

decrease after treatment. These results suggest that Nectin-4 could be a reliable target for the treatment of cancer.

[0004] Accordingly, several anti-Nectin-4 antibodies have been described in the prior art. In particular, Enfortumab Vedotin (ASG-22ME) is an antibody-drug conjugate (ADC) targeting Nectin-4 and is currently in clinical investigation for the treatment of patients suffering from solid tumors.

[0005] The present invention aims at providing anti-Nectin-4 antibodies or antibody fragments with reduced or minimal side effects suitable for therapeutic and diagnostic use, especially for diagnosis and treatment of cancers. Some of these anti-Nectin-4 antibodies or antibody fragments may have a higher binding or binding affinity to Nectin-4 in a tumor microenvironment in comparison with the binding or binding affinity to Nectin-4 present in a non-tumor microenvironment. These anti-Nectin-4 antibodies or antibody fragments typically have at least comparable efficacy to known anti-Nectin-4 antibodies. In addition, the present anti-Nectin-4 antibodies or antibody fragments may exhibit reduced side effects in comparison with monoclonal anti-Nectin-4 antibodies known in the art for having a relatively low binding affinity to Nectin-4 in normal tissues such as a non-tumor microenvironment. These advantages may provide a more selective targeting of the Nectin-4 expressed in a tumor and may permit use of higher dosages of these anti-Nectin-4 antibodies or antibody fragments as a result of the selectivity of the antibodies for Nectin-4 present in a tumor microenvironment, whereby more effective therapeutic treatments can be realized without a corresponding increase in undesirable side effects.

SUMMARY

[0005a] In one aspect, the present disclosure refers to 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.

[0006] In one example, the present disclosure provides an isolated polypeptide that specifically binds to Nectin-4. The polypeptide comprises 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 YISSSSSTIYYADSVKG (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 in combination.

[0007] In another example, the present disclosure includes a product formed by a combination of any of the above-described isolated polypeptides with an isolated polypeptide comprising 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.

[0008] In another example, the present disclosure provides isolated polypeptides comprising a heavy chain variable region and a light chain variable region that specifically bind to Nectin-4, or especially human Nectin-4 protein, wherein the heavy chain variable region includes three complementarity determining regions having sequences H1, H2, and H3, wherein:

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

the H2 sequence is YISSSSSTIYYADSVKG (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; and

the light chain variable region includes three complementarity determining regions 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, with the proviso that the heavy and light chain variable regions cannot be SEQ ID NOS: 18 and 31 in combination.

[0009] In each of the previous examples of paragraphs [0006] to [0008] above, the H1 sequence may be selected from GFTFSSYNMN (SEQ ID NO: 7), and GFTFSSYNDN (SEQ ID NO: 8). The H3 sequence may be selected from AYYYGMDV (SEQ ID NO: 9), AYYYGDDV (SEQ ID NO: 10), and AYYYGMDK (SEQ ID NO: 11).

[0010] In each of the previous examples of paragraphs [0006] to [0009] above, the L1 sequence may be selected from RASQGISGWL A (SEQ ID NO: 12), RASQGISGWEA (SEQ ID NO: 13), and HASQGISGWL A (SEQ ID NO: 14). The L3 sequence may be selected from QQANSFPPT (SEQ ID NO: 15), QQANSEPPT (SEQ ID NO: 16), and QQANSFPDT (SEQ ID NO: 17).

[0011] In each of the previous examples of paragraphs [0006] to [0010] above, the isolated polypeptide may comprise a heavy chain variable region having a sequence selected from SEQ ID NOS: 18-30.

[0012] In each of the foregoing examples of paragraphs [0006] to [0011] above, the isolated polypeptide may comprise a light chain variable region having a sequence selected from SEQ ID NOS: 31-43.

[0013] In another example, isolated polypeptides of the present disclosure comprise a heavy chain variable region and a light chain variable region having any one pair of sequences selected from: SEQ ID NOS: 19 and 32, SEQ ID NOS: 20 and 33, SEQ ID NOS: 21 and 34, SEQ ID NOS: 22 and 35, SEQ ID NOS: 23 and 36, SEQ ID NOS: 24 and 37, SEQ ID NOS: 25 and 38, SEQ ID NOS: 26 and 39, SEQ ID NOS: 27 and 40, SEQ ID NOS: 28 and 41 and SEQ ID NOS: 29 and 42.

[0014] In another example, the isolated polypeptides, of the present disclosure comprise a heavy chain variable region and a light chain variable region each 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 said isolated polypeptides specifically bind to human Nectin-4 protein.

[0015] In another example, the isolated polypeptides of the present disclosure comprise a heavy chain variable region and a light chain variable region each 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: 19 and 32, SEQ ID NOS: 20 and 33, SEQ ID NOS: 21 and 34, SEQ ID NOS: 22 and 35, SEQ ID NOS: 23 and 36, SEQ ID NOS: 24 and 37, SEQ ID NOS: 25 and 38, SEQ ID NOS: 26 and 39, SEQ ID NOS: 27 and 40, SEQ ID NOS: 28 and 41 and SEQ ID NOS: 29 and 42, respectively; with the proviso that the heavy and light chain variable regions cannot be SEQ ID NOS: 18 and 31 in combination and said isolated polypeptides specifically bind to human Nectin-4 protein.

[0016] In each of the previous examples of paragraphs [0006] to [0015], the isolated polypeptide may be an antibody or antibody fragment that specifically binds to Nectin-4, or especially human Nectin-4 protein.

[0017] In a yet another example, the isolated polypeptide is multi-specific and specifically binds to Nectin-4, or especially human Nectin-4 protein and to CD3 and the isolated polypeptide comprises a heavy chain variable region and a light chain variable region,

wherein the heavy chain variable region includes three complementarity determining regions having sequences H1, H2, and H3, wherein:

the H1 sequence is selected from SEQ ID NO: 7 and SEQ ID NO: 8,

the H2 sequence is SEQ ID NO: 2, and

the H3 sequence is selected from SEQ ID NO: 9, SEQ ID NO: 10, and SEQ ID NO: 11; and

the light chain variable region includes three complementarity determining regions 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, and

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.

[0018] In another example of the isolated polypeptide with nine CDRs of paragraph [0017], the L6 sequence is selected from any one of SEQ ID NOs: 50-53, and the L7 sequence is selected from SEQ ID NOs: 54 and 55.

[0019] In a preferred example, the isolated polypeptide with nine CDRs of paragraphs [0017]-[0018] comprises a heavy chain variable region that includes three complementarity determining regions, H1, H2, and H3, wherein:

the H1 sequence is selected from SEQ ID NO: 7 and SEQ ID NO: 8,

the H2 sequence is SEQ ID NO: 2, and

the H3 sequence is selected from SEQ ID NO: 9, SEQ ID NO: 10, and SEQ ID NO: 11; and

the light chain variable region includes three complementarity determining regions having sequences L1, L2, and L3, wherein:

the L1 sequence is selected from SEQ ID NO: 12, SEQ ID NO: 13, and SEQ ID NO: 14,

the L2 sequence is SEQ ID NO: 5,

the L3 sequence is selected from SEQ ID NO: 15, SEQ ID NO: 16, and SEQ ID NO: 17, and 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 selected from HGNFGNSYVSWFAY (SEQ ID NO: 50),
HSNFGNSKVSWFAY (SEQ ID NO: 51), HGNFPNSKVSWFQY (SEQ ID NO: 52), and
HSNFGNSKVSWFAY (SEQ ID NO: 53),
the L7 sequence is selected from RSSTGAVTTSNYAN (SEQ ID NO: 54) and
RSSTGAVTTSNYDN (SEQ ID NO: 55),
the L8 sequence is GTNKRAP (SEQ ID NO: 48), and
the L9 sequence is ALWYSNLWV (SEQ ID NO: 49).

[0020] In another preferred example, the isolated polypeptide with nine CDRs of paragraphs [0017]-[0019] comprises a heavy chain variable region that includes three complementarity determining regions, H1, H2, and H3, wherein:

the H1 sequence is SEQ ID NO: 7,
the H2 sequence is SEQ ID NO: 2, and
the H3 sequence is selected from SEQ ID NO: 9, SEQ ID NO: 10, and SEQ ID NO: 11; and
the light chain variable region includes three complementarity determining regions having sequences L1, L2, and L3, wherein:
the L1 sequence is selected from SEQ ID NO: 12 and SEQ ID NO: 13,
the L2 sequence is SEQ ID NO: 5,
the L3 sequence is SEQ ID NO: 15, and 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 selected from HGNFGNSYVSWFAY (SEQ ID NO: 50),
HSNFGNSKVSWFAY (SEQ ID NO: 51), HGNFPNSKVSWFQY (SEQ ID NO: 52), and
HSNFGNSKVSWFAY (SEQ ID NO: 53),
the L7 sequence is selected from RSSTGAVTTSNYAN (SEQ ID NO: 54) and
RSSTGAVTTSNYDN (SEQ ID NO: 55),
the L8 sequence is GTNKRAP (SEQ ID NO: 48), and
the L9 sequence is ALWYSNLWV (SEQ ID NO: 49).

[0021] In each of the previous examples of paragraphs [0017]-[0020], the isolated polypeptide may comprise a heavy chain variable region having a sequence selected from SEQ ID NOS: 18, 25, 27, and 29.

[0022] In each of the previous examples of paragraphs [0017]-[0021], the isolated polypeptide may comprise a light chain variable region having a sequence selected from SEQ ID NOS: 56-60, with the proviso that the heavy and light chain variable regions cannot be SEQ ID NOS: 18 and 56 combination.

[0023] The isolated polypeptide may comprise a heavy chain variable region having a sequence selected from SEQ ID NOS: 18, 25, 27, and 29, and a light chain variable region having a sequence selected from SEQ ID NOS: 56-60, with the proviso that the heavy and light chain variable regions cannot be SEQ ID NOS: 18 and 56 combination.

[0024] The isolated polypeptide may comprise 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.

[0025] In another example, the isolated polypeptides of the present disclosure comprise a heavy chain variable region and a light chain variable region, each 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, with the proviso that the heavy and light chain variable regions cannot be SEQ ID NOS: 18 and 56 combination; and said isolated polypeptides specifically bind to human Nectin-4 protein.

[0026] In another example, the isolated polypeptides of the present disclosure comprise a heavy chain variable region and a light chain variable region each 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: 25 and 57, SEQ ID NOS: 27 and 58, SEQ ID NOS: 29 and 59, SEQ ID NOS: 29 and 60; and said isolated polypeptides specifically bind to human Nectin-4 protein.

[0027] In each of the previous examples of paragraphs [0017] to [0026], the isolated polypeptide may be a multispecific antibody or antibody fragment that specifically binds to Nectin-4, especially human Nectin-4 protein.

[0028] In a preferred example of paragraphs [0017] to [0027], the isolated polypeptide may be a bispecific antibody or antibody fragment that specifically binds to Nectin-4 and CD3, especially human Nectin-4 protein and CD3.

[0028a] In one aspect, the present disclosure refers to an antibody or antibody fragment comprising any one of the isolated polypeptides disclosed herein.

[0029] In each of the previous examples of paragraphs [0006] to [0028], the isolated polypeptide or antibody or antibody fragment may have a higher binding affinity to Nectin-4 protein, especially human 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. In one example, the condition is pH.

[0030] In each of the previous examples of paragraphs [0006] to [0029], the isolated polypeptide or antibody or antibody fragment may have at least 70% of the antigen binding activity at pH 6.0 as compared to the same antigen binding activity of the parent polypeptide, antibody or antibody fragment at pH 6.0, and the polypeptide or antibody or antibody fragment may have 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 polypeptide or the isolated polypeptide or antibody or antibody fragment at pH 7.4. The antigen binding activity may be binding to Nectin-4 protein.

[0031] In the foregoing examples of paragraphs [0006] to [0030], the isolated polypeptide, antibody or antibody fragment may have a higher binding affinity to Nectin-4 protein, especially human Nectin-4 protein, at a pH in a tumor microenvironment in comparison with a pH that occurs in a non-tumor microenvironment. The pH in the tumor microenvironment may range of from 5.0 to 6.8 and the pH in the non-tumor microenvironment may range from 7.0 to 7.6.

[0032] In each of the previous examples, the antigen binding activity of the isolated polypeptide or antibody or antibody fragment may be measured by an ELISA assay.

[0033] In one aspect, the present disclosure refers to an immunoconjugate comprising the any one of the polypeptides or any one of the antibodies or antibody fragments as disclosed herein. In yet another example, the present disclosure provides an immunoconjugate that includes any of the antibodies or antibody fragments of the disclosure described above. In the immunoconjugate, the antibody or antibody fragment may be conjugated to an agent selected from a chemotherapeutic agent, a radioactive atom, a cytostatic agent and a cytotoxic agent.

[0034] In one aspect, the present disclosure refers to a pharmaceutical composition comprising: any one of the polypeptides disclosed herein, any one of the antibodies or antibody fragments disclosed herein, or any one of the immunoconjugates disclosed herein; and a pharmaceutically acceptable carrier. In yet another example, the present disclosure provides a pharmaceutical composition that includes any of the isolated polypeptides, the antibodies or antibody fragments, or the immunoconjugates of the disclosure described above, together with a pharmaceutically acceptable carrier.

[0034a] In one aspect, the present disclosure refers to a single dose of the pharmaceutical composition disclosed herein, 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, or 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.

[0035] A single dose of the pharmaceutical composition of may include an amount of the isolated polypeptide, the antibody, the 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.

[0036] A single dose of the pharmaceutical composition of may include an amount of the isolated 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.

[0037] Each of the foregoing pharmaceutical compositions may further include an immune checkpoint inhibitor molecule. The immune checkpoint inhibitor molecule may be an antibody or antibody fragment against an immune checkpoint. The immune checkpoint may be selected from LAG3, TIM3, TIGIT, VISTA, BTLA, OX40, CD40, 4-1BB, CTLA4, PD-1, PD-L1, GITR, B7-H3, B7-H4, KIR, A2aR, CD27, CD70, DR3, and ICOS or the immune checkpoint may be CTLA4, PD-1 or PD-L1.

[0038] Each of the foregoing pharmaceutical compositions may further include an antibody or antibody fragment against an antigen selected from PD1, PD-L1, CTLA4, AXL, ROR2, CD3, HER2, B7-H3, ROR1, SFRP4 and a WNT protein. The WNT protein may be selected from WNT1, WNT2, WNT2B, WNT3, WNT4, WNT5A, WNT5B, WNT6, WNT7A, WNT7B, WNT8A, WNT8B, WNT9A, WNT9B, WNT10A, WNT10B, WNT11 and WNT16.

[0038a] In one aspect, the present disclosure refers to any one of the polypeptides disclosed herein, any one of the antibodies or antibody fragments disclosed herein, any one of the immunoconjugates disclosed herein or any one of the pharmaceutical compositions disclosed herein for use as a medicament.

[0038b] In one aspect, the present disclosure refers to use of any one of the polypeptides disclosed herein, any one of the antibodies or antibody fragments disclosed herein, any one of the immunoconjugates disclosed herein or any one of the pharmaceutical compositions disclosed herein, in the manufacture of a medicament for the treatment of cancer.

[0039] In yet another example, the present disclosure provides a kit for diagnosis or treatment including any of the isolated polypeptides, the antibodies or antibody fragments, the immunoconjugates, or the pharmaceutical compositions of the present disclosure described above. In one aspect, the present disclosure refers to a kit for diagnosis or treatment, said kit comprising any one of the polypeptides disclosed herein, any one of the antibodies or antibody fragments disclosed herein, or any one of the immunoconjugates disclosed herein or any one of the pharmaceutical compositions disclosed herein and instructions for using the antibody or antibody fragment, the immunoconjugate and/or the pharmaceutical composition for diagnosis or treatment.

BRIEF DESCRIPTION OF THE DRAWINGS

[0040] FIG. 1 shows the binding activities of exemplary conditionally active anti-Nectin-4 antibodies of the present invention conjugated to a linker payload (hereinafter “CAB ADCs”) to human Nectin-4 at pH 6.0 as measured by an enzyme-linked immunosorbent assay (ELISA). In the figure, BM (benchmark) is a wild type antibody conjugated to the linker payload (hereinafter “WT ADC”).

[0041] FIG. 2 shows the binding activities of exemplary conditionally active anti-Nectin-4 CAB ADCs of the present invention and the WT ADC to human Nectin-4 at pH 7.4 as measured by ELISA.

[0042] FIG. 3 shows the binding activity of further exemplary conditionally active anti-Nectin-4 CAB ADCs of the present invention and the WT ADC to human Nectin-4 at pH 6.0 as measured by ELISA.