQ ID NO: 7, or an amino acid sequence having one, two, three, four, or five amino acid substitutions, insertions, or deletions relative to SEQ ID NO: 7, and/or wherein the VL comprises an amino acid sequence having at least 90% or 95% sequence identity to SEQ ID NO: 8, or an amino acid sequence having one, two, three, four, or five amino acid substitutions, insertions, or deletions relative to SEQ ID NO: 8.

7. The chimeric antigen receptor according to ~~any one of~~ claims 1 ~~to~~ 6, wherein the VH comprises an amino acid sequence of SEQ ID NO: 7 and/or the VL comprises an amino acid sequence of SEQ ID NO: 8.

8. The chimeric antigen receptor according to ~~any one of~~ claims 1 ~~to~~ 6, wherein the extracellular domain comprises an amino acid sequence having at least 90% or 95% sequence identity to SEQ ID NO: 11, or an amino acid sequence having one, two, three, four, or five amino acid substitutions, insertions, or deletions relative to SEQ ID NO: 11, or wherein the extracellular domain comprises an amino acid sequence having at least 90% or 95% sequence identity to SEQ ID NO: 18, or an amino acid sequence having one, two, three, four, or five amino acid substitutions, insertions, or deletions relative to SEQ ID NO: 18.

9. The chimeric antigen receptor according to ~~any one of~~ claims 1 ~~to~~ 8, wherein the transmembrane domain is derived from a polypeptide selected from the group consisting of: alpha or beta chain of the T-cell receptor, CD3 δ , CD3 ϵ , CD3 γ , CD3 ζ , CD4, CD5, CD8 α , CD9, CD16, CD22, CD27, CD28, CD33, CD37, CD45, CD64, CD80, CD86, CD134, CD137 (4-1BB), CD152, CD154, CD278 (ICOS) and PD1.

10. The chimeric antigen receptor according to claim 9, wherein the transmembrane domain is derived from CD8 α .

11. The chimeric antigen receptor according to ~~any one of~~ claims 1 ~~to~~ 10, wherein the one or more intracellular signalling domains is derived from an intracellular signalling molecule selected from the group consisting of: FcR γ , FcR β , CD3 γ , CD3 ϵ , CD3 δ , CD3 ζ , CD22, CD66d, CD79a and CD79b or wherein the one or more intracellular signalling domains is CD3 ζ .

12. The chimeric antigen receptor according to ~~any one of~~ claims 1 ~~to~~ 11, further comprising a co-stimulatory signalling domain.

13. The chimeric antigen receptor according to claim 12, wherein the co-stimulatory domain is derived from a co-stimulatory molecule selected from the group consisting of: CARD11, CD2, CD7, CD27, CD28, CD30, CD40, CD54 (ICAM), CD83, CD134 (OX40), CD137 (4-1BB), CD278 (ICOS), DAP10, LAT, NKD2C, SLP76, TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, TRIM and ZAP70.

14. A chimeric antigen receptor comprising:
- a) an extracellular domain which comprises a claudin-3 binding protein comprising a CDRH1 sequence of SEQ ID NO: 1; a CDRH2 sequence of SEQ ID NO: 2; a CDRH3 sequence of SEQ ID NO: 3; a CDRL1 sequence of SEQ ID NO: 4; a CDRL2 sequence of SEQ ID NO: 5; and a CDRL3 sequence of SEQ ID NO: 6;
 - b) a transmembrane domain derived from CD8 α ;
 - c) a costimulatory domain derived from CD137 (4-1BB); and
 - d) an intracellular signalling domain derived from CD3 ζ .
15. The chimeric antigen receptor according to ~~any one of claims 1 to 14~~, wherein the chimeric antigen receptor comprises an amino acid sequence having at least 90% or 95% sequence identity to SEQ ID NOs: 12, 34, 35, 36, 37, 38, or 39, or an amino acid sequence having one, two, three, four, or five amino acid substitutions, insertions, or deletions relative to SEQ ID NOs: 12, 34, 35, 36, 37, 38, or 39, or wherein the chimeric antigen receptor comprises an amino acid sequence of SEQ ID NOs: 12, 34, 35, 36, 37, 38, or 39.
16. A chimeric antigen receptor that competes for binding with the chimeric antigen receptor according to ~~any one of claims 1 to 15~~.
17. An engineered immune effector cell, comprising the chimeric antigen receptor according to ~~any one of claims 1 to 16~~.
18. The immune effector cell according to claim 17, wherein the immune effector cell is selected from the group consisting of: a T cell, a cytotoxic T lymphocyte, a natural killer T lymphocyte cell, a macrophage, and a natural killer cell.
19. The immune effector cell according to ~~any one of claims 17 to 18~~, further comprising an ablation element.
20. The immune effector cell according to ~~any one of claims 17 to 19~~, wherein the ablation element is derived from a polypeptide selected from the group consisting of: truncated human EGFR polypeptide and CD20, or wherein the ablation element is CD20.
21. A polynucleotide encoding the chimeric antigen receptor according to ~~any one of claims 1 to 16~~.
22. A vector comprising the polynucleotide of claim 21.

23. The vector according to claim 22, wherein the vector is selected from the group consisting of: retroviral vector, lentiviral vector, adeno-associated virus (AAV) vector, human immunodeficiency virus I (HIV-I), human immunodeficiency virus 2 (HIV-2), visna-maedi virus (VMV) virus, caprine arthritis-encephalitis virus (CAEV), equine infectious anemia virus (EIAV), feline immunodeficiency virus (FIV), bovine immune deficiency virus (BIV), and simian immunodeficiency virus.

24. A pharmaceutical composition comprising the engineered immune effector cell according to ~~any one of claims 17 to 20, the polynucleotide according to claim 21, or the vector according to any one of claims 22 to 23,~~ and a pharmaceutically acceptable excipient.

25. Use of the pharmaceutical composition according to claim 24 in the manufacture of a medicament for the treatment of cancer.~~A method of treating cancer in a patient in need thereof, said method comprising administering to the patient a therapeutically effective amount of the pharmaceutical composition according to claim 24.~~

26. The ~~method~~use according to claim 25, wherein the cancer is solid cancer, epithelial cancer, colorectal cancer, pancreatic cancer, breast cancer, triple-negative breast cancer (TNBC), ovarian cancer, lung cancer, non-small cell lung cancer (NSCLC), or prostate cancer.

27. The ~~method~~use according to claim 26, wherein the cancer is colorectal cancer.

28. ~~Use of the chimeric antigen receptor according to any one of claims 1 to 18, the engineered effector cell according to any one of claims 19 to 20, the polynucleotide according to claim 21, the vector according to any one of claims 22 to 23, or the pharmaceutical composition according to claim 24 in the manufacture of a medicament for treatment of cancer.~~

29. ~~The chimeric antigen receptor according to any one of claims 1 to 18, the engineered effector cell according to any one of claims 19 to 20, the polynucleotide according to claim 21, the vector according to any one of claims 22 to 23, or the pharmaceutical composition according to claim 24 for use in therapy.~~

30. ~~The chimeric antigen receptor according to any one of claims 1 to 18, the engineered effector cell according to any one of claims 19 to 20, the polynucleotide according to claim 21, the vector according to any one of claims 22 to 23, or the pharmaceutical composition according to claim 24 for use in the treatment of cancer.~~

~~31~~28. A claudin-3 binding protein, comprising a heavy chain variable region (VH) comprising a heavy chain complementarity determining region 1 (CDRH1) sequence of SEQ ID NO: 1; a heavy chain complementarity determining region 2 (CDRH2) sequence of SEQ ID NO: 2; a heavy chain complementarity determining region 3 (CDRH3) sequence of SEQ ID NO: 3;

~~32.~~ The claudin-3 binding protein according to claim ~~31~~, further comprising and a light chain variable region (VL) comprising a light chain complementarity determining region 1 (CDRL1) sequence of SEQ ID NO: 4; a light chain complementarity determining region 2 (CDRL2) sequence of SEQ ID NO: 5; a light chain complementarity determining region 3 (CDRL3) sequence of SEQ ID NO: 6.

~~33.~~ The claudin-3 binding protein according to claim ~~31~~ or claim ~~32~~, wherein the claudin-3 binding protein is selected from the group consisting of: a monoclonal antibody, a human IgG1 isotype, a Camel Ig, Ig NAR, Fab fragments, Fab' fragments, F(ab)'2 fragments, F(ab)'3 fragments, Fv, single chain variable fragment (scFv), bis-scFv, (scFv)2, minibody, diabody, triabody, tetrabody, disulfide stabilized Fv protein (dsFv), and single domain antibody (sdAb).

~~34.~~ The claudin-3 binding protein according to claims ~~31~~ to ~~33~~, wherein the VL is located at the N terminus of the VH, or wherein the VH is located at the N terminus of the VL, and/or wherein the VL and the VH are directly fused to each other via a peptide bond or linked to each other via a peptide linker.

~~35.~~ The claudin-3 binding protein according to claims ~~31~~ to ~~34~~, wherein the VH comprises an amino acid sequence having at least 90% or 95% sequence identity to SEQ ID NO: 7, or an amino acid sequence having one, two, three, four, or five amino acid substitutions, insertions, or deletions relative to SEQ ID NO: 7, and/or wherein the VL comprises an amino acid sequence having at least 90% or 95% sequence identity to SEQ ID NO: 8, or an amino acid sequence having one, two, three, four, or five amino acid substitutions, insertions, or deletions relative to SEQ ID NO: 8; or wherein the VH comprises an amino acid sequence of SEQ ID NO: 7 and/or the VL comprises an amino acid sequence of SEQ ID NO: 8.

~~36.~~ The claudin-3 binding protein according to claims ~~31~~ to ~~35~~, wherein the claudin-3 binding protein comprises an amino acid sequence having at least 90% or 95% sequence identity to SEQ ID NO: 11, or an amino acid sequence having one, two, three, four, or five amino acid substitutions, insertions, or deletions relative to SEQ ID NO: 11, or wherein the extracellular domain comprises an amino acid sequence having at least 90% or 95% sequence identity to SEQ

~~ID NO: 18, or an amino acid sequence having one, two, three, four, or five amino acid substitutions, insertions, or deletions relative to SEQ ID NO: 18.~~

~~3729.~~ A polynucleotide encoding the claudin-3 binding protein according to ~~any one of claims 31 to 36.~~claim 28.

~~3830.~~ A vector comprising the polynucleotide of claim ~~3729.~~

~~39.~~ The vector according to claim ~~38~~, wherein the vector is selected from the group consisting of: ~~retroviral vector, lentiviral vector, adeno-associated virus (AAV) vector, human immunodeficiency virus I (HIV-1), human immunodeficiency virus 2 (HIV-2), visna-maedi virus (VMV) virus, caprine arthritis-encephalitis virus (CAEV), equine infectious anemia virus (EIAV), feline immunodeficiency virus (FIV), bovine immune deficiency virus (BIV), and simian immunodeficiency virus.~~

~~4031.~~ A pharmaceutical composition comprising the claudin-3 binding protein according to ~~any one of claims 31 to 36.~~28, the polynucleotide according to claim ~~37~~, or the vector according to ~~any one of claims 38 to 39~~, and a pharmaceutically acceptable excipient.

~~4132.~~ Use of the pharmaceutical composition according to claim 31 in the manufacture of a medicament for the treatment of cancer. ~~A method of treating cancer in a patient in need thereof, said method comprising administering to the patient a therapeutically effective amount of the pharmaceutical composition according to claim 40.~~

~~42.~~ The method according to claim ~~41~~, wherein the cancer is a solid cancer.

~~43.~~ The method according to any one of claims ~~41 to 42~~, wherein the cancer is colorectal cancer, epithelial cancer, pancreatic cancer, breast cancer, triple negative breast cancer (TNBC), ovarian cancer, lung cancer, non-small cell lung cancer (NSCLC), or prostate cancer.

~~44.~~ The method according to any one of claims ~~41 to 43~~, wherein the cancer is colorectal cancer.

~~45.~~ Use of the claudin-3 binding protein according to any one of claims 31 to 36, the polynucleotide according to claim 37, or the vector according to any one of claims 38 to 39, or the pharmaceutical composition according to claim 40 in the manufacture of a medicament for treatment of cancer.

46.—The claudin-3 binding protein according to any one of claims 31 to 36, the polynucleotide according to claim 37, or the vector according to any one of claims 38 to 39, or the pharmaceutical composition according to claim 40 for use in therapy.

47.—The claudin-3 binding protein according to any one of claims 31 to 36, the polynucleotide according to claim 37, or the vector according to any one of claims 38 to 39, or the pharmaceutical composition according to claim 40 for use in the treatment of cancer.

4833. An isolated claudin-3 binding protein that binds to a discontinuous epitope on human claudin-3 comprising at least N38 and E153 of SEQ ID NO:13.

49.—The isolated claudin-3 binding protein according to claim 48, wherein the claudin-3 binding protein is chimeric or humanized.

50.—The isolated claudin-3 binding protein according to claim 48 or claim 49, wherein the claudin-3 binding protein is selected from the group consisting of: a monoclonal antibody, a human IgG1 isotype, a Camel Ig, Ig-NAR, Fab fragments, Fab' fragments, F(ab)'2 fragments, F(ab)'3 fragments, Fv, single chain variable fragment (scFv), bis-scFv, (scFv)2, minibody, diabody, triabody, tetrabody, disulfide stabilized Fv protein (dsFv), and single domain antibody (sdAb).

51.—The isolated claudin-3 binding protein according to claims 48 to 50, wherein the isolated claudin-3 binding protein comprises a heavy chain variable region (VH) comprising a heavy chain complementarity determining region 1 (CDRH1) sequence of SEQ ID NO: 1; a heavy chain complementarity determining region 2 (CDRH2) sequence of SEQ ID NO: 2; a heavy chain complementarity determining region 3 (CDRH3) sequence of SEQ ID NO: 3, and/or wherein the isolated claudin-3 binding protein comprises a light chain variable region (VL) comprising a light chain complementarity determining region 1 (CDRL1) sequence of SEQ ID NO: 4; a light chain complementarity determining region 2 (CDRL2) sequence of SEQ ID NO: 5; a light chain complementarity determining region 3 (CDRL3) sequence of SEQ ID NO: 6.

52.—The isolated claudin-3 binding protein according to any one of claims 48 to 51, wherein the VH comprises an amino acid sequence having at least 90% or 95% sequence identity to SEQ ID NO: 7, or an amino acid sequence having one, two, three, four, or five amino acid substitutions, insertions, or deletions relative to SEQ ID NO: 7, and/or wherein the VL comprises an amino acid sequence having at least 90% or 95% sequence identity to SEQ ID NO: 8, or an amino acid

sequence having one, two, three, four, or five amino acid substitutions, insertions, or deletions relative to SEQ ID NO: 8.

5334. A chimeric antigen receptor comprising a polypeptide comprising:

- a) an extracellular domain which comprises the isolated claudin-3 binding protein according to claim 5-48-5233;
- b) a transmembrane domain; and
- c) one or more intracellular signalling domains.

54. — The chimeric antigen receptor according to claim 53, wherein the transmembrane domain is derived from a polypeptide selected from the group consisting of: alpha or beta chain of the T-cell receptor, CD3 δ , CD3 ϵ , CD3 γ , CD3 ζ , CD4, CD5, CD8 α , CD9, CD16, CD22, CD27, CD28, CD33, CD37, CD45, CD64, CD80, CD86, CD134, CD137 (4-1BB), CD152, CD154, CD278 (ICOS) and PD1.

55. — The chimeric antigen receptor according to any one of claims 53 to 54, wherein the transmembrane domain is derived from CD8 α .

56. — The chimeric antigen receptor according to any one of claims 53 to 55, wherein the one or more intracellular signalling domains is derived from an intracellular signalling molecule selected from the group consisting of: FcR γ , FcR β , CD3 γ , CD3 ϵ , CD3 δ , CD3 ζ , CD22, CD66d, CD79a and CD79b.

57. — The chimeric antigen receptor according to claim 56, wherein the one or more intracellular signalling domains is CD3 ζ .

58. — The chimeric antigen receptor according to any one of claims 53 to 57, further comprising a co-stimulatory signalling domain.

59. — The chimeric antigen receptor according to claim 58, wherein the co-stimulatory domain is derived from a co-stimulatory molecule selected from the group consisting of: CARD11, CD2, CD7, CD27, CD28, CD30, CD40, CD54 (ICAM), CD83, CD134 (OX40), CD137 (4-1BB), CD278 (ICOS), DAP10, LAT, NKD2C, SLP76, TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, TRIM and ZAP70.

60. — The chimeric antigen receptor according to any one of claims 58 to 59, wherein the co-stimulatory domain is CD137 (4-1BB).

~~61~~35. An engineered immune effector cell, comprising the chimeric antigen receptor according to any one of claim ~~5~~53 to ~~60~~34.

~~62~~.—The immune effector cell according to claim ~~61~~, wherein the immune effector cell is selected from the group consisting of: a T cell, a cytotoxic T lymphocyte, a natural killer T lymphocyte cell, a macrophage, and a natural killer cell.

~~63~~.—The immune effector cell according to any one of claims ~~61~~ to ~~62~~, further comprising an ablation element.

~~64~~.—The immune effector cell according to any one of claims ~~61~~ to ~~63~~, wherein the ablation element is derived from a polypeptide selected from the group consisting of: truncated human EGFR polypeptide and CD20, or wherein the ablation element is CD20.

~~65~~36. A pharmaceutical composition comprising the isolated claudin-3 binding protein according to any one of claims ~~48~~ to ~~52~~ or the engineered immune effector cell according to any one of claims ~~61~~ to ~~64~~33, and a pharmaceutically acceptable excipient.

~~66~~37. Use of the pharmaceutical composition according to claim 36 in the manufacture of a medicament for the treatment of cancer. A method of treating cancer in a patient in need thereof, said method comprising administering to the patient a therapeutically effective amount of the pharmaceutical composition according to claim 65.

~~67~~.—The method according to claim ~~66~~, wherein the cancer is solid cancer, epithelial cancer, colorectal cancer, pancreatic cancer, breast cancer, triple negative breast cancer (TNBC), ovarian cancer, lung cancer, non-small cell lung cancer (NSCLC), or prostate cancer.

~~68~~.—The method according to claim ~~67~~, wherein the cancer is colorectal cancer.

~~69~~.—Use of the isolated claudin-3 binding protein according to any one of claims ~~48~~ to ~~52~~, the chimeric antigen receptor according to any one of claims ~~53~~ to ~~61~~, the engineered effector cell according to any one of claims ~~63~~ to ~~64~~, or the pharmaceutical composition according to claim ~~65~~ in the manufacture of a medicament for treatment of cancer.

~~70~~.—The isolated claudin-3 binding protein according to any one of claims ~~48~~ to ~~52~~, the chimeric antigen receptor according to any one of claims ~~53~~ to ~~61~~, the engineered effector cell according

to any one of claims 63 to 64, or the pharmaceutical composition according to claim 65 for use in therapy.

71. — The isolated claudin-3 binding protein according to any one of claims 48 to 52, the chimeric antigen receptor according to any one of claims 53 to 61, the engineered effector cell according to any one of claims 63 to 64, or the pharmaceutical composition according to claim 65 for use in the treatment of cancer.