Claims

1. An in vitro method of interfering with Programmed Cell Death 1 protein (PD-1) and T-cell immunoglobulin and mucin-domain containing-3 (TIM-3) mediated inhibition in a PD-1 and/or TIM-3 positive cell, the method comprising contacting said cell with an antibody or a functional part, derivative and/or analogue thereof that comprises:

- 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 TIM-3, thereby inhibiting PD-1 and/or TIM-3 mediated activity in said cell,

wherein said variable domain that can bind to an extracellular part of PD-1 comprises a heavy chain variable region with a CDR1, CDR2 and CDR3 region that comprises the amino acid sequence of the CDR1, CDR2 and CDR3 of a heavy chain variable region of one of the VH depicted for MF6076; MF6236; MF6256; MF6226; MF6930; MF6932; MF6935; MF6936; MF6972; MF6974; or MF6982, in Figure 3;

wherein said variable domain that can bind to an extracellular part of TIM-3 comprises a heavy chain variable region with a CDR1, CDR2 and CDR3 region that comprises the amino acid sequence of the CDR1, CDR2 and CDR3 of a heavy chain variable region of one of the VH depicted for MF7676; MF7677; MF7678; or MF7679, of Figure 3; and

wherein the variable domains that can bind to an extracellular part of PD-1 and TIM-3 comprise a light chain variable region comprising a light chain CDR1 comprising sequence QSISSY, CDR2 comprising sequence AAS and CDR3 comprising sequence QQSYSTPPT.

2. The method of claim 1, wherein the binding of said PD-1 binding variable domain to PD-1 blocks the binding of PD-1 to PD-L1 and/or PD-L2.

3. An in vitro method of stimulating the formation, stability and/or activity of an immunological synapse comprising providing a system that comprises at least two cells capable of associating with each other via an immunological synapse and providing said system with an antibody or a functional part, derivative and/or analogue thereof that comprises:

- 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 TIM-3,

thereby stimulating the formation, stability and/or activity of an immunological synapse between said at least two cells,

wherein said variable domain that can bind to an extracellular part of PD-1 comprises a heavy chain variable region with a CDR1, CDR2 and CDR3 region that comprises the amino acid sequence of the CDR1, CDR2 and CDR3 of a heavy chain variable region of one of the VH depicted for MF6076; MF6236; MF6256; MF6226; MF6930; MF6932; MF6935; MF6936; MF6972; MF6974; or MF6982, in Figure 3;

wherein said variable domain that can bind to an extracellular part of TIM-3 comprises a heavy chain variable region with a CDR1, CDR2 and CDR3 region that comprises the amino acid sequence of the CDR1, CDR2 and CDR3 of a heavy chain variable region of one of the VH depicted for MF7676; MF7677; MF7678; or MF7679, of Figure 3; and

wherein the variable domains that can bind to an extracellular part of PD-1 and TIM-3 comprise a light chain comprising a light chain CDR1 comprising sequence QSISSY, CDR2 comprising sequence AAS and CDR3 comprising sequence QQSYSTPPT.

4. An antibody or variant thereof that comprises 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 TIM-3,

wherein said variable domain that can bind to an extracellular part of PD-1 comprises a heavy chain variable region with a CDR1, CDR2 and CDR3 region that comprises the amino acid sequence of the CDR1, CDR2 and CDR3 of a heavy chain variable region of one of the VH depicted for MF6076; MF6236; MF6256; MF6226; MF6930; MF6932; MF6935; MF6936; MF6972; MF6974; or MF6982, in Figure 3;

wherein said variable domain that can bind to an extracellular part of TIM-3 comprises a heavy chain variable region with a CDR1, CDR2 and CDR3 region that comprises the amino acid sequence of the CDR1, CDR2 and CDR3 of a heavy chain variable region of one of the VH depicted for MF7676; MF7677; MF7678; or MF7679, of Figure 3; and

wherein the variable domains that can bind to an extracellular part of PD-1 and TIM-3 comprise a light chain comprising a light chain CDR1 comprising sequence QSISSY, CDR2 comprising sequence AAS and CDR3 comprising sequence QQSYSTPPT.

5. The antibody or variant thereof of claim 4,

wherein said variable domain that can bind to an extracellular part of PD-1 comprises a heavy chain variable region with a CDR1, CDR2 and CDR3 region that comprises the amino acid sequence of the CDR1, CDR2 and CDR3 of a heavy chain variable region of one of the VH depicted for MF6076; MF6256; MF6930; or MF6974, in Figure 3;

wherein said variable domain that can bind to an extracellular part of TIM-3 comprises a heavy chain variable region with a CDR1, CDR2 and CDR3 region that comprises the amino acid sequence of the CDR1, CDR2 and CDR3 of a heavy chain variable region of one of the VH depicted for MF7677; or MF7678, of Figure 3.

6. The antibody or variant thereof of claim 4 or 5, wherein the variable domain that binds PD-1 blocks the binding of PD-1 to PD-L1 and/or PD-L2.

7. The antibody or variant thereof of any one of claims 4 to 6, wherein the variable domain that binds TIM-3, blocks the binding of TIM-3 to Galectin-9.

8. The antibody or variant thereof of any one of claims 4 to 7, wherein the variable domain that binds an extracellular part of PD-1 is defined as a variable region that when in a bivalent monospecific antibody that comprises two of said variable domains that bind PD-1, inhibits PD-1/PD-L1 inhibition in a Jurkat cell in a range of 20-150% when compared to the inhibition obtained with the antibody Nivolumab on a Jurkat cell.

9. The antibody or variant thereof of any one of claims 4 to 8, 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 MF6076; MF6236; MF6256; MF6226; MF6930; MF6932; MF6935; MF6936; MF6972; MF6974; or MF6982 (as depicted in Figure 3) 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 the MF.

10. The antibody or variant thereof of any one of claims 4 to 9, 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 MF6076; MF6236; MF6256; MF6226; MF6930; MF6932; MF6935; MF6936; MF6972; MF6974; or MF6982 (as depicted in Figure 3).

11. The antibody or variant thereof of claim 9, wherein the variable domain that can bind to an extracellular part of TIM-3 comprises a heavy chain variable region that comprises the amino acid sequence of the heavy chain variable region as depicted for MF7676; MF7677; MF7678; or MF7679 (of Figure 3) 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 indicated MF.

12. The antibody or variant thereof of claim 10, wherein the variable domain that can bind to an extracellular part of TIM-3 comprises a heavy chain variable region that comprises the amino acid sequence of the heavy chain variable region as depicted for MF7676; MF7677; MF7678; or MF7679 (of Figure 3).

13. The antibody or variant thereof of any one of claims 4 to 8, wherein:

the variable domain that can bind to an extracellular part of TIM-3 comprises a heavy chain variable region that comprises the amino acid sequence of the heavy chain variable region as depicted for MF7677 (of Figure 3) 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 indicated MF; and

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 MF6930 (as depicted in Figure 3) 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 the MF, wherein said insertions,

deletions, or substitutions are not in the CDR1, CDR2 or CDR3 of the heavy chain variable region.

14. The antibody or variant thereof of any one of claims 4 to 8, wherein:

the variable domain that can bind to an extracellular part of TIM-3 comprises a heavy chain variable region that comprises the amino acid sequence of the heavy chain variable region as depicted for MF7678 (of Figure 3) 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 indicated MF; and

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 MF6930 (as depicted in Figure 3) 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 the MF, wherein said insertions, deletions, or substitutions are not in the CDR1, CDR2 or CDR3 of the heavy chain variable region.

15. The antibody or variant thereof of any one of claims 4 to 8, wherein:

the variable domain that can bind to an extracellular part of TIM-3 comprises a heavy chain variable region that comprises the amino acid sequence of the heavy chain variable region as depicted for MF7677 (of Figure 3) 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 indicated MF; and

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 MF6256 (as depicted in Figure 3) 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 the MF, wherein said insertions, deletions, or substitutions are not in the CDR1, CDR2 or CDR3 of the heavy chain variable region.

16. The antibody or variant thereof of any one of claims 4 to 8, wherein:

the variable domain that can bind to an extracellular part of TIM-3 comprises a heavy chain variable region that comprises the amino acid sequence of the heavy chain variable region as depicted for MF7678 (of Figure 3) 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 indicated MF; and

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 MF6256 (as depicted in Figure 3) 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 the MF, wherein said insertions, deletions, or substitutions are not in the CDR1, CDR2 or CDR3 of the heavy chain variable region.

17. The antibody or variant thereof of any one of claims 4 to 8, wherein:

the variable domain that can bind to an extracellular part of TIM-3 comprises a heavy chain variable region that comprises the amino acid sequence of the heavy chain variable region as depicted for MF7677 (of Figure 3) 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 indicated MF; and

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 MF6076 (as depicted in Figure 3) 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 the MF, wherein said insertions, deletions, or substitutions are not in the CDR1, CDR2 or CDR3 of the heavy chain variable region.

18. The antibody or variant thereof of any one of claims 4 to 8, wherein:

the variable domain that can bind to an extracellular part of TIM-3 comprises a heavy chain variable region that comprises the amino acid sequence of the heavy chain variable region as depicted for MF7678 (of Figure 3) 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 indicated MF; and

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 MF6076 (as depicted in Figure 3) 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 the MF, wherein said insertions, deletions, or substitutions are not in the CDR1, CDR2 or CDR3 of the heavy chain variable region.

19. The antibody or variant thereof of any one of claims 4 to 8, wherein:

the variable domain that can bind to an extracellular part of TIM-3 comprises a heavy chain variable region that comprises the amino acid sequence of the heavy chain variable region as depicted for MF7677 (of Figure 3) 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 indicated MF; and

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) 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 the MF, wherein said insertions, deletions, or substitutions are not in the CDR1, CDR2 or CDR3 of the heavy chain variable region.

20. The antibody or variant thereof of any one of claims 4 to 8, wherein:

the variable domain that can bind to an extracellular part of TIM-3 comprises a heavy chain variable region that comprises the amino acid sequence of

the heavy chain variable region as depicted for MF7678 (of Figure 3) 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 indicated MF; and

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) 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 the MF, wherein said insertions, deletions, or substitutions are not in the CDR1, CDR2 or CDR3 of the heavy chain variable region.

21. An antibody or variant thereof that comprises 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 TIM-3,

wherein said variable domain that can bind TIM-3 comprises a heavy chain variable region that comprises the amino acid sequence of the heavy chain variable region as depicted for MF7676; MF7677; MF7678; or MF7679 (of Figure 3) 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 indicated MF, wherein said insertions, deletions, or substitutions are not in the CDR1, CDR2 or CDR3 of the heavy chain variable region,

and wherein the variable domain that can bind TIM-3 comprises a light chain comprising a light chain CDR1 comprising sequence QSISSY, CDR2 comprising sequence AAS and CDR3 comprising sequence QQSYSTPPT.

22. The antibody or variant thereof of claim 21, wherein said variable domain that can bind TIM-3 comprises a heavy chain variable region that comprises the amino acid sequence of the heavy chain variable region as depicted for MF7676 (of Figure 3).

23. The antibody or variant thereof of claim 21, wherein said variable domain that can bind TIM-3 comprises a heavy chain variable region that comprises the amino acid sequence of the heavy chain variable region as depicted for MF7677 (of Figure 3).

24. The antibody or variant thereof of claim 21, wherein said variable domain that can bind TIM-3 comprises a heavy chain variable region that comprises the amino acid sequence of the heavy chain variable region as depicted for MF7678 (of Figure 3).

25. The antibody or variant thereof of claim 21, wherein said variable domain that can bind TIM-3 comprises a heavy chain variable region that comprises the amino acid sequence of the heavy chain variable region as depicted for MF7679 (of Figure 3).

26. The antibody or variant thereof of any one of claims 4 to 25 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 or Figure 1A and comprises the CDR1, CDR2 and CDR3 sequences of the light chain as depicted in Figure 1B or 1D.

27. The antibody or variant thereof of any one of claims 4 to 26 which comprises a light chain variable region having a sequence that is identical to the amino acid sequence as depicted in Figure 1B or Figure 1A.

28. The method of any one of claims 1 to 3, wherein the antibody or variant thereof is an antibody according to any one of claims 4 to 27.

29. The antibody or variant thereof of any one of claims 4 to 27, for use as a medicament.

30. Use of an antibody or variant thereof of any one of claims 4 to 27 in the manufacture of a medicament for treating cancer or an infection with a pathogen, preferably a virus or parasite.

31. A composition or kit of parts comprising at least one antibody or variant thereof according to any one of claims 4 to 27.

32. A pharmaceutical composition comprising at least one antibody or variant thereof of any one of claims 4 to 27 and a pharmaceutically acceptable carrier, diluent or excipient.

33. A nucleic acid molecule encoding an antibody or variant of any one of claims 4 to 27.

34. A vector comprising a nucleic acid molecule or functional equivalent thereof according to claim 33.

35. An isolated or recombinant cell, or a non-human animal, comprising a nucleic acid molecule according to claim 33 or a vector according to claim 34.

36. A method of producing an antibody or variant thereof of any one of claims 4 to 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:
- a cell having a) a first nucleic acid molecule encoding a IgG heavy chain that specifically recognizes an extracellular part of a 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 TIM3, and that contains a 2nd CH3 domain, wherein said nucleic acid sequences encode residues for preferential pairing of said 1st and 2nd CH3 domains,
wherein said cell has a third nucleic acid molecule encoding a light chain, comprising a light chain CDR1 comprising sequence QSISSY, CDR2 comprising sequence AAS and CDR3 comprising sequence QQSYSTPPT,
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.

37. The method according to claim 36, wherein the light chain is the rearranged germline human kappa light chain IgVκ1-39*01/IGJκ1*01.

38. The method according to claim 36 or 37, wherein said first nucleic acid encodes said first CH3 domain comprising the amino acid substitutions L351K and T366K (numbering according to the EU numbering) and wherein said second nucleic acid encodes said second CH3 domain comprising 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.

39. Use of antibody or a functional part, derivative and/or analogue thereof in the manufacture of a medicament for interfering with Programmed Cell Death 1 protein (PD-1) and T-cell immunoglobulin and mucin-domain containing-3 (TIM-3) mediated inhibition in a PD-1 and/or TIM-3 positive cell, wherein the antibody or a functional part, derivative and/or analogue thereof comprises:

- 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 TIM-3, thereby inhibiting PD-1 and/or TIM-3 mediated activity in said cell,

wherein said variable domain that can bind to an extracellular part of PD-1 comprises a heavy chain variable region with a CDR1, CDR2 and CDR3 region that comprises the amino acid sequence of the CDR1, CDR2 and CDR3 of a heavy chain variable region of one of the VH depicted for MF6076; MF6236; MF6256; MF6226; MF6930; MF6932; MF6935; MF6936; MF6972; MF6974; or MF6982, in Figure 3;

wherein said variable domain that can bind to an extracellular part of TIM-3 comprises a heavy chain variable region with a CDR1, CDR2 and CDR3 region that comprises the amino acid sequence of the CDR1, CDR2 and CDR3 of a heavy chain variable region of one of the VH depicted for MF7676; MF7677; MF7678; or MF7679, of Figure 3; and

wherein the variable domains that can bind to an extracellular part of PD-1 and TIM-3 comprise a light chain, comprising a light chain CDR1 comprising sequence QSISSY, CDR2 comprising sequence AAS and CDR3 comprising sequence QQSYSTPPT.

40. The use of claim 39, wherein the binding of said PD-1 binding variable domain to PD-1 blocks the binding of PD-1 to PD-L1 and/or PD-L2.

41. Use of an antibody or a functional part, derivative and/or analogue thereof in the manufacture of a medicament for stimulating the formation, stability and/or activity of an immunological synapse, wherein the antibody or a functional part, derivative and/or analogue thereof that comprises:

- 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 TIM-3,

thereby stimulating the formation, stability and/or activity of an immunological synapse between said at least two cells,

wherein said variable domain that can bind to an extracellular part of PD-1 comprises a heavy chain variable region with a CDR1, CDR2 and CDR3 region that comprises the amino acid sequence of the CDR1, CDR2 and CDR3 of a heavy chain variable region of one of the VH depicted for MF6076; MF6236; MF6256; MF6226; MF6930; MF6932; MF6935; MF6936; MF6972; MF6974; or MF6982, in Figure 3;

wherein said variable domain that can bind to an extracellular part of TIM-3 comprises a heavy chain variable region with a CDR1, CDR2 and CDR3 region that comprises the amino acid sequence of the CDR1, CDR2 and CDR3 of a heavy chain variable region of one of the VH depicted for MF7676; MF7677; MF7678; or MF7679, of Figure 3; and

wherein the variable domains that can bind to an extracellular part of PD-1 and TIM-3 comprise a light chain, comprising a light chain CDR1 comprising sequence QSISSY, CDR2 comprising sequence AAS and CDR3 comprising sequence QQSYSTPPT.