ANTI-B7-H4 ANTIBODIES AND IMMUNOCONJUGATES

CROSS REFERENCE TO RELATED APPLICATIONS

[001] This application claims priority to U.S. Provisional Application No. 61/784,877, filed March 14, 2013; U.S. Provisional Application No. 61/785,811, filed March 14, 2013; and U.S. Provisional Application No. 61/874,175, filed September 5, 2013; each of which is incorporated by reference herein in its entirety for any purpose.

FIELD OF THE INVENTION

[002] The present invention relates to anti-B7-H4 antibodies and immunoconjugates and methods of using the same.

BACKGROUND

[003] B7-H4 is a Type I transmembrane protein and is a member of the B7 superfamily of proteins that provides co-signal in conjunction with a T-cell receptor antigenic signal. B7-H4 is a negative regulator of T-cell function and ligation of T-cells inhibits their growth, cytokine secretion and cytotoxicity. Elimination of B7-H4 in mice does not affect immune cell homeostasis and no signs of autoimmunity. Zhu et al., Blood, 113(8): 1759-1767 (2009); Suh et al., *Molecular and Cellular Biology, 26(17): 6403-6411 (2006).The receptor for* B7-H4 is unknown and unidentified.

[004] Human B7-H4 is a 282 amino acid protein (including the amino-terminal signal sequence), of which ~227 amino acids are predicted to be in the extracellular space following cleavage of the amino-terminal signal sequence. B7-H4 comprises an Ig-like V-domain, an Ig-like C domain, a transmembrane domain and a short cytoplasmic tail.

[005] There is a need in the art for agents that target B7-H4 for the diagnosis and treatment of B7-H4-associated conditions, such as cancer. The invention fulfills that need and provides other benefits.

SUMMARY

[006] The ~~invention~~ disclosure provides anti-B7-H4 antibodies and immunoconjugates and methods of using the same.

[007] Disclosed is~~In some embodiments,~~ an isolated antibody that binds to B7-H4 ~~is provided~~. In some embodiments, the antibody binds to B7-H4 and has at least one of the following characteristics: (a) binds to an epitope within all or a portion of the B7-H4 Ig-V containing

domain (amino acids 29-157 of SEQ ID NO: 73); or binds to an epitope within all or a portion of the B7-H4 Ig-C containing domain (amino acids 158-250 of SEQ ID NO: 73); or binds to an epitope within all or a portion of the B7-H4 Ig-V and Ig-C domains (amino acids 29-250 of SEQ ID NO:73); or binds to an epitope within all or a portion of SEQ ID NO: 74 (mature human B7-H4) or binds to an epitope within all or a portion of SEQ ID NO: 73 (precursor human B7-H4). In some embodiments, the antibody binds to B7-H4 with an affinity of $\leq$ 50 nM. In some embodiments, the antibody is a monoclonal antibody. In some embodiments, the antibody is a human, humanized, or chimeric antibody. In some embodiments, the antibody is an IgG1, IgG2a or IgG2b antibody. In some embodiments, the antibody is an antibody fragment that binds B7-H4. In some embodiments, B7-H4 is human B7-H4 has the sequence of SEQ ID NO: 73.

[007a] In another aspect, there is provided an isolated antibody that binds to B7-H4, wherein the antibody has at least one of the following characteristics: (a) binds to an epitope within all or a portion of the B7-H4 Ig-V containing domain (amino acids 29-157 of SEQ ID NO: 73); or binds to an epitope within all or a portion of the B7-H4 Ig-C containing domain (amino acids 158-250 of SEQ ID NO: 73); or binds to an epitope within all or a portion of the B7-H4 Ig-V and Ig-C domains (amino acids 29-250 of SEQ ID NO:73); or binds to an epitope within all or a portion of SEQ ID NO: 74 (mature human B7-H4); or binds to an epitope within all or a portion of SEQ ID NO: 73 (precursor human B7-H4), and (b) binds to B7-H4 with an affinity of $\leq$ 50 nM.

[008] In some embodiments, the antibody comprises (a) (i) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 7, (ii) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 10, and (iii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 6; or (b) (i) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 15, (ii) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 18, and (iii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 14; or (c) (i) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 23, (ii) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26, and (iii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 22; or (d) (i) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 31, (ii) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 34, and (iii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 30; or (e) (i) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 41, (ii) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 44, and (iii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 40; or (f) (i) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 60, (ii) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 63, and (iii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 59.

[009] In some embodiments, the antibody comprises (a) (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 5, (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 6, and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 7; or (b) (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 13, (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 14, and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 15; or (c) (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 21, (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 22, and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 23; or (d) (i) HVR-H1

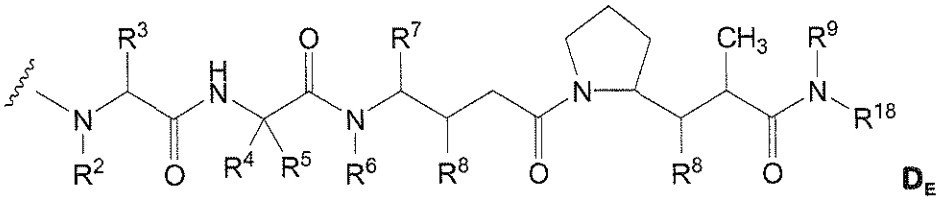
SEQ ID NO: 4, 28, a VH sequence of SEQ ID NO: 36, a VH sequence of SEQ ID NO: 37, a VH sequence of SEQ ID NO: 38, or a VH sequence of SEQ ID NO: 56.

[016] In some embodiments, the antibody comprises a VL sequence of SEQ ID NO: 3, a VL sequence of SEQ ID NO: 11, a VL sequence of SEQ ID NO: 19, a VL sequence of SEQ ID NO: 27, a VL sequence of SEQ ID NO: 35, a VL sequence of SEQ ID NO: 55, or a VL sequence of SEQ ID NO: 57.

[017] In another aspect, there is provided~~some embodiments,~~ an isolated antibody comprising~~es~~ (a) a VH sequence of SEQ ID NO: 4 and a VL sequence of SEQ ID NO: 3; or (b) a VH sequence of SEQ ID NO: 20 and a VL sequence of SEQ ID NO: 19; or (c) a VH sequence of SEQ ID NO: 28 and a VL sequence of SEQ ID NO: 27; or (d) a VH sequence of SEQ ID NO: 36 and a VL sequence of SEQ ID NO: 35; or (e) a VH sequence of SEQ ID NO: 37 and a VL sequence of SEQ ID NO: 35; or (f) a VH sequence of SEQ ID NO: 38 and a VL sequence of SEQ ID NO: 35; or (g) a VH sequence of SEQ ID NO: 56 and a VL sequence of SEQ ID NO: 55; or (h) a VH sequence of SEQ ID NO: 56 and a VL sequence of SEQ ID NO: 57.

[018] In another aspect, there is provided~~some embodiments,~~ an isolated nucleic acid ~~that~~ encoding~~es~~ the~~an~~ antibody described herein ~~is provided~~. In another aspect, there is provided~~some embodiments,~~ a host cell comprising the nucleic acid described herein~~is provided~~. ~~In some embodiments,~~Disclosed is a method of producing an antibody described herein ~~is provided~~. In another aspect, there is provided a method of producing an antibody comprising culturing the host cell described herein so that the antibody is produced.In some embodiments, the method comprises culturing the host cell comprising the nucleic acid that encodes an antibody.

[019] ~~In some embodiments,~~Disclosed are immunoconjugates ~~are provided~~. In another aspect, there is provided an immunoconjugate comprising the antibody described herein and a cytotoxic agent. In some embodiments, an immunoconjugate comprises an anti-B7-H4 antibody and a cytotoxic agent. In some embodiments, an immunoconjugate has the formula Ab-(L-D)p, wherein: (a) Ab is the an antibody described herein; (b) L is a linker; (c) D is a drug selected from a maytansinoid, an auristatin, a calicheamicin, a pyrrolobenzodiazepine, and a nemorubicin derivative; and (d) p ranges from 1-8. In some embodiments, D is an auristatin. In some such embodiments, D has formula D_E



D_E

wherein R^2 and R^6 are each methyl, R^3 and R^4 are each isopropyl, R^5 is H, R^7 is sec-butyl, each R^8 is independently selected from CH_3, O-CH_3, OH, and H; R^9 is H; and R^{18} is $-C(R^8)_2-C(R^8)_2$–aryl. In some embodiments, D is MMAE having the structure:

SEQ ID NO: 60; (l) (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 61, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 62, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 63.

[027a] In another aspect, there is provided a pharmaceutical formulation comprising the immunoconjugate described herein and a pharmaceutically acceptable carrier.

[028] In some embodiments, pharmaceutical formulations are provided. In some such embodiments, a pharmaceutical formulation comprises an immunoconjugate comprising an antibody that binds B7-H4, e.g., as described herein. In some embodiments, a pharmaceutical formulation further comprises an additional therapeutic agent. In some embodiments, the additional therapeutic agent is Avastin® (bevacizumab).

[28a] In another aspect, there is provided use of the immunoconjugate of any one of the embodiments described herein in the manufacture of a medicament for treating an individual having a B7-H4-positive cancer.

[029] Disclosed are~~In some embodiments,~~ methods of treating individuals having B7-H4 positive cancers~~ are provided~~. In some such embodiments, a method comprises administering a pharmaceutical formulation comprising an immunoconjugate comprising an antibody that binds B7-H4, e.g., as described herein. In some such embodiments, the method comprises administering a pharmaceutical formulation comprising an immunoconjugate comprising an antibody that bind to the extracellular domain of B7-H4. In some embodiments, the B7-H4-positive cancer is selected from breast cancer, ovarian cancer, and endometrial cancer. In some embodiments, a method comprises administering an additional therapeutic agent to the individual. In some such embodiments, the additional therapeutic agent is Avastin® (bevacizumab).

[030] Disclosed are~~In some embodiments,~~ methods of inhibiting proliferation of a B7-H4-positive cell~~ are provided~~. In another aspect, there is provided a method of inhibiting proliferation of an B7-H4-positive cell, the method comprising exposing the cell to the immunoconjugate described herein under conditions permissive for binding of the immunoconjugate to B7-H4 on the surface of the cell, thereby inhibiting proliferation of the cell. In some embodiments, the method comprising exposing the cell to an immunoconjugate comprising an antibody that binds B7-H4 under conditions permissive for binding of the immunoconjugate to B7-H4 on the surface of the cell. In some embodiments, an antibody that binds B7-H4 is an antibody described herein. In some embodiments, proliferation of the cell is thereby inhibited. In some embodiments, the cell is a breast, ovarian, or endometrial cancer cell.

[031] In some embodiments, an antibody that binds B7-H4 is conjugated to a label. In some embodiments, an antibody that binds B-H4 is an antibody described herein. In some embodiments, the label is a positron emitter. In some embodiments, the positron emitter is ^{89}Zr.

[032] Disclosed is~~In some embodiments,~~ a method of detecting human B7-H4 in a biological sample~~ is provided~~. In one aspect, there is provided a method of detecting human B7-H4 in a biological sample comprising contacting the biological sample with the anti-B7-H4 antibody described herein under conditions permissive for binding of the anti-B7-H4 antibody to a naturally occurring human B7-H4, and detecting whether a complex is formed between the anti-B7-H4 antibody and a naturally occurring human B7-H4 in the biological sample.In some embodiments, a method comprises contacting the biological sample with an anti-B7-H4 antibody under conditions permissive for binding of the anti-B7-H4 antibody to a naturally occurring human B7-H4, and detecting whether a complex is formed between the anti-B7-H4 antibody and a naturally occurring human B7-H4 in the biological sample. In some embodiments, an anti-B7-H4 antibody is an antibody described

herein. In some embodiments, the biological sample is a breast cancer sample, ovarian cancer sample, or endometrial cancer sample.

[033] Disclosed is~~In some embodiments,~~ a method for detecting an B7-H4-positive cancer~~is provided~~. In one aspect, there is provided a method for detecting an B7-H4-positive cancer comprising (i) administering a labeled anti-B7-H4 antibody to a subject having or suspected of having a B7-H4-positive cancer, wherein the labeled anti-B7-H4 antibody comprises the anti-B7-H4 antibody described herein, and (ii) detecting the labeled anti-B7-H4 antibody in the subject, wherein detection of the labeled anti-B7-H4 antibody indicates a B7-H4-positive cancer in the subject. In some such embodiments, a method comprises (i) administering a labeled anti-B7-H4 antibody to a subject having or suspected of having an B7-H4-positive cancer, and (ii) detecting the labeled anti-B7-H4 antibody in the subject, wherein detection of the labeled anti- B7-H4 antibody indicates a B7-H4-positive cancer in the subject. In some embodiments, an anti-B7-H4 antibody is an antibody described herein. In some such embodiments, the labeled anti-B7-H4 antibody comprises an anti-B7-H4 antibody conjugated to a positron emitter. In some embodiments, the positron emitter is ^{89}Zr.

BRIEF DESCRIPTION OF THE FIGURES

[034] **Figure 1** shows a graphic representation of the levels of human B7-H4 gene expression in various tissues, as described in Example A.

[035] **Figure 2** shows expression of B7-H4 in breast carcinoma samples by in situ hybridization, as described in Example B.

[036] **Figure 3** shows **(A)** the prevalence of B7-H4 expression in all breast cancer subtypes, **(B)** the prevalence of 0, 1+, 2+, and 3+ levels of B7-H4 staining in breast cancer subtypes, **(C)** the overall expression of B7-H4 in breast tumors (by Western analysis), **(D)** the expression of B7-H4 in primary breast tumors (by Western analysis), **(E)** the expression of B7-H4 in ovarian tumors, as described in Example B.

[037] **Figure 4 (A)** shows the properties of certain anti-B7-H4 monoclonal antibodies developed as described in the Examples. **(B)** shows the epitope grouping of the anti-B7-H4 monoclonal antibodies described herein.

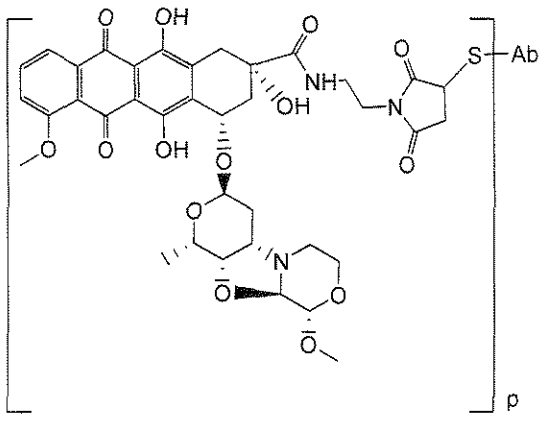
[038] **Figure 5** shows an alignment of the light chain and heavy chain variable regions sequences of murine antibodies 1D11, 32D6, 9B9 and 22C10.

[039] **Figure 6** shows an alignment of the light chain variable region sequences of murine antibody mu1D11 and humanized variants thereof.

[040] **Figure 7** shows an alignment of the heavy chain variable region sequences of murine antibody mu1D11 and humanized variants thereof.

[041] **Figure 8** shows the light chain variable region sequences of murine antibody mu22C10 and humanized variants thereof.

[042] **Figure 9** shows the heavy chain variable region sequences of murine antibody mu22C10 and humanized variants thereof.



36. The immunoconjugate of any one of claims 21 to 35, wherein p ranges from 2-5.

37. The immunoconjugate of any one of claims 21 to 36, comprising the antibody of claim 9.

38. The immunoconjugate of any one of claims 21 to 36, comprising the antibody of claim 15.

39. A pharmaceutical formulation comprising the immunoconjugate of any one of claims 21 to 38 and a pharmaceutically acceptable carrier.

40. The pharmaceutical formulation of claim 39, further comprising an additional therapeutic agent.

41. The pharmaceutical formulation of claim 40, wherein the additional therapeutic agent is Avastin® (bevacizumab).

42. Use of the immunoconjugate of any one of claims 21 to 38 in the manufacture of a medicament for ~~A method of~~ treating an individual having a B7-H4-positive cancer~~, the method comprising administering to the individual an effective amount of the immunoconjugate of any one of claims 21 to 38~~.

43. The use~~method~~ of claim 42, wherein the B7-H4-positive cancer is selected from breast cancer, ovarian cancer, and endometrial cancer.

44. The use~~method~~ of claim 43, further comprising administering an additional therapeutic agent to the individual.

45. The use~~method~~ of claim 44, wherein the additional therapeutic agent is Avastin® (bevacizumab).

46. A method of inhibiting proliferation of an B7-H4-positive cell, the method comprising exposing the cell to the immunoconjugate of any one of claims 21 to 38 under