

ANTI-NECTIN4 ANTIBODIES AND MULTI-SPECIFIC PROTEIN COMPLEXES COMPRISING SUCH

CROSS REFERENCE TO RELATED APPLICATIONS

5 This application claims the benefit of the filing date of U.S. Provisional Application No. 63/216,276, filed June 29, 2021, the entire contents of which are incorporated by reference herein.

BACKGROUND OF THE INVENTION

10 Nectins and nectin-like molecules are cell adhesion molecules involved in calcium-independent cellular adhesion. Nectin Cell Adhesion Molecule 4 (nectin-4), also known as PVRL4, is a member of the nectin family, which is within the immunoglobulin superfamily. Nectin4 has been reported as a tumor associated antigen in various cancer tissues, including pancreatic cancer, ovarian cancer, lung cancer, and breast cancer. Accordingly, nectin4 may
15 be a promising target in cancer therapy.

SUMMARY

The present disclosure is based, at least in part, on the development of antibodies binding to nectin4 with high binding affinity and specificity, as well as multi-specific protein
20 complexes comprising such (*e.g.*, bi-specific antibodies and protein complexes comprising an anti-nectin4 moiety and a cytokine (*e.g.*, IL2) moiety). In one aspect, the present disclosure refers to an isolated antibody that binds Nectin Cell Adhesion Molecule 4 (nectin-4), wherein the antibody binds the same epitope as a reference antibody or competes against the reference antibody from binding to nectin-4, and wherein the reference antibody is selected from the
25 group consisting of 2020EP034-B09, 2020EP034-H09, 2020EP034-E01, 2020EP47-F02, 2021EP030-B10, 2021EP030-C11, 2021EP023-D06, 2021EP023-E10, 2021EP030-F02, 2021EP023-H06, 2021EP029-C04, 2021EP032-D10, and 2021EP032-E06; wherein: (a) 2020EP034-B09 comprises a V_H set forth as SEQ ID NO: 14 and V_L set forth as SEQ ID NO: 19; (b) 2020EP034-H09 comprises a heavy chain variable region (V_H) set forth as SEQ ID
30 NO: 4 and a light chain variable region (V_L) set forth as SEQ ID NO: 9; (c) 2020EP034-E01 comprises a V_H set forth as SEQ ID NO: 23 and V_L set forth as SEQ ID NO: 28; (d) 2020EP47-F02 comprises a V_H set forth as SEQ ID NO: 33 and V_L set forth as SEQ ID NO: 37; (e) 2021EP030-B10 comprises a V_H set forth as SEQ ID NO: 49 and V_L set forth as SEQ

ID NO: 53; (f) 2021EP030-C11 comprises a V_H set forth as SEQ ID NO: 57 and V_L set forth as SEQ ID NO: 61; (g) 2021EP023-D06 comprises a V_H set forth as SEQ ID NO: 71 and V_L set forth as SEQ ID NO: 75; (h) 2021EP023-E10 comprises a V_H set forth as SEQ ID NO: 65 and V_L set forth as SEQ ID NO: 69; (i) 2021EP030-F02 comprises a V_H set forth as SEQ ID NO: 41 and V_L set forth as SEQ ID NO: 45; (j) 2021EP023-H06 comprises a V_H set forth as SEQ ID NO: 79 and V_L set forth as SEQ ID NO: 83; (k) 2021EP029-C04 comprises a V_H set forth as SEQ ID NO: 87 and V_L set forth as SEQ ID NO: 91; (l) 2021EP032-D10 comprises a V_H set forth as SEQ ID NO: 93 and V_L set forth as SEQ ID NO: 97; and (m) 2021EP032-E06 comprises a V_H set forth as SEQ ID NO: 99 and V_L set forth as SEQ ID NO: 103.

Accordingly, some examples of the present disclosure provide an isolated antibody that binds Nectin Cell Adhesion Molecule 4 (nectin-4) (“anti-nectin4 antibody”). The anti-nectin4 antibody binds the same epitope as a reference antibody or competes against the reference antibody from binding to nectin-4. The reference antibody is one of the following: 2020EP034-H09 (*a.k.a.*, EP034-H09), 2020EP034-B09 (*a.k.a.*, EP034-B09), 2020EP034-E01 (*a.k.a.*, EP034-E01), 2020EP47-F02 (*a.k.a.*, EP047-F02), 2021EP030-B10 (*a.k.a.*, EP030-B10), 2021EP030-C11 (*a.k.a.*, EP030-C11), 2021EP030-D06 (*a.k.a.*, EP030-D06), 2021EP030-E10 (*a.k.a.*, EP030-E10), 2021EP030-F02 (*a.k.a.*, EP034-F02), 2021EP030-H06 (*a.k.a.*, EP030-H06), 2021EP029-C04 (*a.k.a.*, EP029-C04), 2021EP032-D10 (*a.k.a.*, EP032-D10), and 2021EP032-E06 (*a.k.a.*, EP032-E06). In specific examples, the reference antibody is EP034-B09.

In some examples, the anti-nectin4 antibody may comprise: (a) a heavy chain complementary determining region 1 (HC CDR1), a heavy chain complementary determining region 2 (HC CDR2), and a heavy chain complementary determining region 3 (HC CDR3), wherein the HC CDR1, HC CDR2, and HC CDR3 collectively are at least 80% identical to the heavy chain CDRs of the reference antibody; and/or (b) a light chain complementary determining region 1 (LC CDR1), a light chain complementary determining region 2 (LC CDR2), and a light chain complementary determining region 3 (LC CDR3), wherein the LC CDR1, LC CDR2, and LC CDR3 collectively are at least 80% identical to the light chain CDRs of the reference antibody.

In some instances, the anti-nectin4 antibody disclosed herein may comprise HC CDRs that collectively contain no more than 8 amino acid residue variations as compared with the HC CDRs of the reference antibody. Alternatively or in addition, the anti-nectin4 antibody may comprise LC CDRs that collectively contain no more than 8 amino acid residue variations as compared with the LC CDRs of the reference antibody. In some instances, the anti-nectin4 antibody may comprise a V_H that is at least 85% identical to the V_H of the reference antibody, and/or a V_L that is at least 85% identical to the V_L of the reference antibody. In some instances, the anti-nectin4 antibody disclosed herein may have a binding affinity of less than about 25 nM to nectin-4 expressed on cell surface. For example, the binding affinity may be less than 10 nM. In some examples, the binding affinity may be less than 1 nM.

In specific examples, the anti-nectin4 antibodies disclosed herein may comprise the same heavy chain complementary determining regions (HC CDRs) and the same light chain complementary determining regions (LC CDRs) as the reference antibodies. In some examples, the anti-nectin4 antibodies comprise the same V_H and the same V_L as the reference antibodies.

Any of the anti-nectin4 antibodies disclosed herein may be a human antibody or a humanized antibody. In some examples, the antibody may be a single-chain antibody (scFv). Alternatively, the antibody may be a multi-chain molecule comprising at least two polypeptides. In some examples, each of the at least two polypeptides comprise an Fc fragment.

In another aspect, the present disclosure refers to a multi-specific antibody, comprising: a first binding moiety that binds nectin-4, wherein the first binding moiety is set forth as disclosed herein; and a second binding moiety that binds CD3. In other examples, the present disclosure features a multi-specific antibody, comprising: (a) a first binding moiety

that binds nectin-4; and (b) a second binding moiety that binds CD3. In some instances, the first binding moiety that binds nectin-4 can be any anti-nectin4

antibodies disclosed herein (e.g., derived from clone EP034-B09). In some instances, the second binding moiety that binds CD3 can be derived from clone EP500 or a variant thereof (e.g., EP695, EP696, or EP697). In some examples, the first binding moiety, the second binding moiety, or both are in single-chain variable fragment (scFv) format. Alternatively, the first binding moiety, the second binding moiety, or both are in immunoglobulin (Ig) format. In one example, one of the first binding moiety and the second binding moiety is in scFv format and the other binding moiety is in Ig format.

In some examples, (i) the first binding moiety comprises a first heavy chain and a first light chain, wherein the first heavy chain comprises a first heavy chain variable region (V_H) and a first heavy chain constant region, which comprises a first Fc fragment; and wherein the first light chain comprises a first light chain variable region (V_L) and a first light chain constant region; and (ii) the second binding moiety comprises a second heavy chain and a second light chain, wherein the second heavy chain comprises a second heavy chain variable region (V_H) and a second heavy chain constant region, which comprises a second Fc fragment; and wherein the second light chain comprises a second light chain variable region (V_L) and a second light chain constant region. The first Fc fragment and the second Fc fragment form a dimer.

In some examples, (i) the first binding moiety comprises a first heavy chain, a second heavy chain, and a light chain, wherein the first heavy chain comprises V_H and a first heavy chain constant region, which comprises a first Fc fragment, wherein the second heavy chain comprises the V_H and a second heavy chain constant region, which comprises a second Fc fragment, and wherein the light chain comprises a V_L and a light chain constant region; and (ii) the second binding moiety is an scFv fragment, which is fused with either the first heavy chain or the second heavy chain of (i), optionally wherein the scFv fragment is fused with the first or second heavy chain between the first or second Fc fragment and the V_H . The first Fc fragment and the second Fc fragment form a dimer.

In some examples, (i) the first binding moiety comprises a first heavy chain, a second heavy chain, and a light chain, wherein the first heavy chain comprises V_H and a first heavy chain constant region, which comprises a first Fc fragment, and wherein the second heavy chain comprises the V_H and a second heavy chain constant region, which comprises a second Fc fragment, and wherein the light chain comprises a V_L and a light chain constant region; and (ii) the second binding moiety is a heavy chain only fragment (VHH), which is fused with either the first heavy chain or the second heavy chain of (i), optionally wherein the VHH

fragment is fused with the first or second heavy chain between the first or second Fc fragment and the V_H. The first Fc fragment and the second Fc fragment form a dimer.

In some examples, (i) the first binding moiety comprises a first heavy chain and a first light chain, wherein the first heavy chain comprises a first heavy chain variable region (V_H) and a first heavy chain constant region, which comprises a first Fc fragment; and wherein the first light chain comprises a first light chain variable region (V_L) and a first light chain constant region; and (ii) the second binding moiety is an scFv fragment fused to a second Fc fragment. The first Fc fragment and the second Fc fragment form a dimer.

Any of the multi-specific antibodies disclosed herein may further comprise a cytokine, which optionally is IL-2. In some examples, the cytokine is fused to the C-terminus of the first Fc fragment. In some examples, the cytokine is fused to the C-terminus of the second Fc fragment. In some examples, the cytokine is fused to both the C-terminus of the first Fc fragment and the C-terminus of the second Fc fragment.

In some examples, the multi-specific antibody disclosed herein may further comprise a third binding moiety, which binds a T cell co-stimulatory receptor. Examples include, but are not limited to, ICOS, 4-1BB, CD28, or CD86.

In another aspect, the present disclosure refers to a protein complex, comprising a first moiety that binds nectin-4 and a second moiety that comprises a cytokine, wherein the first moiety that binds nectin-4 is set forth as disclosed herein. In another example, the present disclosure features a protein complex, comprising a first moiety that binds nectin-4 and a second moiety that comprises a cytokine, *e.g.*, IL-2. The first moiety that binds nectin-4 may be any of the anti-nectin4 antibodies disclosed herein. In some examples, the first moiety comprises an scFv fragment fused to a first Fc fragment; and the second moiety comprises the cytokine fused to a second Fc fragment. The first Fc fragment and the second Fc fragment form a dimer.

In some examples, the first moiety comprises a first polypeptide, which comprises an scFv fragment fused to a first Fc fragment, and a second polypeptide, which comprises the scFv fragment fused to a second Fc fragment. In some examples, the cytokine of the second moiety is fused to the C-terminus of the first Fc fragment. In some examples, the cytokine of the second moiety is fused to the C-terminus of the second Fc fragment. In some examples, the cytokine of the second moiety is fused to both the C-terminus of the first Fc fragment and the C-terminus of the second Fc fragment. The first Fc fragment and the second Fc fragment form a dimer.

In some examples, (i) the first moiety comprises a heavy chain comprising a V_H and a heavy chain constant region, which comprises a first Fc fragment, and a light chain comprising a V_L and a light chain constant region; and (ii) the second moiety comprises the

cytokine fused to a second Fc fragment. The first Fc fragment and the second Fc fragment form a dimer.

In some examples, the first moiety comprises a first heavy chain comprising a V_H and a first heavy chain constant region, which comprises a first Fc fragment, a second heavy chain comprising the V_H and a second heavy chain constant region, which comprises a second Fc fragment, and a light chain comprising a V_L and a light chain constant region. In some examples, the cytokine of the second moiety is fused to the C-terminus of the first Fc fragment. In some examples, the cytokine of the second moiety is fused to the C-terminus of the second Fc fragment. In some examples, the cytokine of the second moiety is fused to both the C-terminus of the first Fc fragment and the C-terminus of the second Fc fragment. The first Fc fragment and the second Fc fragment form a dimer.

In any of the multi-specific antibodies and protein complexes disclosed herein, the first Fc fragment and the second Fc fragment comprise mutations that enhances heterodimerization over homodimerization as relative to the wild-type counterpart. In some examples, the mutations are knob-hole mutations. For example, the knob mutation may comprise S354C, T366W and/or K409A. The hole mutation may comprise S354C, Y349C, T366S, L368A, F405K, and/or Y407V.

In addition, in a further aspect, the present disclosure provides a nucleic acid or a set of nucleic acids, which collectively encodes any of the nectin4 antibodies disclosed here or any of the multi-specific antibodies or protein complexes as also disclosed herein. In some examples, the nucleic acid or the set of nucleic acids can be a vector or a set of vectors. In some examples, the vector is an expression vector. Further, in another aspect, provided herein is a host cell comprising any of the encoding nucleic acid or the set of nucleic acids as disclosed herein.

Moreover, in another aspect, provided herein is a pharmaceutical composition comprising any of the anti-nectin4 or multi-specific antibodies disclosed herein, any of the protein complexes disclosed herein, any of the encoding nucleic acid or nucleic acids, or the host cell comprising such, and a pharmaceutically acceptable carrier.

In yet another aspect, the present disclosure refers to a pharmaceutical composition for use as a medicament, wherein the pharmaceutical composition is set forth as disclosed herein. In yet another aspect, the present disclosure refers to a use of a pharmaceutical composition in the manufacture of a medicament for inhibiting nectin-4 or nectin-4⁺ cells in a subject, wherein the pharmaceutical composition is set forth as disclosed herewith. In other examples, the present disclosure features a method for inhibiting nectin-4 or nectin-4⁺ cells in

a subject, comprising administering to a subject in need thereof any effective amount of the pharmaceutical composition disclosed herein. In some examples, the subject is a human patient having nectin-4⁺ pathogenic cells. In some examples, the subject is a human patient having nectin-4 positive cancer. Examples include breast cancer,

bladder cancer, ovary cancer, cervical cancer, pancreatic cancer, lung cancer, or head and neck cancer.

In yet another aspect, the present disclosure refers to an *in vitro* method for detecting presence of nectin, comprising: (i) contacting an antibody as disclosed herewith with a sample suspected of containing nectin-4 expressed on cell surface, and (ii) detecting binding of the antibody to nectin-4. Further, the present disclosure features a method for detecting presence of nectin, comprising: (i) contacting an antibody that binds nectin4 as disclosed herein with a sample suspected of containing nectin-4, and (ii) detecting binding of the antibody to nectin-4. In some instances, the antibody is conjugated to a detectable label. In some instances, the nectin-4 is expressed on cell surface. In some instances, the contacting step is performed by administering the antibody to a subject.

In yet another aspect, the present disclosure refers to a method of producing an antibody binding to nectin-4 or a multi-specific antibody or protein complex comprising such, comprising: (i) culturing the host cell as disclosed herein under conditions allowing for expression of the antibody that binds nectin-4, the multi-specific antibody comprising such, or the protein complex comprising such; and (ii) harvesting the antibody, the multi-specific antibody, or the protein complex thus produced from the cell culture. In addition, the present disclosure provides a method of producing an antibody binding to nectin-4 or a multi-specific antibody or protein complex comprising such, comprising: (i) culturing the host cell comprising nucleic acid(s) encoding the anti-nectin4 antibody, the multi-specific antibody, or the protein complex as disclosed herein under conditions allowing for expression of the antibody that binds nectin-4, the multi-specific antibody comprising such, or the protein complex comprising such; and (ii) harvesting the antibody, the multi-specific antibody, or the protein complex thus produced from the cell culture.

Also within the scope of the present disclosure are any of the anti-nectin4 antibodies, the multi-specific antibodies, and the protein complexes disclosed herein for use in treating a target disease (*e.g.*, a disease or disorder associated with nectin4⁺ cells such as nectin4⁺ cancer cells) and use of such antibody, multi-specific antibody, or protein complex for manufacturing a medicament for use in treating the target disease.

The details of one or more examples of the disclosure are set forth in the description below. Other features or advantages of the present disclosure will be apparent from the following drawings and detailed description of several examples, and also from the appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present disclosure, which can be better understood by reference to the drawing in combination with the detailed description of specific

5 embodiments presented herein.

Figure 1 is a diagram showing antibody-dependent cell cytotoxicity (ADCC) of anti-nectin4 IgG antibodies against cells expressing nectin4.

WHAT IS CLAIMED IS:

1. An isolated antibody that binds Nectin Cell Adhesion Molecule 4 (nectin-4), wherein the antibody binds the same epitope as a reference antibody or competes against the reference antibody from binding to nectin-4, and wherein the reference antibody is selected from the group consisting of 2020EP034-B09, 2020EP034-H09, 2020EP034-E01, 2020EP47-F02, 2021EP030-B10, 2021EP030-C11, 2021EP023-D06, 2021EP023-E10, 2021EP030-F02, 2021EP023-H06, 2021EP029-C04, 2021EP032-D10, and 2021EP032-E06;

wherein:

(a) 2020EP034-B09 comprises a V_H set forth as SEQ ID NO: 14 and V_L set forth as SEQ ID NO: 19;

(b) 2020EP034-H09 comprises a heavy chain variable region (V_H) set forth as SEQ ID NO: 4 and a light chain variable region (V_L) set forth as SEQ ID NO: 9;

(c) 2020EP034-E01 comprises a V_H set forth as SEQ ID NO: 23 and V_L set forth as SEQ ID NO: 28;

(d) 2020EP47-F02 comprises a V_H set forth as SEQ ID NO: 33 and V_L set forth as SEQ ID NO: 37;

(e) 2021EP030-B10 comprises a V_H set forth as SEQ ID NO: 49 and V_L set forth as SEQ ID NO: 53;

(f) 2021EP030-C11 comprises a V_H set forth as SEQ ID NO: 57 and V_L set forth as SEQ ID NO: 61;

(g) 2021EP023-D06 comprises a V_H set forth as SEQ ID NO: 71 and V_L set forth as SEQ ID NO: 75;

(h) 2021EP023-E10 comprises a V_H set forth as SEQ ID NO: 65 and V_L set forth as SEQ ID NO: 69;

(i) 2021EP030-F02 comprises a V_H set forth as SEQ ID NO: 41 and V_L set forth as SEQ ID NO: 45;

(j) 2021EP023-H06 comprises a V_H set forth as SEQ ID NO: 79 and V_L set forth as SEQ ID NO: 83;

(k) 2021EP029-C04 comprises a V_H set forth as SEQ ID NO: 87 and V_L set forth as SEQ ID NO: 91;

(l) 2021EP032-D10 comprises a V_H set forth as SEQ ID NO: 93 and V_L set forth as SEQ ID NO: 97; and

(m) 2021EP032-E06 comprises a V_H set forth as SEQ ID NO: 99 and V_L set forth as SEQ ID NO: 103.

2. The isolated antibody of claim 1, wherein the antibody comprises:

(a) a heavy chain complementary determining region 1 (HC CDR1), a heavy chain complementary determining region 2 (HC CDR2), and a heavy chain complementary determining region 3 (HC CDR3), wherein the HC CDR1, HC CDR2, and HC CDR3 collectively are at least 80% identical to the heavy chain CDRs of the reference antibody; and

(b) a light chain complementary determining region 1 (LC CDR1), a light chain complementary determining region 2 (LC CDR2), and a light chain complementary determining region 3 (LC CDR3), wherein the LC CDR1, LC CDR2, and LC CDR3 collectively are at least 80% identical to the light chain CDRs of the reference antibody.

3. The isolated antibody of claim 1 or claim 2, wherein the HC CDRs of the antibody collectively contain no more than 8 amino acid residue variations as compared with the HC CDRs of the reference antibody; and wherein the LC CDRs of the antibody collectively contain no more than 8 amino acid residue variations as compared with the LC CDRs of the reference antibody; or wherein the antibody comprises a V_H that is at least 85% identical to the V_H of the reference antibody, and/or a V_L that is at least 85% identical to the V_L of the reference antibody.

4. The isolated antibody of any one of claims 1-3, wherein the antibody has a binding affinity of less than about 10 nM to nectin-4 expressed on cell surface.

5. The isolated antibody of claim 1, which comprises the same heavy chain complementary determining regions (HC CDRs) and the same light chain complementary determining regions (LC CDRs) as the reference antibody.
6. The isolated antibody of claim 5, which comprises the same V_H and the same V_L as the reference antibody.
7. The isolated antibody of any one of claims 1-6, wherein the antibody is a human antibody or a humanized antibody.
8. The isolated antibody of any one of claims 1-7, wherein the antibody is a single-chain antibody (scFv).
9. The isolated antibody of any one of claims 1-8, wherein the antibody is a multi-chain molecule comprising at least two polypeptides.
10. The isolated antibody of claim 9, wherein each of the at least two polypeptides comprise an Fc fragment.
11. A multi-specific antibody, comprising:
 - a first binding moiety that binds nectin-4, wherein the first binding moiety is set forth in any one of claims 2-6; and
 - a second binding moiety that binds CD3.
12. A protein complex, comprising a first moiety that binds nectin-4 and a second moiety that comprises a cytokine, wherein the first moiety that binds nectin-4 is set forth in any one of claims 2-6.

13. A nucleic acid or a set of nucleic acids, which collectively encodes the antibody of any one of claims 1-10, the multi-specific antibody of claim 11, or the protein complex of claim 12.
14. The nucleic acid or the set of nucleic acids of claim 13, which is a vector or a set of vectors.
15. The nucleic acid or the set of nucleic acids of claim 14, wherein the vector is an expression vector.
16. A host cell comprising the nucleic acid or the set of nucleic acids of any one of claims 13-15.
17. A pharmaceutical composition comprising the antibody of any one of claims 1-10, the multi-specific antibody of claim 11, the protein complex of claim 12, the nucleic acid or nucleic acids of any one of claims 13-15, or the host cell of claim 16, and a pharmaceutically acceptable carrier.
18. A pharmaceutical composition for use as a medicament, wherein the pharmaceutical composition is set forth in claim 17.
19. Use of a pharmaceutical composition in the manufacture of a medicament for inhibiting nectin-4 or nectin-4⁺ cells in a subject, wherein the pharmaceutical composition is set forth in claim 17.
20. The use of claim 19, wherein the use is for treating a nectin-4 positive cancer, which is breast cancer, bladder cancer, ovary cancer, cervical cancer, pancreatic cancer, lung cancer, or head and neck cancer.
21. An *in vitro* method for detecting presence of nectin, comprising:

(i) contacting an antibody of any one of claims 1-10 with a sample suspected of containing nectin-4 expressed on cell surface, and

(ii) detecting binding of the antibody to nectin-4.

22. A method of producing an antibody binding to nectin-4 or a multi-specific antibody or protein complex comprising such, comprising:

(i) culturing the host cell of claim 16 under conditions allowing for expression of the antibody that binds nectin-4, the multi-specific antibody comprising such, or the protein complex comprising such; and

(ii) harvesting the antibody, the multi-specific antibody, or the protein complex thus produced from the cell culture.