

CLAIMS

1. An antigen binding protein comprising a first polypeptide chain and a second polypeptide chain that together form two antigen binding sites,

5 wherein the first polypeptide chain comprises a structure represented by the formula:

$$V_3-L_1-V_4[I]$$

wherein V_3 is an antibody variable light chain domain; V_4 is a T cell receptor (TCR) beta variable domain comprising a complementary determining region (CDR)1 comprising the amino acid sequence of SEQ ID NO: 62, a CDR2 comprising the amino acid sequence of SEQ ID No: 65, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 71; and; L_1 is a linker; and wherein the second polypeptide chain comprises a structure represented by the formula:

$$V_5-L_3-V_6 [II]$$

wherein V_5 is a TCR alpha variable domain comprising a CDR1 comprising the amino acid sequence of SEQ ID NO: 5, a CDR2 comprising the amino acid sequence of SEQ ID NO: 56, and a CDR3 comprising the amino acid sequence of SEQ ID NO: 35; V_6 is an antibody variable heavy chain domain; and L_3 is a linker; and wherein the antibody variable light chain domain (V_3) and the antibody variable heavy chain domain (V_6) form together one antigen binding site that binds specifically to the human TCR-CD3 complex, and wherein the TCR beta variable domain (V_4) and the TCR alpha variable domain (V_5) form together one antigen binding site that binds specifically to a MAGE-A antigenic peptide consisting of the amino acid sequence KVLEHVVRV according to SEQ ID NO: 1 in a complex with a human major histocompatibility complex (MHC) protein.

- 25 2. The antigen binding protein according to claim 1, wherein the first polypeptide chain comprises a structure represented by the formula :

$$V_3-L_1-V_4-L_5-F_{C1} \quad [III]$$

and the second polypeptide chain comprises a structure represented by the formula :

$$V_5-L_3-V_6-L_6-F_{C2} \quad [IV]$$

30 wherein V_3 , L_1 , V_4 , V_5 , L_3 and V_6 , , are as defined in claim 1 and wherein L_5 and L_6 are linkers and present or absent and F_{C1} , and F_{C2} are Fc-domains and wherein F_{C1} and F_{C2} are the same or different.

3. The antigen binding protein according to claim 1 or 2, wherein V_3 and V_6 are humanized.

- 35 4. The antigen binding protein according to any one of claims 1-3, wherein the human MHC protein is HLA-A*02.

5. The antigen binding protein according to any one of claims 1-4, wherein
V₃ comprises complementarity determining regions (CDRs) CDR1, CDR2 and CDR3 of
SEQ ID NO: 159; and
5 V₆ comprises CDR1, CDR2 and CDR3 of SEQ ID NO: 160.

6. The antigen binding protein according to any one of claims 1-5, wherein
V₃ comprises the amino acid sequence of SEQ ID NO: 159 or an amino acid sequence
at least 95% identical thereto; and
10 V₆ comprises the amino acid sequence of SEQ ID NO: 160 or an amino acid sequence
at least 95% identical thereto.

7. The antigen binding protein according to claim 6, wherein
V₃ comprises the amino acid sequence of SEQ ID NO: 159; and
15 V₆ comprises the amino acid sequence of SEQ ID NO: 160.

8. The antigen binding protein according to any one of claims 1-7, wherein
V₅ comprises one or more framework regions selected from the group consisting of FR1-
a, FR2-a, FR3-a and FR4-a, wherein
20 - FR1-a comprises the amino acid sequence of SEQ ID NO: 83 or an amino acid
sequence at least 90% identical thereto,
- FR2-a comprises the amino acid sequence of SEQ ID NO: 84 or an amino acid
sequence at least 90% identical thereto,
- FR3-a comprises the amino acid sequence of SEQ ID NO: 85 or an amino acid
25 sequence at least 90% identical thereto, and/or
- FR4-a comprises the amino acid sequence of SEQ ID NO: 86 or an amino acid
sequence at least 90% identical thereto, and
V₄ comprises one or more framework regions selected from the group consisting of FR1-
b, FR2-b, FR3-b and FR4-b, and wherein
30 - FR1-b comprises the amino acid sequence of SEQ ID NO: 87 or an amino acid
sequence at least 90% identical thereto,
- FR2-b comprises the amino acid sequence of SEQ ID NO: 88 or an amino acid
sequence at least 90% identical thereto,
- FR3-b comprises the amino acid sequence of SEQ ID NO: 89 or an amino acid
35 sequence at least 90% identical thereto, and/or
- FR4-b comprises the amino acid sequence of SEQ ID NO: 90 or an amino acid
sequence at least 90% identical thereto.

9. The antigen binding protein according to any one of claims 1-8, wherein the framework region(s) comprise(s) at least one amino acid substitution selected from the group of amino acid substitutions comprising:
- 5 - an amino acid substitution at position 19 in FR1-a, wherein the amino acid substitution is optionally S19A, S19V,
 - an amino acid substitution at position 48 and/or 50 in FR2-a, wherein the amino acid substitution at position 48 is optionally A48K and the amino acid substitution at position 50 is optionally L50P,
 - 10 - an amino acid substitution at position 46, 47 and/or 54 in FR2-b, wherein the amino acid substitution at position 46, 47 and 54 is optionally M46P, M47G and I54F, respectively,
 - an amino acid substitution at position 66 in FR3-b, wherein the amino acid substitution at position 66 is optionally I66C, and
 - 15 wherein the positions of the substitutions are given according to the IMGT nomenclature.
10. The antigen binding protein according to any one of claims 1-9, wherein V₅ comprises one or more framework regions selected from the group consisting of FR1-a, FR2-a, FR3-a and FR4-a, wherein
- 20 - FR1-a comprises the amino acid sequence of SEQ ID NO: 91 or an amino acid sequence at least 95% identical to SEQ ID NO: 91, wherein optionally the amino acid sequence at least 95% identical to SEQ ID NO: 91 comprises the amino acid 19V,
 - FR2-a comprises the amino acid sequence of SEQ ID NO: 92 or an amino acid sequence at least 95% identical to SEQ ID NO: 92, wherein optionally the amino acid sequence at least 95% identical to SEQ ID NO: 92 comprises the amino acid 48K and optionally an amino acid substitution at position 50, optionally L50P,
 - 25 - FR3-a comprises the amino acid sequence of SEQ ID NO: 85 or an amino acid sequence at least 95% identical to SEQ ID NO: 85, and/or
 - 30 - FR4-a comprises the amino acid sequence of SEQ ID NO: 86 or an amino acid sequence at least 95% identical to SEQ ID NO: 86, and
- V₄ comprises one or more framework regions selected from the group consisting of FR1-b, FR2-b, FR3-b and FR4-b, wherein
- 35 - FR1-b comprises the amino acid sequence of SEQ ID NO: 87 or an amino acid sequence at least 95% identical to SEQ ID NO: 87,

- FR2-b comprises the amino acid sequence of SEQ ID NO: 93 or an amino acid sequence at least 95% identical to SEQ ID NO: 93, wherein optionally the amino acid sequence at least 95% identical to SEQ ID NO: 93 comprises optionally the amino acid 54F, and optionally an amino acid substitution at position 46 and/or 47, wherein the amino acid substitution at position 46 and/or 47 is optionally M46P and/or M47G, respectively,
 - FR3-b comprises the amino acid sequence of SEQ ID NO: 89 or an amino acid sequence at least 95% identical to SEQ ID NO: 89, or of the amino acid sequence of SEQ ID NO: 94 or an amino acid sequence at least 95% identical to SEQ ID NO: 94, wherein optionally the amino acid sequence at least 95% identical to SEQ ID NO: 94 comprises the amino acid 66C, and/or
 - FR4-b comprises the amino acid sequence of SEQ ID NO: 90 or an amino acid sequence at least 95% identical to SEQ ID NO: 90.
11. The antigen binding protein according to any one of claims 1-10, wherein V₅ comprises one or more framework regions selected from the group consisting of FR1-a, FR2-a, FR3-a and FR4-a, wherein
- FR1-a comprises the amino acid sequence of SEQ ID NO: 91,
 - FR2-a comprises the amino acid sequence of SEQ ID NO: 92,
 - FR3-a comprises the amino acid sequence of SEQ ID NO: 85, and/or
 - FR4-a comprises the amino acid sequence of SEQ ID NO: 86; and
- V₄ comprises one or more framework regions selected from the group consisting of FR1-b, FR2-b, FR3-b and FR4-b, and wherein
- FR1-b comprises the amino acid sequence of SEQ ID NO: 87,
 - FR2-b comprises the amino acid sequence of SEQ ID NO: 93,
 - FR3-b comprises the amino acid sequence of SEQ ID NO: 94, and/or
 - FR4-b comprises the amino acid sequence of SEQ ID NO: 90.
12. The antigen binding protein according to any one of claims 1-10, wherein V₅ comprises framework regions FR1-a, FR2-a, FR3-a and FR4-a, wherein
- FR1-a comprises the amino acid sequence of SEQ ID NO: 91,
 - FR2-a comprises the amino acid sequence of SEQ ID NO: 92,
 - FR3-a comprises the amino acid sequence of SEQ ID NO: 85, and
 - FR4-a comprises the amino acid sequence of SEQ ID NO: 86; and
- V₄ comprises framework regions FR1-b, FR2-b, FR3-b and FR4-b, and wherein
- FR1-b comprises the amino acid sequence of SEQ ID NO: 87,
 - FR2-b comprises the amino acid sequence of SEQ ID NO: 93,

FR3-b comprises the amino acid sequence of SEQ ID NO: 94, and
FR4-b comprises the amino acid sequence of SEQ ID NO: 90.

13. The antigen binding protein according to any one of claims 1-12, wherein
 5 V₃ comprises the amino acid sequence of SEQ ID NO: 159 or an amino acid sequence
 at least 95% identical thereto;
 V₄ comprises the amino acid sequence of SEQ ID NO: 150 or an amino acid sequence
 at least 95% identical thereto;
 V₅ comprises the amino acid sequence of SEQ ID NO: 151 or an amino acid sequence
 10 at least 95% identical thereto; and
 V₆ comprises the amino acid sequence of SEQ ID NO: 160 or an amino acid sequence
 at least 95% identical thereto.

14. The antigen binding protein according to claim 13, wherein
 15 V₃ comprises the amino acid sequence of SEQ ID NO: 159;
 V₄ comprises the amino acid sequence of SEQ ID NO: 150;
 V₅ comprises the amino acid sequence of SEQ ID NO: 151; and
 V₆ comprises the amino acid sequence of SEQ ID NO: 160.

- 20 15. The antigen binding protein according to any one of claims 1-14, wherein L₁ and L₃ each
 separately comprises the amino acid sequence of GGGSGGGG (SEQ ID NO: 96).

16. The antigen binding protein according to any one of claims 1-15, wherein F_{C1} comprises
 the amino acid sequence of SEQ ID NO: 112 and F_{C2} comprises the amino acid
 25 sequence of SEQ ID NO: 113.

17. The antigen binding protein according to any one of claims 1-16, wherein
 the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 136 or an
 amino acid sequence at least 95% identical thereto, and
 30 the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 137
 or an amino acid sequence at least 95% identical thereto.

18. The antigen binding protein according to any one of claims 1-17, wherein
 the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 136, and
 35 the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 137.

19. The antigen binding protein of claim 18, wherein

the first polypeptide chain consists of the amino acid sequence of SEQ ID NO: 136, and the second polypeptide chain consists of the amino acid sequence of SEQ ID NO: 137.

- 5 20. The antigen binding protein according to any one of claims 1-19, wherein the first polypeptide and the second polypeptide are covalently bonded by a disulfide bond.
- 10 21. An antigen binding protein comprising a first polypeptide chain and a second polypeptide chain that together form two antigen binding sites, wherein the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 136, and the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 137, wherein one antigen binding site binds specifically to the human TCR-CD3 complex and another antigen binding site binds specifically to an antigenic peptide consisting of the amino acid sequence KVLEHVVRV (SEQ ID NO: 1) in a complex with human major histocompatibility complex (MHC) protein HLA-A*02.
- 15 22. An antigen binding protein comprising a first polypeptide chain and a second polypeptide chain that together form two antigen binding sites, wherein the first polypeptide chain consists of the amino acid sequence of SEQ ID NO: 136, and the second polypeptide chain consists of the amino acid sequence of SEQ ID NO: 137, wherein one antigen binding site binds specifically to the human TCR-CD3 complex and another antigen binding site binds specifically to an antigenic peptide consisting of the amino acid sequence KVLEHVVRV (SEQ ID NO: 1) in a complex with human major histocompatibility complex (MHC) protein HLA-A*02.
- 20 23. An antigen binding protein consisting of a first polypeptide chain and a second polypeptide chain that together form two antigen binding sites, wherein the first polypeptide chain comprises the amino acid sequence of SEQ ID NO: 136, and the second polypeptide chain comprises the amino acid sequence of SEQ ID NO: 137, wherein one antigen binding site binds specifically to the human TCR-CD3 complex and the other antigen binding site binds specifically to an antigenic peptide consisting of the amino acid sequence KVLEHVVRV (SEQ ID NO: 1) in a complex with human major histocompatibility complex (MHC) protein HLA-A*02.
- 25 30 35 24. An antigen binding protein consisting of a first polypeptide chain and a second polypeptide chain that together form two antigen binding sites, wherein the first polypeptide chain consists of the amino acid sequence of SEQ ID NO: 136, and

the second polypeptide chain consists of the amino acid sequence of SEQ ID NO: 137, wherein one antigen binding site binds specifically to the human TCR-CD3 complex and the other antigen binding site binds specifically to an antigenic peptide consisting of the amino acid sequence KVLEHVVRV (SEQ ID NO: 1) in a complex with human major histocompatibility complex (MHC) protein HLA-A*02.

25. An isolated nucleic acid or isolated nucleic acids comprising a sequence encoding an antigen binding protein according to any one of claims 1 to 24, or a nucleic acid vector comprising said isolated nucleic acid or isolated nucleic acids.

26. A recombinant host cell comprising an antigen binding protein according to any one of claims 1 to 24, or an isolated nucleic acid, isolated nucleic acids or a vector according to claim 25, wherein said host cell is

- a) a lymphocyte, preferably a T lymphocyte or T lymphocyte progenitor cell: or
- b) a cell for recombinant expression, preferably a Chinese Hamster Ovary (CHO) cell.

27. The recombinant host cell according to claim 26, wherein the T lymphocyte is a CD4 or CD8 positive T cell.

28. A composition comprising the antigen binding protein of according to any one of claims 1 to 24, or the isolated nucleic acid or the isolated nucleic acids of claim 25.

29. A pharmaceutical composition comprising the antigen binding protein according to any one of claims 1 to 24, the isolated nucleic acid, isolated nucleic acids or vector according to claim 25, or the host cell according to claim 26 or 27, and a pharmaceutically acceptable carrier, diluent stabilizer, and/or excipient.

30. A method of producing the antigen binding protein according to any one of claims 1 to 24, comprising

- a. providing a suitable host cell,
- b. providing a genetic construct comprising a coding sequence encoding the antigen binding protein according to any of claims 1 to 24,
- c. introducing said genetic construct into said suitable host cell, and
- d. expressing said genetic construct by said suitable host cell.

31. The method according to claim 30, further comprising the isolation and purification of the antigen binding protein from the suitable host cell and, optionally, reconstitution of the antigen binding protein in a T cell.
- 5 32. The antigen binding protein according to any one of claims 1 to 24, the nucleic acid or vector according to claim 25, the host cell according to claim 26 or 27 or the pharmaceutical composition according to claim 29 for use in medicine.
- 10 33. The antigen binding protein according to any one of claims 1 to 24, the isolated nucleic acid, isolated nucleic acids or vector according to claim 25, the host cell according to claim 26 or 27, or the pharmaceutical composition according to claim 29 for use in the diagnosis of a proliferative disease, preferably cancer, more preferably a MAGEA4 and/or MAGEA8 positive cancer.
- 15 34. The antigen binding protein, the isolated nucleic acid, isolated nucleic acids or vector, the host cell, or the pharmaceutical composition for use according to claim 33, wherein said cancer is selected from the group consisting of lung cancer, liver cancer, head and neck cancer, skin cancer, renal cell cancer, brain cancer, gastric cancer, colorectal cancer, pancreatic cancer, prostate cancer, leukemia, breast cancer, Merkel cell carcinoma, ovarian cancer, urinary bladder cancer, uterine cancer, gallbladder cancer, bile duct cancer, osteosarcoma cancer, and esophageal cancer.
- 20 35. The antigen binding protein, the isolated nucleic acid, isolated nucleic acids or vector, the host cell; or the pharmaceutical composition for use according to claim 34, wherein
 - 25 (i) lung cancer is non-small cell lung cancer (NSCLC), preferably non-small cell lung cancer adenocarcinoma or squamous cell non-small cell lung cancer (SNSCLC); or small cell lung cancer (SCLC);
 - (ii) skin cancer is melanoma;
 - (iii) head and neck cancer is head and neck squamous cell carcinoma (HNSCC);
 - 30 (iv) liver cancer is hepatocellular cancer (HCC);
 - (v) esophageal cancer is gastroesophageal junction cancer.
- 35 36. Use of the antigen binding protein according to any one of claims 1 to 24, the isolated nucleic acid, isolated nucleic acids or vector according to claim 25, the host cell according to claim 26 or 27, or the pharmaceutical composition according to claim 29 in the manufacture of a medicament for the treatment of a proliferative disease, wherein the medicament is for the diagnosis of the proliferative disease, and wherein the

proliferative disease is preferably cancer, more preferably a MAGEA4 and/or MAGEA8 positive cancer.

37. Use of the antigen binding protein according to any one of claims 1 to 24, the isolated
5 nucleic acid, isolated nucleic acids or vector according to claim 25, the host cell according to claim 26 or 27, or the pharmaceutical composition according to claim 29 in the manufacture of a medicament for the prevention, and/or treatment of a proliferative disease, preferably cancer, more preferably a MAGEA4 and/or MAGEA8 positive cancer.
- 10 38. Use of the antigen binding protein, the isolated nucleic acid, isolated nucleic acids or vector, the host cell, or the pharmaceutical composition according to claim 36 or 37, wherein said cancer is selected from the group consisting of lung cancer, liver cancer, head and neck cancer, skin cancer, renal cell cancer, brain cancer, gastric cancer, colorectal cancer, pancreatic cancer, prostate cancer, leukemia, breast cancer, Merkel
15 cell carcinoma, ovarian cancer, urinary bladder cancer, uterine cancer, gallbladder cancer, bile duct cancer, osteosarcoma cancer, and esophageal cancer.
39. Use of the antigen binding protein, the isolated nucleic acid, isolated nucleic acids or vector, the host cell; or the pharmaceutical composition according to claim 38, wherein
20 (i) lung cancer is non-small cell lung cancer (NSCLC), preferably non-small cell lung cancer adenocarcinoma or squamous cell non-small cell lung cancer (SNSCLC); or small cell lung cancer (SCLC);
(ii) skin cancer is melanoma;
(iii) head and neck cancer is head and neck squamous cell carcinoma (HNSCC);
25 (iv) liver cancer is hepatocellular cancer (HCC);
(v) esophageal cancer is gastroesophageal junction cancer.