

CLAIMS

1. A multispecific antibody, or variant thereof that maintains the binding specificity of the antibody, wherein the antibody or variant thereof comprises a binding domain that specifically binds to an extracellular domain of LAG-3 and a binding domain that specifically binds to an extracellular domain of a protein of the B7 family,

wherein the LAG-3 binding domain comprises a heavy chain variable region comprising:

a) heavy chain CDR1 (HCDR1), heavy chain CDR2 (HCDR2), and heavy chain CDR3 (HCDR3), of a heavy chain variable region having an amino acid sequence as set forth in SEQ ID NO: 1;

b) heavy chain CDR1 (HCDR1), heavy chain CDR2 (HCDR2), and heavy chain CDR3 (HCDR3), of a heavy chain variable region having an amino acid sequence as set forth in SEQ ID NO: 2;

c) heavy chain CDR1 (HCDR1), heavy chain CDR2 (HCDR2), and heavy chain CDR3 (HCDR3), of a heavy chain variable region having an amino acid sequence as set forth in SEQ ID NO: 3;

d) heavy chain CDR1 (HCDR1), heavy chain CDR2 (HCDR2), and heavy chain CDR3 (HCDR3), of a heavy chain variable region having an amino acid sequence as set forth in SEQ ID NO: 4;

e) heavy chain CDR1 (HCDR1), heavy chain CDR2 (HCDR2), and heavy chain CDR3 (HCDR3), of a heavy chain variable region having an amino acid sequence as set forth in SEQ ID NO: 5;

f) heavy chain CDR1 (HCDR1), heavy chain CDR2 (HCDR2), and heavy chain CDR3 (HCDR3), of a heavy chain variable region having an amino acid sequence as set forth in SEQ ID NO: 6;

g) heavy chain CDR1 (HCDR1), heavy chain CDR2 (HCDR2), and heavy chain CDR3 (HCDR3), of a heavy chain variable region having an amino acid sequence as set forth in SEQ ID NO: 7;

h) heavy chain CDR1 (HCDR1), heavy chain CDR2 (HCDR2), and heavy chain CDR3 (HCDR3), of a heavy chain variable region having an amino acid sequence as set forth in SEQ ID NO: 8;

i) heavy chain CDR1 (HCDR1), heavy chain CDR2 (HCDR2), and heavy chain CDR3 (HCDR3), of a heavy chain variable region having an amino acid sequence as set forth in SEQ ID NO: 9;

j) heavy chain CDR1 (HCDR1), heavy chain CDR2 (HCDR2), and heavy chain CDR3 (HCDR3), of a heavy chain variable region having an amino acid sequence as set forth in SEQ ID NO: 10;

k) heavy chain CDR1 (HCDR1), heavy chain CDR2 (HCDR2), and heavy chain CDR3 (HCDR3), of a heavy chain variable region having an amino acid sequence as set forth in SEQ ID NO: 11;

l) heavy chain CDR1 (HCDR1), heavy chain CDR2 (HCDR2), and heavy chain CDR3 (HCDR3), of a heavy chain variable region having an amino acid sequence as set forth in SEQ ID NO: 12;

m) heavy chain CDR1 (HCDR1), heavy chain CDR2 (HCDR2), and heavy chain CDR3 (HCDR3), of a heavy chain variable region having an amino acid sequence as set forth in SEQ ID NO: 13;

n) heavy chain CDR1 (HCDR1), heavy chain CDR2 (HCDR2), and heavy chain CDR3 (HCDR3), of a heavy chain variable region having an amino acid sequence as set forth in SEQ ID NO: 14;

o) heavy chain CDR1 (HCDR1), heavy chain CDR2 (HCDR2), and heavy chain CDR3 (HCDR3), of a heavy chain variable region having an amino acid sequence as set forth in SEQ ID NO: 15;

p) heavy chain CDR1 (HCDR1), heavy chain CDR2 (HCDR2), and heavy chain CDR3 (HCDR3), of a heavy chain variable region having an amino acid sequence as set forth in SEQ ID NO: 16;

q) heavy chain CDR1 (HCDR1), heavy chain CDR2 (HCDR2), and heavy chain CDR3 (HCDR3), of a heavy chain variable region having an amino acid sequence as set forth in SEQ ID NO: 17,

r) heavy chain CDR1 (HCDR1), heavy chain CDR2 (HCDR2), and heavy chain CDR3 (HCDR3), of a heavy chain variable region having an amino acid sequence as set forth in SEQ ID NO: 52,

s) heavy chain CDR1 (HCDR1), heavy chain CDR2 (HCDR2), and heavy chain CDR3 (HCDR3), of a heavy chain variable region having an amino acid sequence as set forth in SEQ ID NO: 53,

t) heavy chain CDR1 (HCDR1), heavy chain CDR2 (HCDR2), and heavy chain CDR3 (HCDR3), of a heavy chain variable region having an amino acid sequence as set forth in SEQ ID NO: 54,

u) heavy chain CDR1 (HCDR1), heavy chain CDR2 (HCDR2), and heavy chain CDR3 (HCDR3), of a heavy chain variable region having an amino acid sequence as set forth in SEQ ID NO: 55,

v) heavy chain CDR1 (HCDR1), heavy chain CDR2 (HCDR2), and heavy chain CDR3 (HCDR3), of a heavy chain variable region having an amino acid sequence as set forth in SEQ ID NO: 56,

w) heavy chain CDR1 (HCDR1), heavy chain CDR2 (HCDR2), and heavy chain CDR3 (HCDR3), of a heavy chain variable region having an amino acid sequence as set forth in SEQ ID NO: 57,

x) heavy chain CDR1 (HCDR1), heavy chain CDR2 (HCDR2), and heavy chain CDR3 (HCDR3), of a heavy chain variable region having an amino acid sequence as set forth in SEQ ID NO: 58, or

y) heavy chain CDR1 (HCDR1), heavy chain CDR2 (HCDR2), and heavy chain CDR3 (HCDR3), of a heavy chain variable region having an amino acid sequence as set forth in SEQ ID NO: 59,

wherein each of the HCDRs may comprise at most three, two, or one amino acid substitution.

2. The multispecific antibody or variant thereof according to claim 1, wherein the LAG-3 binding domain comprises a heavy chain variable region having an amino acid sequence as set forth in any one of SEQ ID NO: 1-17 or SEQ ID NO: 52-59, or having at least 80%, preferably 85%, more preferably 90%, or most preferably 95% sequence identity thereto.

3. The multispecific antibody or variant thereof according to claim 1 or 2, wherein the LAG-3 binding domain further comprises a light chain variable region, in particular wherein the LAG-3 binding domain further comprises a CH1 and CL region.

4. The multispecific antibody or variant thereof according to any one of claims 1-3, wherein the antibody or variant is monovalent for binding to LAG-3.

5. The multispecific antibody or variant thereof according to any one of claims 1-4, wherein the protein of the B7 family is selected from the group consisting of PD-L1, PD-L2, CD80, CD86, B7-H4, TNFRSF14, and B7-H7, in particular wherein the protein of the B7 family is PD-L1.

6. The multispecific antibody or variant thereof according to any one of claims 1-5, wherein the variable domain that specifically binds to an extracellular domain of a protein of the B7 family blocks the binding of PD-L1 to its receptor, in particular wherein the receptor is PD-1 and/or CD80.

7. The multispecific antibody or variant thereof according to any one of claims 1-6, wherein the binding domain that binds to PD-L1 comprises a heavy chain variable region comprising:

a) heavy chain CDR1 (HCDR1), heavy chain CDR2 (HCDR2), and heavy chain CDR3 (HCDR3), of a heavy chain variable region having an amino acid sequence as set forth in SEQ ID NO: 20;

b) heavy chain CDR1 (HCDR1), heavy chain CDR2 (HCDR2), and heavy chain CDR3 (HCDR3), of a heavy chain variable region having an amino acid sequence as set forth in SEQ ID NO: 21;

c) heavy chain CDR1 (HCDR1), heavy chain CDR2 (HCDR2), and heavy chain CDR3 (HCDR3), of a heavy chain variable region having an amino acid sequence as set forth in SEQ ID NO: 22;

d) heavy chain CDR1 (HCDR1), heavy chain CDR2 (HCDR2), and heavy chain CDR3 (HCDR3), of a heavy chain variable region having an amino acid sequence as set forth in SEQ ID NO: 23; or

e) heavy chain CDR1 (HCDR1), heavy chain CDR2 (HCDR2), and heavy chain CDR3 (HCDR3), of a heavy chain variable region having an amino acid sequence as set forth in SEQ ID NO: 24,

wherein each of the HCDRs may comprise at most three, two, or one amino acid substitution.

8. The multispecific antibody or variant thereof according to any one of claims 1-7, wherein the PD-L1 binding domain comprises a heavy chain variable region having an amino acid sequence as set forth in any one of SEQ ID NO: 20-24, or having at least 80%, preferably 85%, more preferably 90%, or most preferably 95% sequence identity thereto.

9. The multispecific antibody or variant thereof according to any one of claims 1-8, wherein the PD-L1 binding domain further comprises a light chain variable region, in particular wherein the PD-L1 binding domain further comprises a CH1 and CL region.

10. The multispecific antibody or variant thereof according to any one of claims 1-9, wherein the antibody or variant is monovalent for binding to PD-L1.

11. The multispecific antibody or variant thereof according to any one of claims 1-10, wherein the antibody or variant has comparable, or equal or higher, potency than a combination of reference antibodies, wherein the combination of reference antibodies comprises a reference antibody comprising two heavy chain variable regions having an amino acid sequence as set forth in SEQ ID NO: 27 and two light chain variable regions having an amino acid sequence as set forth in SEQ ID NO: 28, and a reference antibody comprising two heavy chain variable regions having an amino acid sequence as set forth in SEQ ID NO: 25 and two light chain variable regions having an amino acid sequence as set forth in SEQ ID NO: 26.

12. The multispecific antibody or variant thereof according to claim 11, wherein the potency is determined by measuring tumor volume reduction in an *in vivo* mouse study.

13. The multispecific antibody or variant thereof according to claim 11 or 12, wherein a comparable potency includes a 5 to 2 fold, preferably a 3 to 2 fold, deviation from the tumor volume reduction of the combination of reference antibodies.

14. The multispecific antibody or variant thereof according to claim 11, wherein the potency is determined by the potency in blocking ligand or receptor binding to LAG-3 and/or PD-L1, in particular wherein the potency in blocking ligand binding to LAG-3 and/or PD-L1 is measured in a PD-1/LAG-3 reporter assay.

15. The multispecific antibody or variant thereof according to claim 14, wherein a comparable potency is a potency in blocking ligand or receptor binding to LAG-3 and/or PD-L1, within a 5 fold range of the potency in blocking ligand or receptor binding to LAG-3 and/or PD-L1 of the combination of reference antibodies, and includes a 5 to 2 fold, preferably 3 to 2 fold, deviation from the LAG-3 and PD-L1 blocking activity of the combination of reference antibodies.

16. The multispecific antibody or variant thereof according to any one of claims 1-15, wherein the antibody or variant is a full length antibody, in particular a full length bispecific antibody.

17. A pharmaceutical composition comprising the multispecific antibody or variant thereof according to any one of claims 1-16 and a pharmaceutical acceptable carrier, diluent or excipient.

18. The multispecific antibody or variant thereof according to any one of claims 1-16, or pharmaceutical composition according to claim 17, for use as a medicament.

19. The multispecific antibody or variant thereof according to any one of claims 1-16, or the pharmaceutical composition as claimed in claim 17, for use in the treatment of a disease associated with a suppressed immune system.

20. The multispecific antibody or variant thereof according to any one of claims 1-16, or the pharmaceutical composition as claimed in claim 17, for use in the treatment of cancer.

21. Use of an effective amount of the multispecific antibody or variant thereof as claimed in any one of claims 1-16, or the pharmaceutical composition as claimed in claim 17 in the manufacture of a medicament for treating a disease.

22. Use of an effective amount of the multispecific antibody or variant thereof as claimed in any one of claims 1-16, or the pharmaceutical composition as claimed in claim 17 in the manufacture of a medicament for treating a disease associated with a suppressed immune system, in particular cancer.

23. Use of an effective amount of the multispecific antibody or variant thereof as claimed in any one of claims 1-16, or the pharmaceutical composition as claimed in claim 17 for treating cancer.

24. A vector comprising a nucleic acid sequence encoding the heavy chain variable region of a LAG-3 binding domain as defined in any one of claims 1-4 and a nucleic acid sequence encoding the heavy chain variable region of a PD-L1 binding domain as defined in any one of claims 7-9.

25. The vector according to claim 24, wherein the vector further comprises a nucleic acid sequence encoding a CH1 region and preferably a hinge, CH2 and CH3 region.

26. The vector according to claim 24 or 25, further comprising at least one nucleic acid sequence encoding a light chain variable region, and preferably a CL region.

27. The vector according to claim 26, wherein the light chain variable region is a light chain variable region of a light chain that is capable of pairing with multiple heavy chains having different epitope specificities.

28. A cell comprising a nucleic acid sequence encoding the heavy chain variable region of a LAG-3 binding domain as defined in any one of claims 1-4 and a nucleic acid sequence encoding the heavy chain variable region of a PD-L1 binding domain as defined in any one of claims 7-9.

29. The cell according to claim 28, wherein the cell further comprises a nucleic acid sequence encoding a CH1 region and preferably a hinge, CH2 and CH3 region.

30. The cell according to claim 28 or 29, further comprising at least one nucleic acid sequence encoding a light chain variable region, and preferably a CL region.

31. The cell according to claim 30, wherein the light chain variable region is a light chain variable region of a light chain that is capable of pairing with multiple heavy chains having different epitope specificities.

32. A cell producing a multispecific antibody or variant thereof according to any one of claims 1-16, in particular wherein the cell is a recombinant cell, which has been transformed with a vector as claimed in any one of claims 24-27.