

Claims

1. An antibody, or a variant of said antibody that maintains the binding specificity of the antibody that comprises:

5 a variable domain that can bind to an extracellular part of a PD-1 and a variable domain that can bind to an extracellular part of a PD-L1 ;

wherein the variable domain that can bind to an extracellular part of a PD-1 comprises a heavy chain variable region that comprises the amino acid sequence of the CDR1, CDR2 and CDR3 of MF7686 as depicted in figure 13 and the variable domain
10 that can bind to an extracellular part of a PD-L1 comprises a heavy chain variable region that comprises the amino acid sequence of the CDR1, CDR2 and CDR3 of MF7703 as depicted in figure 13, or

wherein the variable domain that can bind to an extracellular part of a PD-1 comprises a heavy chain variable region that comprises the amino acid sequence of the
15 CDR1, CDR2 and CDR3 of MF6974 as depicted in figure 3 and/or figure 13 and the variable domain that can bind to an extracellular part of a PD-L1 comprises a heavy chain variable region that comprises the amino acid sequence of the CDR1, CDR2 and CDR3 of MF7689 as depicted in figure 13; and

wherein the variable domains that bind PD-1 and PD-L1 comprise a light chain comprising a light chain variable region CDR1 comprising sequence QSISSY, CDR2 comprising sequence AAS and CDR3 comprising sequence QQSYSTPPT;

wherein PD-1 and PD-L1 are binding partners (i.e. a ligand and receptor pair), and wherein the binding of the variable domain that can bind to an extracellular part of PD-1 blocks the binding of PD-1 to PD-L1 and/or the binding of the variable domain that
25 can bind to an extracellular part of PD-L1 blocks the binding of PD-1 to PD-L1.

2. The antibody or variant thereof of claim 1, wherein the binding of the antibody or variant thereof reduces an inhibitory activity of the binding of PD-1 and PD-L1.

30 3. The antibody or variant thereof of claim 1 or 2, wherein the binding of the variable domain that binds PD-1 blocks the binding of PD-1 to PD-L1 and the binding of the variable domain that binds PD-L1 blocks the binding of PD-L1 to PD-1.

4. The antibody or variant thereof of any one of claims 1-3, wherein the binding of the variable domain that binds to an extracellular part of PD-1 blocks the binding of PD-1 to PD-L2.

5. The antibody or variant thereof of any one of claims 1-4, wherein the binding of the variable domain that binds to an extracellular part of PD-L1 blocks the binding of PD-L1 to CD80.

6. The antibody or variant thereof of any one of claims 1-5, wherein the antibody is able to activate T cells in an antigen-specific CD4⁺ T cell assay more strongly than benchmark antibody 5C4 or benchmark antibody YW243.55.S70 or a combination of benchmark antibodies 5C4 and YW243.55.S70.

7. The antibody or variant thereof of any one of claims 1-6, wherein the antibody has a stronger CD4⁺ T cell activation potential in a mixed lymphocyte reaction (MLR) assay as compared to benchmark antibody 5C4 or benchmark antibody YW243.55.S70.

8. The antibody or variant thereof of any one of claims 1-7, that comprises a variable domain that has a binding affinity for an extracellular part of PD-L1 with an equilibrium dissociation constant (K_D) of lower than or equal to 4.27 nM, preferably of lower than or equal to 1.31 nM, preferably of lower than or equal to 1.27 nM, as measured by SPR.

9. The antibody or variant thereof of any one of claims 1-8, wherein the antibody is capable of enhancing the proliferation of CD4⁺ and/or CD8⁺ tumor-infiltrating T cells.

10. The antibody or variant thereof of any one of claims 1-9, wherein the antibody is capable of inducing a stronger T cell mediated anti-tumor response *in vivo* as compared to a combination of benchmark antibodies MK-3475 and YW243.55.S70.

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11. The antibody or variant thereof of any one of claims 1-10, wherein the variable domain that binds an extracellular part of PD-1 is defined as a variable domain that when in a bivalent monospecific antibody format that comprises two of said variable domains that bind PD-1, inhibits PD-1/PD-L1 mediated inhibition of T cell receptor

mediated activation of a Jurkat cell in a range of 20-150% when compared to the inhibition obtained with the antibody Nivolumab on a Jurkat cell.

12. The antibody or variant thereof of any one of claims 1-11, wherein the variable domain that binds an extracellular part of PD-L1 is defined as a variable domain that when in a bispecific antibody that has a second variable domain that binds an irrelevant antigen such as Tetanus Toxoid, provides the bispecific antibody with a K_d of 0.1-14 nM for PD-L1 binding (as measured by biacore).

13. The antibody or variant thereof of claim 1, wherein the variable domain that can bind to an extracellular part of PD-1 comprises a heavy chain variable region that comprises the amino acid sequence of the heavy chain variable region of MF7686 (as depicted in figure 13) having at most 15, preferably 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 and preferably having 0, 1, 2, 3, 4 or 5 amino acid insertions, deletions, substitutions or a combination thereof with respect to the amino acid sequence of the VH of MF7686, wherein said insertions, deletions, or substitutions or a combination thereof are not in the CDR1, CDR2 or CDR3 of the heavy chain variable region.

14. The antibody or variant thereof of claim 1, wherein the variable domain that can bind to an extracellular part of PD-1 comprises a heavy chain variable region that comprises the amino acid sequence of the heavy chain variable region of MF7686.

15. The antibody or variant thereof of claim 1, wherein the variable domain that can bind to an extracellular part of PD-1 comprises a heavy chain variable region that comprises the amino acid sequence of the heavy chain variable region as depicted for MF6974 (as depicted in figure 3 and/or figure 13) having at most 15, preferably 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 and preferably having 0, 1, 2, 3, 4 or 5 amino acid insertions, deletions, substitutions or a combination thereof with respect to the amino acid sequence of the VH of MF6974, wherein said insertions, deletions, or substitutions or a combination thereof are not in the CDR1, CDR2 or CDR3 of the heavy chain variable region.

16. The antibody or variant thereof of claim 1, wherein the variable domain that can bind to an extracellular part of PD-1 comprises a heavy chain variable region that

comprises the amino acid sequence of the heavy chain variable region as depicted for MF6974.

17. The antibody or variant thereof of claim 13, wherein the variable domain that
5 can bind to an extracellular part of PD-L1 comprises a heavy chain variable region that
comprises the amino acid sequence of the heavy chain variable region of MF7703 (as
depicted in figure 13) having at most 15, preferably 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 and
preferably having 0, 1, 2, 3, 4 or 5 amino acid insertions, deletions, substitutions or a
combination thereof with respect to the amino acid sequence of MF7703, wherein said
10 insertions, deletions, or substitutions or a combination thereof are not in the CDR1,
CDR2 or CDR3 of the variable heavy chain region.

18. The antibody or variant thereof of claim 14, wherein the variable domain that
can bind to an extracellular part of PD-L1 comprises a heavy chain variable region that
15 comprises the amino acid sequence of the heavy chain variable region as depicted for
MF7703.

19. The antibody or variant thereof of claim 15, wherein the variable domain that
can bind to an extracellular part of PD-L1 comprises a heavy chain variable region that
20 comprises the amino acid sequence of the heavy chain variable region of MF7689 (as
depicted in figure 13) having at most 15, preferably 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 and
preferably having 0, 1, 2, 3, 4 or 5 amino acid insertions, deletions, substitutions or a
combination thereof with respect to the amino acid sequence of MF7689, wherein said
insertions, deletions, or substitutions or a combination thereof are not in the CDR1,
25 CDR2 or CDR3 of the variable heavy chain region.

20. The antibody or variant thereof of claim 16, wherein the variable domain that
can bind to an extracellular part of PD-L1 comprises a heavy chain variable region that
comprises the amino acid sequence of the heavy chain variable region of MF7689.

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21. The antibody or variant thereof of claim 1, wherein
said variable domain that can bind to an extracellular part of PD-L1 comprises a
heavy chain variable region that comprises the amino acid sequence of MF7689 having
at most 15, preferably 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 and preferably having 0, 1, 2, 3, 4 or
35 5 amino acid insertions, deletions, substitutions or a combination thereof with respect to

the amino acid sequence of MF7689, wherein said insertions, deletions, or substitutions or a combination thereof are not in the CDR1, CDR2 or CDR3 of the variable heavy chain region;

wherein the variable domain that can bind to an extracellular part of PD-1 comprises a heavy chain variable region that comprises the amino acid sequence of MF6974 having at most 15, preferably 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 and preferably having 0, 1, 2, 3, 4 or 5 amino acid insertions, deletions, substitutions or a combination thereof with respect to the amino acid sequence of MF6974, wherein said insertions, deletions, or substitutions or a combination thereof are not in the CDR1, CDR2 or CDR3 of the variable heavy chain region, and

wherein the antibody or variant thereof comprises a light chain variable region CDR1 comprising sequence QSISSY, CDR2 comprising sequence AAS and CDR3 comprising sequence QQSYSTPPT.

22. The antibody or variant thereof of claim 1, wherein

said variable domain that can bind to an extracellular part of PD-L1 comprises a heavy chain variable region that comprises the amino acid sequence of MF7703 having at most 15, preferably 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 and preferably having 0, 1, 2, 3, 4 or 5 amino acid insertions, deletions, substitutions or a combination thereof with respect to the amino acid sequence of MF7703, wherein said insertions, deletions, or substitutions or a combination thereof are not in the CDR1, CDR2 or CDR3 of the variable heavy chain region;

wherein the variable domain that can bind to an extracellular part of PD-1 comprises a heavy chain variable region that comprises the amino acid sequence of MF7686 having at most 15, preferably 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 and preferably having 0, 1, 2, 3, 4 or 5 amino acid insertions, deletions, substitutions or a combination thereof with respect to the amino acid sequence of MF7686, wherein said insertions, deletions, or substitutions or a combination thereof are not in the CDR1, CDR2 or CDR3 of the variable heavy chain region, and

wherein the antibody or variant thereof comprises a light chain variable region CDR1 comprising sequence QSISSY, CDR2 comprising sequence AAS and CDR3 comprising sequence QQSYSTPPT.

23. The antibody or variant thereof of any one of claims 13-22 which comprises a light chain variable region having a sequence that is at least 80% identical to the amino acid sequence as depicted in Figure 1B, comprising light chain variable region CDR1 comprising sequence QSISSY, CDR2 comprising sequence AAS and CDR3 comprising sequence QQSYSTPPT.

24. The antibody or variant thereof of any one of claims 13-23 which comprises a light chain variable region having a sequence that is identical to the amino acid sequence as depicted in Figure 1B.

25. The antibody or variant thereof of any one of claims 1-24, wherein the antigen binding sites of said antibody or variant thereof consist of one immunoglobulin variable domain that can bind an extracellular part of PD-1 and one immunoglobulin variable domain that can bind an extracellular part of PD-L1.

26. The antibody of any one of claims 1-25, wherein said antibody is a full length bispecific antibody.

27. The antibody of any one of claims 1-26, wherein said antibody is an IgG.

28. A method of inhibiting a biological activity in a first or second cell mediated by the binding of PD-1 on a first cell to PD-L1 on a second cell, wherein PD-1 and PD-L1 are binding partners (i.e. a ligand and receptor pair), the method comprising

- providing a system comprising said first and second cell with an antibody or a variant of said antibody that maintains the binding specificity of the antibody comprising a variable domain that can bind to an extracellular part of PD-1 and a variable domain that can bind to an extracellular part of PD-L1; and

- incubating said system under conditions that are permissive for the first or second cell to express said biological activity mediated by the binding of PD-1 to PD-L1 in the absence of said antibody or variant thereof;

- wherein the binding of the variable domain that can bind to an extracellular part of PD-1 blocks the binding of PD-1 to PD-L1 and/or the binding of the variable domain that can bind to an extracellular part of PD-L1 blocks the binding of PD-1 to PD-L1,

wherein said antibody or variant thereof is an antibody or variant thereof of any one of claims 1-27.

29. The method of claim 28, wherein the binding of the antibody or variant thereof
5 reduces an inhibitory activity of the binding of PD-1 and PD-L1 in said first cell.

30. A method for producing an antibody or variant thereof of any one of claims 1-27 from a single cell, wherein said antibody or variant thereof comprises two CH3 domains that are capable of forming an interface, said method comprising providing:

10 - a cell having a) a first nucleic acid molecule encoding a IgG heavy chain that specifically recognizes an extracellular part of PD-1, and that contains a 1st CH3 domain, and b) a second nucleic acid sequence encoding a IgG heavy chain that specifically recognizes an extracellular part of PD-L1, and that contains a 2nd CH3 domain, wherein said nucleic acid sequences are provided with mutations for
15 preferential pairing of said 1st and 2nd CH3 domains, said method further comprising the step of culturing said cell and allowing for expression of said nucleic acid sequences and harvesting said antibody or variant thereof from the culture.

31. The method according to claim 30, wherein said cell has a third nucleic acid
20 sequence encoding a common light chain, preferably the rearranged germline human kappa light chain IgV κ 1-39*01/IGJ κ 1*01.

32. The method according to claim 30 or 31, wherein said first CH3 domain comprises the amino acid substitutions L351K and T366K (numbering according to the
25 EU numbering) and wherein said second CH3 domain comprises the amino acid substitutions L351D and L368E (numbering according to the EU numbering), said method further comprising the step of culturing said cell and allowing for expression of said nucleic acid sequences and harvesting said antibody or variant thereof from the culture.

33. An antibody or variant thereof of any one of claims 1-27 for use as a medicament.

34. An antibody or variant thereof of any one of claims 1-27 for use in a method for the treatment of cancer or an infection with a pathogen, preferably a virus or parasite.

35. A composition or kit of parts comprising at least one antibody or variant thereof according to any one of claims 1-27.

5 36. A pharmaceutical composition comprising at least one antibody or variant thereof of any one of claims 1-27 and a pharmaceutically acceptable carrier, diluent or excipient.

37. A nucleic acid molecule encoding an antibody or variant of any one of claims 1-27.

10 38. A vector comprising a nucleic acid molecule according to claim 37.

39. An isolated or recombinant cell, or a non-human animal, comprising a nucleic acid molecule according to claim 37 or a vector according to claim 38.

15 40. Use of an antibody or variant of any one of claims 1-27, a composition according to claim 35 or 36, a nucleic acid molecule according to claim 37, or a vector according to claim 38 in the manufacture of a medicament for treating a cancer or an infection with a pathogen.