

ANTIBODY MOLECULES THAT BIND TO NKP30 AND USES THEREOF

RELATED APPLICATION

This application claims priority to U.S. Serial No. 62/808,582 filed February 21, 2019, the contents of which are incorporated herein by reference in their entireties.

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on February 20, 2020, is named E2070-7025WO_SL.txt and is 517,567 bytes in size.

BACKGROUND

Natural Killer (NK) cells recognize and destroy tumors and virus-infected cells in an antibody-independent manner. The regulation of NK cells is mediated by activating and inhibiting receptors on the NK cell surface. One family of activating receptors is the natural cytotoxicity receptors (NCRs) which include NKp30, NKp44 and NKp46.

Given the importance of immune checkpoint pathways in regulating an immune response, the need exists for developing novel agents that modulate the activity of immunoinhibitory proteins, such as PD-1, thus leading to activation of the immune system. Such agents can be used, *e.g.*, for cancer immunotherapy and treatment of other conditions, such as chronic infection.

SUMMARY

Disclosed herein are antibody molecules (*e.g.*, humanized antibody molecules) that bind to NKp30 with high affinity and specificity. Nucleic acid molecules encoding the antibody molecules, expression vectors, host cells and methods for making the antibody molecules are also provided. Multi- or bispecific or multifunctional antibody molecules and pharmaceutical compositions comprising the antibody molecules are also provided. The anti- NKp30 antibody molecules disclosed herein can be used (alone or in combination with other agents or therapeutic modalities) to treat, prevent and/or diagnose disorders, such as cancerous disorders (*e.g.*, solid

and soft-tissue tumors), as well as autoimmune and infectious diseases. Thus, compositions and methods for detecting NKp30, as well as methods for treating various disorders including cancer, autoimmune and/or infectious diseases, using the anti- NKp30 antibody molecules are disclosed herein.

Accordingly, in one example, the disclosure features an antibody molecule (*e.g.*, an isolated or recombinant antibody molecule), comprising one or more sequences according to the following enumerated examples. Additional features of any of the disclosed antibody molecules, multifunctional molecules, nucleic acids, vectors, host cells, or methods include one or more of the following enumerated examples.

In one aspect, the present disclosure refers to an antibody or an antigen-binding fragment thereof that binds to NKp30 comprising:

- (a) a heavy chain variable region (VH) comprising
 - (i) a heavy chain complementarity determining region 1 (VHCDR1) amino acid sequence of SEQ ID NO: 7313,
 - (ii) a VHCDR2 amino acid sequence of SEQ ID NO: 6001, and
 - (iii) a VHCDR3 amino acid sequence of SEQ ID NO: 7315; and/or
- (b) a light chain variable region (VL) comprising
 - (i) a light chain complementarity determining region 1 (VLCDR1) amino acid sequence of SEQ ID NO: 7326,
 - (ii) a VLCDR2 amino acid sequence of SEQ ID NO: 7327, and
 - (iii) a VLCDR3 amino acid sequence of SEQ ID NO: 7329.

In another aspect, the present disclosure refers to an antibody or an antigen-binding fragment thereof that binds to NKp30 comprising:

- (a) a heavy chain variable region (VH) comprising
 - (i) a heavy chain complementarity determining region 1 (VHCDR1) amino acid sequence of SEQ ID NO: 6000,
 - (ii) a VHCDR2 amino acid sequence of SEQ ID NO: 6001, and
 - (iii) a VHCDR3 amino acid sequence of SEQ ID NO: 6002; and/or
- (b) a light chain variable region (VL) comprising
 - (i) a light chain complementarity determining region 1 (VLCDR1) amino acid sequence of SEQ ID NO: 6063,

- (ii) a VLCDR2 amino acid sequence of SEQ ID NO: 6064, and
- (iii) a VLCDR3 amino acid sequence of SEQ ID NO: 7239.

In another aspect, the present disclosure refers to an antibody or an antigen-binding fragment thereof that binds to NKp30 comprising:

- (a) a heavy chain variable region (VH) comprising
 - (i) a heavy chain complementarity determining region 1 (VHCDR1) amino acid sequence of SEQ ID NO: 6007,
 - (ii) a VHCDR2 amino acid sequence of SEQ ID NO: 6008, and
 - (iii) a VHCDR3 amino acid sequence of SEQ ID NO: 6009; and/or
- (b) a light chain variable region (VL) comprising
 - (i) a light chain complementarity determining region 1 (VLCDR1) amino acid sequence of SEQ ID NO: 6070,
 - (ii) a VLCDR2 amino acid sequence of SEQ ID NO: 6071, and
 - (iii) a VLCDR3 amino acid sequence of SEQ ID NO: 6072.

In another aspect, the present disclosure refers to a multispecific molecule comprising the antibody or an antigen-binding fragment thereof as described herein.

In another aspect, the present disclosure refers to a polynucleotide comprising a sequence encoding the antibody or an antigen-binding fragment thereof as described herein or the multispecific molecule as described herein.

In another aspect, the present disclosure refers to an expression vector comprising a sequence encoding the antibody or an antigen-binding fragment thereof as described herein or the multispecific molecule as described herein.

In yet another aspect, the present disclosure refers to a host cell comprising the polynucleotide as described herein or the expression vector as described herein.

In yet another aspect, the present disclosure refers to a method of making the antibody or an antigen-binding fragment thereof as described herein or the multispecific molecule as described herein comprising culturing the host cell as described herein under suitable conditions for gene expression and/or homo- or heterodimerization.

In yet another aspect, the present disclosure refers to a medicament comprising the antibody or an antigen-binding fragment thereof as described herein or the multispecific molecule as described herein, and a pharmaceutically acceptable carrier, excipient, or stabilizer.

In yet another aspect, the present disclosure refers to a use of the pharmaceutical composition comprising the antibody or an antigen-binding fragment thereof as described herein or the multispecific molecule as described herein in the manufacture of a medicament for treating a disease or condition in a subject in need thereof,

optionally, wherein the disease or condition is cancer, an autoimmune or inflammatory disorder, or an infectious disorder.

In yet another aspect, the present disclosure refers to a pharmaceutical composition comprising the antibody or an antigen-binding fragment thereof as described herein or the multispecific molecule as described herein for use in therapy.

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific examples of the disclosure described herein. Such equivalents are intended to be encompassed by the following enumerated examples.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a graph showing binding of NKp30 antibodies to NK92 cells. Data was calculated as the percent-AF747 positive population.

FIG. 2 is a graph showing activation of NK92 cells by NKp30 antibodies. Data were generated using hamster anti-NKp30 mAbs.

Enumerated Embodiments

1. An isolated antibody molecule that binds to NKp30, comprising:
 - (i) a heavy chain variable region (VH) comprising a heavy chain complementarity determining region 1 (VHCDR1) amino acid sequence of SEQ ID NO: 7313 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions), a VHCDR2 amino acid sequence of SEQ ID NO: 6001 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions, and/or a VHCDR3 amino acid sequence of SEQ ID NO: 7315 (or a sequence with no more than 1, 2, 3, or 4 mutations, e.g., substitutions, additions, or deletions; and/or
 - (ii) a light chain variable region (VL) comprising a light chain complementarity determining region 1 (VLCDR1) amino acid sequence of SEQ ID NO: 7326 (or a sequence with

What is claimed is:

1. An antibody or an antigen-binding fragment thereof that binds to NKp30 comprising:
 - (a) a heavy chain variable region (VH) comprising
 - (i) a heavy chain complementarity determining region 1 (VHCDR1) amino acid sequence of SEQ ID NO: 7313,
 - (ii) a VHCDR2 amino acid sequence of SEQ ID NO: 6001, and
 - (iii) a VHCDR3 amino acid sequence of SEQ ID NO: 7315; and/or
 - (b) a light chain variable region (VL) comprising
 - (i) a light chain complementarity determining region 1 (VLCDR1) amino acid sequence of SEQ ID NO: 7326,
 - (ii) a VLCDR2 amino acid sequence of SEQ ID NO: 7327, and
 - (iii) a VLCDR3 amino acid sequence of SEQ ID NO: 7329.
2. The antibody or an antigen-binding fragment thereof of claim 1, wherein
 - (a) the VH comprises an amino acid sequence with at least 75% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 7298 and 7300-7304; and
 - (b) the VL comprising an amino acid sequence with at least 85% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 7299 or 7305-7309.
3. The antibody or an antigen-binding fragment thereof of claim 1 or 2, wherein
 - (a) the VH comprises an amino acid sequence with at least 75% sequence identity to SEQ ID NO: 7302; and
 - (b) the VL comprises an amino acid sequence with at least 75% sequence identity to SEQ ID NO: 7305.
4. The antibody or an antigen-binding fragment thereof of any one of claims 1-3, wherein
 - (a) the VH comprises an amino acid sequence with at least 75% sequence identity to SEQ ID NO: 7302; and

(b) the VL comprises an amino acid sequence with at least 75% sequence identity to SEQ ID NOs: 7305 or SEQ ID NO: 7309.

5. The antibody or an antigen-binding fragment thereof of any one of claims 1-4, wherein the antibody or an antigen-binding fragment thereof comprises an amino acid sequence with at least 75% sequence identity to SEQ ID NO: 7310 or SEQ ID NO: 7311.

6. An antibody or an antigen-binding fragment thereof that binds to NKp30 comprising:

(a) a heavy chain variable region (VH) comprising

(i) a heavy chain complementarity determining region 1 (VHCDR1) amino acid sequence of SEQ ID NO: 6000,

(ii) a VHCDR2 amino acid sequence of SEQ ID NO: 6001, and

(iii) a VHCDR3 amino acid sequence of SEQ ID NO: 6002; and/or

(b) a light chain variable region (VL) comprising

(i) a light chain complementarity determining region 1 (VLCDR1) amino acid sequence of SEQ ID NO: 6063,

(ii) a VLCDR2 amino acid sequence of SEQ ID NO: 6064, and

(iii) a VLCDR3 amino acid sequence of SEQ ID NO: 7239.

7. The antibody or an antigen-binding fragment thereof of claim 6, wherein

(a) the VH comprises an amino acid sequence with at least 75% sequence identity to SEQ ID NO: 6121; and/or

(b) the VL comprises an amino acid sequence with at least 85% sequence identity to SEQ ID NO: 7294.

8. The antibody or an antigen-binding fragment thereof of claim 6 or 7, wherein

(a) the VH comprises:

(i) a heavy chain framework region 1 (VHFWR1) amino acid sequence with at least 90% sequence identity to SEQ ID NO: 6003,

(ii) a VHFWR2 amino acid sequence with at least 90% sequence identity to SEQ ID NO: 6004,

(iii) a VHFWR3 amino acid sequence with at least 90% sequence identity to SEQ ID NO: 6005,

- (iv) a VHFWR4 amino acid sequence with at least 90% sequence identity to SEQ ID NO: 6006; and
 - (b) the VL comprises
 - (i) a light chain framework region 1 (VLFWR1) amino acid sequence with at least 90% sequence identity to SEQ ID NO: 6066,
 - (ii) a VLFWR2 amino acid sequence with at least 90% sequence identity to SEQ ID NO: 6067,
 - (iii) a VLFWR2 amino acid sequence with at least 90% sequence identity to SEQ ID NO: 7292, and
 - (iv) a VLFWR2 amino acid sequence with at least 90% sequence identity to SEQ ID NO: 6069.
9. An antibody or an antigen-binding fragment thereof that binds to NKp30 comprising:
- (a) a heavy chain variable region (VH) comprising
 - (i) a heavy chain complementarity determining region 1 (VHCDR1) amino acid sequence of SEQ ID NO: 6007,
 - (ii) a VHCDR2 amino acid sequence of SEQ ID NO: 6008, and
 - (iii) a VHCDR3 amino acid sequence of SEQ ID NO: 6009; and/or
 - (b) a light chain variable region (VL) comprising
 - (i) a light chain complementarity determining region 1 (VLCDR1) amino acid sequence of SEQ ID NO: 6070,
 - (ii) a VLCDR2 amino acid sequence of SEQ ID NO: 6071, and
 - (iii) a VLCDR3 amino acid sequence of SEQ ID NO: 6072.
10. The antibody or an antigen-binding fragment thereof of claim 9, wherein
- (a) the VH comprises an amino acid sequence with at least 75% sequence identity to SEQ ID NO: 6122; and
 - (b) the VL comprises an amino acid sequence with at least 85% sequence identity to SEQ ID NOs: 7305 or SEQ ID NO: 6136.
11. The antibody or an antigen-binding fragment thereof of claim 9 or 10, wherein
- (a) the VH comprises:

- (i) a heavy chain framework region 1 (VHFWR1) amino acid sequence with at least 90% sequence identity to SEQ ID NO: 6010,
 - (ii) a VHFWR2 amino acid sequence with at least 90% sequence identity to SEQ ID NO: 6011,
 - (iii) a VHFWR3 amino acid sequence with at least 90% sequence identity to SEQ ID NO: 6012,
 - (iv) a VHFWR4 amino acid sequence with at least 90% sequence identity to SEQ ID NO: 6013; and
- (b) the VL comprises
- (i) a light chain framework region 1 (VLFWR1) amino acid sequence with at least 90% sequence identity to SEQ ID NO: 6073,
 - (ii) a VLFWR2 amino acid sequence with at least 90% sequence identity to SEQ ID NO: 6074,
 - (iii) a VLFWR2 amino acid sequence with at least 90% sequence identity to SEQ ID NO: 6075, and
 - (iv) a VLFWR2 amino acid sequence with at least 90% sequence identity to SEQ ID NO: 6076.

12. A multispecific molecule comprising the antibody or an antigen-binding fragment thereof of any one of claims 1-11.

13. The multispecific molecule of claim 12, wherein the multispecific molecule further comprises one, two, three, four or more of:

- (a) a tumor targeting moiety;
- (b) a cytokine molecule;
- (c) a T cell engager; and
- (d) a stromal modifying moiety.

14. The multispecific molecule of claim 12 or 13, wherein the multispecific molecule further comprises

- (a) a T cell engager that binds to an antigen present on the surface of an autoreactive T cell that is associated with an inflammatory or autoimmune disorder, or

(b) a binding moiety that binds to an antigen present on the surface of a cell infected by a virus or a bacteria.

15. The multispecific molecule of any one of claims 12-14, wherein the multispecific molecule comprises an immunoglobulin chain constant region.

16. The multispecific molecule of claim 15, wherein the immunoglobulin chain constant region is an IgG1 chain constant region that comprises an amino acid substitution at a position selected from one or more of 347, 349, 350, 351, 366, 368, 370, 392, 394, 395, 397, 398, 399, 405, 407, and 409.

17. The multispecific molecule of claim 15 or 16, wherein the multispecific molecule comprises an IgG1 chain constant region that comprises an amino acid substitution at a position selected from the group consisting of T366S, L368A, Y407V, T366W, and any combination thereof.

18. The multispecific molecule of any one of claims 13-17, wherein the multispecific molecule comprises a linker between one or more of:

- (a) the targeting moiety and the cytokine molecule or the stromal modifying moiety,
- (b) the targeting moiety and the immune cell engager, the cytokine molecule, or the stromal modifying moiety,
- (c) the immune cell engager, the cytokine molecule, or the stromal modifying moiety and the immunoglobulin chain constant region,
- (d) the targeting moiety and the immunoglobulin chain constant region, and
- (e) the immune cell engager and the immunoglobulin chain constant region.

19. A polynucleotide comprising a sequence encoding the antibody or an antigen-binding fragment thereof of any one of claims 1-11 or the multispecific molecule of any one of claims 12-18.

20. An expression vector comprising a sequence encoding the antibody or an antigen-binding fragment thereof of any one of claims 1-11 or the multispecific molecule of any one of claims 12-18.

21. A host cell comprising the polynucleotide of claim 19 or the expression vector of claim 20.

22. A method of making the antibody or an antigen-binding fragment thereof of any one of claims 1-11 or the multispecific molecule of any one of claims 12-18 comprising culturing the host cell of claim 21 under suitable conditions for gene expression and/or homo- or heterodimerization.

23. A medicament comprising the antibody or an antigen-binding fragment thereof of any one of claims 1-11 or the multispecific molecule of any one of claims 12-18, and a pharmaceutically acceptable carrier, excipient, or stabilizer.

24. Use of the pharmaceutical composition comprising the antibody or an antigen-binding fragment thereof of any one of claims 1-11 or the multispecific molecule of any one of claims 12-18 in the manufacture of a medicament for treating a disease or condition in a subject in need thereof,

optionally, wherein the disease or condition is cancer, an autoimmune or inflammatory disorder, or an infectious disorder.

25. The use of claim 24, wherein the disease or condition is cancer, and the cancer is a solid tumor cancer, a metastatic lesion, or a hematological cancer,

optionally, wherein the solid tumor cancer is one or more of pancreatic, breast, colorectal, lung, skin, ovarian, and liver cancer.

26. A pharmaceutical composition comprising the antibody or an antigen-binding fragment thereof of any one of claims 1-11 or the multispecific molecule of any one of claims 12-18 for use in therapy.