

WHAT IS CLAIMED IS:

1. An isolated polypeptide that specifically binds to Nectin-4 comprising a heavy chain variable region including three complementarity determining regions (CDRs) having sequences H1, H2, and H3, wherein:

the H1 sequence is GFTFSSYNX₁N (SEQ ID NO: 1);

the H2 sequence is ISSSSSTIYYADSVKG (SEQ ID NO: 2); and

the H3 sequence is AYYYGX₂DX₃ (SEQ ID NO: 3);

wherein X₁ is M or D; X₂ is M or D; X₃ is V or K, with the proviso that the heavy and light chain variable regions cannot be SEQ ID NOS: 18 and 31 or SEQ ID NOS: 18 and 56, in combination and a light chain variable region including three CDRs having sequences L1, L2, and L3, wherein:

the L1 sequence is X₄ASQGISGWX₅A (SEQ ID NO: 4);

the L2 sequence is AASTLQS (SEQ ID NO: 5); and

the L3 sequence is QQANSX₆PX₇T (SEQ ID NO: 6),

wherein X₄ is R or H; X₅ is L or E; X₆ is F or E; and X₇ is P or D, and with the proviso that X₁, X₂, X₃, X₄, X₅, X₆ and X₇ cannot simultaneously be, M, M, V, R, L, F and P, respectively.

2. The isolated polypeptide of claim 1, further comprising six anti-CD3 complementarity determining regions L4, L5, L6, L7, L8, and L9, wherein:

the L4 sequence is GFTFNTYAMN (SEQ ID NO: 44),

the L5 sequence is RIRSKYNNYATYYADSVKD (SEQ ID NO: 45),

the L6 sequence is HX₁₁NFX₁₂NSX₁₃VSWFX₁₄Y (SEQ ID NO: 46),

the L7 sequence is RSSTGAVTTSNYX₁₅N (SEQ ID NO: 47),

the L8 sequence is GTNKRAP (SEQ ID NO: 48), and

the L9 sequence is ALWYSNLWV (SEQ ID NO: 49),

wherein X₁₁ is G or S, X₁₂ is G or P, X₁₃ is Y or K, X₁₄ is A or Q and X₁₅ is A or D.

3. The isolated polypeptide as claimed in any one of claims 1-2, wherein the H1 sequence is selected from GFTFSSYNMN (SEQ ID NO: 7), and GFTFSSYNDN (SEQ ID NO: 8).

4. The isolated polypeptide as claimed in any one of claims 1-3, wherein the H3 sequence is selected from AYYYGMDV (SEQ ID NO: 9), AYYYGDDV (SEQ ID NO: 10), and AYYYGMDK (SEQ ID NO: 11).
5. The isolated polypeptide as claimed in any one of claims 1-4, wherein the L1 sequence is selected from RASQGISGWL A (SEQ ID NO: 12), RASQGISGWEA (SEQ ID NO: 13), and HASQGISGWL A (SEQ ID NO: 14).
6. The isolated polypeptide as claimed in claim 1, wherein the L3 sequence is selected from QQANSFPPT (SEQ ID NO: 15), QQANSEPPT (SEQ ID NO: 16), and QQANSFPDT (SEQ ID NO: 17).
7. The isolated polypeptide as claimed in any one of claims 2 and 3-6 when dependent on claim 2, wherein the L6 sequence is selected from HGNFGNSYVSWFAY (SEQ ID NO: 50), HSNFGNSKVSWFAY (SEQ ID NO: 51), HGNFPNSKVSWFQY (SEQ ID NO: 52), and HSNFGNSKVSWFAY (SEQ ID NO: 53).
8. The isolated polypeptide as claimed in any one of claims 2 and 3-7 when dependent on claim 2, wherein the L7 sequence is selected from RSSTGAVTTSNYAN (SEQ ID NO: 54) and RSSTGAVTTSNYDN (SEQ ID NO: 55).
9. The isolated polypeptide as claimed in claim 1, wherein the heavy chain variable region has a sequence selected from SEQ ID NOS: 18-30.
10. The isolated polypeptide as claimed in claim 1, wherein the light chain variable region has a sequence selected from SEQ ID NOS: 31-43.
11. The isolated polypeptide as claimed in claim 1, comprising a heavy chain variable region and a light chain variable region having any one pair of sequences selected from: SEQ ID NOS: 32 and 19, SEQ ID NOS: 33 and 20, SEQ ID NOS: 34 and 21, SEQ ID NOS: 35 and 22, SEQ ID NOS: 36 and 23, SEQ ID NOS: 37 and 24, SEQ ID NOS: 38 and 25, SEQ ID NOS: 39 and 26, SEQ ID NOS: 40 and 27, SEQ ID NOS: 41 and 28 and SEQ ID NOS: 42 and 29.

12. The isolated polypeptide as claimed in claim 1, comprising a heavy chain variable region and a light chain variable region, each said region independently having at least 80%, 85%, 90%, 95%, 98% or 99% identity to a combination of amino acid sequences selected from one of SEQ ID NOS: 18-30 in combination with one of SEQ ID NOS: 31-43; with the proviso that the heavy and light chain variable regions cannot be SEQ ID NOS: 18 and 31 in combination; and wherein said isolated polypeptides specifically bind to human Nectin-4 protein.

13. The isolated polypeptide as claimed in claim 2, comprising a heavy chain variable region and a light chain variable region, each said region independently having at least 80%, 85%, 90%, 95%, 98% or 99% identity to a pair of amino acid sequences selected from: SEQ ID NOS: 32 and 19, SEQ ID NOS: 33 and 20, SEQ ID NOS: 34 and 21, SEQ ID NOS: 35 and 22, SEQ ID NOS: 36 and 23, SEQ ID NOS: 37 and 24, SEQ ID NOS: 38 and 25, SEQ ID NOS: 39 and 26, SEQ ID NOS: 40 and 27, SEQ ID NOS: 41 and 28 and SEQ ID NOS: 42 and 29, respectively; and said isolated polypeptides specifically bind to human Nectin-4 protein.

14. The isolated polypeptide as claimed in claim 2, wherein the heavy chain variable region has a sequence selected from SEQ ID NOS: 18, 25, 27, and 29.

15. The isolated polypeptide as claimed in claim 2, wherein the light chain variable region has a sequence selected from SEQ ID NOS: 56-60.

16. The isolated polypeptide as claimed in claim 2, comprising a heavy chain variable region and a light chain variable region having any one pair of sequences selected from: SEQ ID NO: 25 and SEQ ID NO: 57, SEQ ID NO: 27 and SEQ ID NO: 58, SEQ ID NO: 29 and SEQ ID NO: 59, and SEQ ID NO: 29 and SEQ ID NO: 60.

17. The isolated polypeptide as claimed in claim 2, comprising a heavy chain variable region and a light chain variable region, each said region independently having at least 80%, 85%, 90%, 95%, 98% or 99% identity to a combination of amino acid sequences selected

from one of SEQ ID NOS: 18, 25, 27, and 29 in combination with one of SEQ ID NOS: 56-60 and wherein said isolated polypeptides specifically bind to human Nectin-4 protein.

18. The isolated polypeptide as claimed in claim 2, comprising a heavy chain variable region and a light chain variable region, each said region independently having at least 80%, 85%, 90%, 95%, 98% or 99% identity to a pair of amino acid sequences selected from: SEQ ID NO: 25 and SEQ ID NO: 57, SEQ ID NO: 27 and SEQ ID NO: 58, SEQ ID NO: 29 and SEQ ID NO: 59, and SEQ ID NO: 29 and SEQ ID NO: 60; and wherein said isolated polypeptides specifically bind to human Nectin-4 protein.

19. An antibody or antibody fragment comprising the isolated polypeptide as claimed in any one of claims 1-18.

20. The antibody or antibody fragment of claim 19 having a higher binding affinity to Nectin-4 protein at a value of a condition in a tumor microenvironment in comparison with a different value of the same condition that occurs in a non-tumor microenvironment.

21. The antibody or antibody fragment of claim 20, wherein the condition is pH.

22. The antibody or antibody fragment of claim 21, wherein the pH in the tumor microenvironment is in a range of from 5.0 to 6.8 and the pH in the non-tumor microenvironment is in a range of from 7.0 to 7.6.

23. The antibody or antibody fragment of claim 19 having at least 70% of the antigen binding activity at pH 6.0 as compared to the same antigen binding activity of the parent antibody or antibody fragment at pH 6.0.

24. The antibody or antibody fragment of claim 19 having less than 50%, or less than 40%, or less than 30%, or less than 20% or less than 10% of the antigen binding activity at pH 7.4 as compared to the same antigen binding activity of the parent antibody or antibody fragment at pH 7.4.

25. The antibody or antibody fragment of any one of claims 23-24, wherein the antigen binding activity is binding to Nectin-4 protein.
26. The antibody or antibody fragment of any one of claims 23-25, wherein the antigen binding activity is measured by an ELISA assay.
27. The antibody or antibody fragment of any one of claims 19-26, wherein the antibody or antibody fragment has a ratio of binding activity to the Nectin-4 protein at a value of a condition in a tumor microenvironment to a binding activity to the Nectin-4 protein at a different value of the same condition in a non-tumor microenvironment of at least about 1.5:1, at least about 2:1, at least about 3:1, at least about 4:1, at least about 5:1, at least about 6:1, at least about 7:1, at least about 8:1, at least about 9:1, at least about 10:1, at least about 20:1, at least about 30:1, at least about 50:1, at least about 70:1, or at least about 100:1.
28. The antibody or antibody fragment of any one of claims 19-27, wherein the antibody is a multi-specific antibody or antibody fragment.
29. The antibody or antibody fragment of any one of claims 19-28, wherein the antibody is a bispecific antibody or antibody fragment.
30. An immunoconjugate comprising the antibody or antibody fragment of any one of claims 19-29.
31. The immunoconjugate of claim 30, wherein the immunoconjugate comprises at least one agent selected from a chemotherapeutic agent, a radioactive atom, a cytostatic agent and a cytotoxic agent.
32. The immunoconjugate of claim 31, comprising at least two said agents.
33. The immunoconjugate of any one of claims 31-32, wherein the at least one agent is a radioactive agent.
34. The immunoconjugate of claim 33, wherein the radioactive agent is selected from an alpha emitter, a beta emitter and a gamma emitter.

35. The immunoconjugate of any one of claims 31-33, wherein the antibody or antibody fragment and the at least one agent are covalently bonded to a linker molecule.
36. The immunoconjugate of any one of claims 31-32, wherein the at least one agent is selected from maytansinoids, auristatins, dolastatins, calicheamicin, pyrrolobenzodiazepines, and anthracyclines.
37. A pharmaceutical composition comprising:
the polypeptide of any one of claims 1-18, the antibody or antibody fragment of any one of claims 19-29, or the immunoconjugate of any one of claims 30-36; and
a pharmaceutically acceptable carrier.
38. A single dose of the pharmaceutical composition of claim 37, comprising an amount of the polypeptide, the antibody or antibody fragment, or the immunoconjugate of about 135 mg, 235 mg, 335 mg, 435 mg, 535 mg, 635 mg, 735 mg, 835 mg, 935 mg, 1035 mg, 1135 mg, 1235 mg, or 1387 mg.
39. A single dose of the pharmaceutical composition of claim 37, comprising an amount of the polypeptide, the antibody or antibody fragment, or the immunoconjugate in a range of 135-235 mg, 235-335 mg, 335-435 mg, 435-535 mg, 535-635 mg, 635-735 mg, 735-835 mg, 835-935 mg, 935-1035 mg, 1035-1135 mg, 1135-1235 mg, or 1235-1387 mg.
40. The pharmaceutical composition of any one of claims 37-39, further comprising an immune checkpoint inhibitor molecule.
41. The pharmaceutical composition of claim 40, wherein the immune checkpoint inhibitor molecule is an antibody or antibody fragment against an immune checkpoint.
42. The pharmaceutical composition of claim 40, wherein the immune checkpoint is selected from CTLA4, LAG3, TIM3, TIGIT, VISTA, BTLA, OX40, CD40, 4-1BB, PD-1, PD-L1, GITR, B7-H3, B7-H4, KIR, A2aR, CD27, CD70, DR3, and ICOS.
43. The pharmaceutical composition of claim 42, wherein the immune checkpoint is CTLA4, PD-1 or PD-L1.

44. The pharmaceutical composition of any one of claims 40-43, further comprising an antibody or antibody fragment against an antigen selected from CTLA4, PD1, PD-L1, AXL, ROR2, CD3, HER2, B7-H3, ROR1, SFRP4 and a WNT protein.

45. A method of treating cancer comprising a step of administering the polypeptides of any one of claims 1-18, the antibody or antibody fragment of any one of claims 19-29, the immunoconjugate of any one of claims 30-36 or the pharmaceutical composition of any one of claims 37-44 to a patient with cancer.

46. Use of the polypeptides of any one of claims 1-18, the antibody or antibody fragment of any one of claims 19-29, the immunoconjugate of any one of claims 30-36 or the pharmaceutical composition of any one of claims 37-44, for the treatment of cancer.

47. A kit for diagnosis or treatment, said kit comprising the polypeptides of any one of claims 1-18, the antibody or antibody fragment of any one of claims 19-29, or the immunoconjugate of any one of claims 30-36 or the pharmaceutical composition of any one of claims 37-44 and instructions for using the antibody or antibody fragment, the immunoconjugate and/or the pharmaceutical composition for diagnosis or treatment.