

comprising a PD-1 targeting binding domain. However, each of these antibodies has its own challenges in the production of an effective therapeutic drug. There thus remains a need for the development of novel PD-1 binding domains in order to produce effective PD-1 targeting therapeutic antibodies.

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SUMMARY

One of the objects of the present disclosure is to provide a new pharmaceutical agent for the treatment of human disease, in particular for the treatment of cancer. This object is met by the provision of novel anti-human PD-1 binding domains, and in particular by binding moieties, such as antibodies, comprising such anti-human PD-1 binding domains.

In certain [embodiment](#)[example](#)s, the present disclosure provides an anti-human PD-1 binding domain having higher binding affinity for human PD-1 than a reference anti-human PD-1 binding domain, wherein the reference anti-human PD-1 binding domain comprises a heavy chain variable region having an amino acid sequence as set forth in SEQ ID NO: 20 and a light chain variable region having an amino acid sequence as set forth in SEQ ID NO: 21.

In certain [embodiment](#)[example](#)s, the present disclosure provides an anti-human PD-1 binding domain, which when monovalently present in a bivalent antibody, provides at least comparable, or equal or higher, potency in blocking ligand binding to PD-1 than a reference anti-human PD-1 antibody, wherein the reference anti-human PD-1 antibody comprises two heavy chain variable regions having an amino acid sequence as set forth in SEQ ID NO: 20 and two light chain variable regions having an amino acid sequence as set forth in SEQ ID NO: 21.

In certain [embodiment](#)[example](#)s, the present disclosure provides a binding moiety comprising a PD-1 binding domain as described herein, and in particular a monospecific or multispecific binding moiety, such as a monospecific or multispecific antibody, comprising such PD-1 binding domain.

In certain [embodiment](#)[example](#)s, the present disclosure provides a pharmaceutical composition comprising an effective amount of an anti-human PD-1 binding domain or binding moiety as described herein.

In certain [embodiment](#)[example](#)s, the present disclosure provides for a PD-1 binding domain, binding moiety comprising the PD-1 binding domain, and pharmaceutical composition as

described herein, for use (a) in therapy, or (b) in the treatment of a disease associated with a suppressed immune system, or c) in the treatment of cancer.

In certain [embodiment examples](#), the present disclosure provides a method for treating a disease, comprising administering an effective amount of a PD-1 binding domain, a binding moiety comprising a PD-1 binding domain, or pharmaceutical composition, as described herein, to an individual in need thereof.

In certain [embodiment examples](#), the present disclosure provides a method for treating cancer, comprising administering an effective amount of a PD-1 binding domain, a binding moiety comprising a PD-1 binding domain, or pharmaceutical composition, as described herein, to an individual in need thereof.

In certain [embodiment examples](#), the present disclosure provides a nucleic acid sequence encoding the heavy chain variable region of an anti-human PD-1 binding domain as described herein, a vector and cell comprising such nucleic acid sequence, and a cell producing an anti-human PD-1 binding domain, or the binding moiety, as described herein.

In certain [embodiment examples](#), the present disclosure provides a method for producing an anti-human PD-1 binding domain as described herein, as well as a method for producing variants thereof.

In one aspect, the present disclosure relates to an anti-human PD-1 binding domain having higher binding affinity for human PD-1 than a reference anti-human PD-1 binding domain, wherein the reference anti-human PD-1 binding domain comprises a heavy chain variable region having an amino acid sequence as set forth in SEQ ID NO: 20 and a light chain variable region having an amino acid sequence as set forth in SEQ ID NO: 21, in particular wherein the binding affinity is measured by surface plasmon resonance.

In another aspect, the present disclosure relates to an anti-human PD-1 binding domain, which when monovalently present in a bivalent antibody, provides comparable, or equal or higher, potency in blocking ligand binding to PD-1 than a reference anti-human PD-1 antibody, wherein the reference anti-human PD-1 antibody comprises two heavy chain variable regions having an amino acid sequence as set forth in SEQ ID NO: 20 and two light chain variable regions having an amino acid sequence as set forth in SEQ ID NO: 21, in particular wherein the potency in blocking ligand binding to PD-1 is measured in a PD-1/PD-L1 reporter assay.

In another aspect, the present disclosure relates to an anti-human PD-1 binding domain comprising a heavy chain variable region, wherein the heavy chain variable region comprises a heavy chain CDR1 (HCDR1) from a heavy chain variable region having an amino acid sequence from the group consisting of SEQ ID NO: 1-9, a heavy chain CDR2 (HCDR2) from a heavy chain variable region having an amino acid sequence from the group consisting of SEQ ID NO: 1-9, and a heavy

chain CDR3 (HCDR3) from a heavy chain variable region having an amino acid sequence from the group consisting of SEQ ID NO: 1-9.

In another aspect, the present disclosure relates to a binding moiety comprising an anti-PD-1 binding domain disclosed herein, in particular wherein the binding moiety is a monospecific binding moiety, preferably a bivalent monospecific antibody.

In yet another aspect, the present disclosure relates to a pharmaceutical composition comprising an effective amount of the anti-human PD-1 binding domain as disclosed herein, or the binding moiety as disclosed herein, and a pharmaceutically acceptable carrier.

In yet another aspect, the present disclosure relates to the anti-human PD-1 binding domain as disclosed herein, the binding moiety as disclosed herein or the pharmaceutical composition as disclosed herein, for use in therapy.

In another aspect, the present disclosure relates to use of the anti-human PD-1 binding domain as disclosed herein, the binding moiety as disclosed herein, or the pharmaceutical composition as disclosed herein, in the manufacture of a medicament for the treatment of a disease associated with a suppressed immune system.

In another aspect, the present disclosure relates to use of the anti-human PD-1 binding domain as disclosed herein, the binding moiety as disclosed herein, or the pharmaceutical composition as disclosed herein, in the manufacture of a medicament for the treatment of cancer.

In another aspect, the present disclosure relates to a nucleic acid sequence encoding the heavy chain variable region of the anti-human PD-1 binding domain as disclosed herein.

In another aspect, the present disclosure relates to a vector comprising a nucleic acid as disclosed herein.

In another aspect, the present disclosure relates to a cell comprising a nucleic acid sequence encoding the heavy chain variable region of the anti-human PD-1 binding domain as disclosed herein.

In yet another aspect, the present disclosure relates to a cell producing the anti-human PD-1 binding domain as disclosed herein, or a binding moiety as disclosed herein, in particular wherein the cell is a recombinant cell, which has been transformed with the vector as disclosed herein.

DETAILED DESCRIPTION

In certain embodiments, the present disclosure provides several anti-human PD-1 binding domains, the heavy chain variable region having an amino acid sequence as set forth in SEQ ID NO: 1-9. In certain embodiments, the anti-human PD-1 binding domain comprises a heavy chain variable region, wherein the heavy chain variable region comprises the heavy chain CDR1 (HCDR1), heavy chain CDR2 (HCDR2), and heavy chain CDR3 (HCDR3) of

any one of the heavy chain variable regions having an amino acid sequence as set forth in SEQ ID NO: 1-9.

5 Programmed Cell Death 1 protein (PD-1) is a cell surface receptor that belongs to the CD28 family of receptors and is expressed on T cells and pro-B cells. PD-1 is presently known to bind two ligands, PD-L1 and PD-L2. PD-1, functioning as an immune checkpoint, plays an important role in down regulating the immune system by inhibiting the activation of T-cells, which in turn reduces autoimmunity and promotes self-tolerance. The inhibitory effect of PD-1 is thought to be accomplished through a dual mechanism of promoting

CLAIMS

1. An anti-human PD-1 binding domain having higher binding affinity for human PD-1 than a reference anti-human PD-1 binding domain, wherein the reference anti-human PD-1 binding domain comprises a heavy chain variable region having an amino acid sequence as set forth in SEQ ID NO: 20 and a light chain variable region having an amino acid sequence as set forth in SEQ ID NO: 21, in particular wherein the binding affinity is measured by surface plasmon resonance.

2. An anti-human PD-1 binding domain, which when monovalently present in a bivalent antibody, provides comparable, or equal or higher, potency in blocking ligand binding to PD-1 than a reference anti-human PD-1 antibody, wherein the reference anti-human PD-1 antibody comprises two heavy chain variable regions having an amino acid sequence as set forth in SEQ ID NO: 20 and two light chain variable regions having an amino acid sequence as set forth in SEQ ID NO: 21, in particular wherein the potency in blocking ligand binding to PD-1 is measured in a PD-1/PD-L1 reporter assay.

3. The anti-human PD-1 binding domain according to claim 1 or 2, wherein the anti-human PD-1 binding domain comprises at least a heavy chain variable region and a light chain variable region, and wherein the light chain variable region preferably is a light chain variable region of a light chain that is capable of pairing with multiple heavy chains having different epitope specificities.

~~4. The anti human PD-1 binding domain according to claim 1 or 3, wherein the binding affinity is measured by surface plasmon resonance.~~

~~5~~4. The anti-human PD-1 binding domain according to any one of claims 1-~~4~~3, wherein the anti-human PD-1 binding domain has at least a ten-fold higher binding affinity for human PD-1 than the reference anti-human PD-1 binding domain, or wherein the anti-human PD-1 binding domain has a ten-fold higher binding affinity for human PD-1 than the reference anti-human PD-1 binding domain.

~~6. The anti-human PD-1 binding domain according to any one of claims 1-4, wherein the anti-human PD-1 binding domain has a ten-fold higher binding affinity for human PD-1 than the reference anti-human PD-1 binding domain.~~

~~7~~5. The anti-human PD-1 binding domain according to any one of claims 1-~~6~~4, wherein the anti-human PD-1 binding domain has a binding affinity for human PD-1 in a range of about 0.1-1.0 nM, in particular in a range of about 0.3-0.8 nM, more in particular in a range of about 0.38-0.78 nM.

~~8~~6. The anti-human PD-1 binding domain according to any one of claims 1, and 3-~~7~~5, wherein the binding affinity is measured with both the anti-human PD-1 binding domain and the reference anti-human PD-1 binding domain in a bivalent monospecific IgG format, or wherein the binding affinity is measured with the anti-human PD-1 binding domain in a bivalent bispecific IgG format and the reference anti-human PD-1 binding domain in a bivalent monospecific IgG format.

~~9. The anti-human PD-1 binding domain according to any one of claims 1, and 3-7, wherein the binding affinity is measured with the anti-human PD-1 binding domain in a bivalent bispecific IgG format and the reference anti-human PD-1 binding domain in a bivalent monospecific IgG format.~~

~~10. The anti-human PD-1 binding domain according to claim 2, 3, or 5-9, wherein the potency in blocking ligand binding to PD-1 is measured in a PD-1/PD-L1 reporter assay.~~

~~11~~7. The anti-human PD-1 binding domain according to any one of claims ~~2, 3 or 5-10~~6, wherein a comparable potency in blocking ligand binding to PD-1 activity is a potency within a 5 fold range of the potency in blocking ligand binding to PD-1 of the reference anti-human PD-1 antibody, including a 5, 4, 3, and 2 fold deviation from the potency in blocking ligand binding to PD-1 of the reference anti-human PD-1 antibody.

128. The anti-human PD-1 binding domain according to any one of claims 1-117, wherein the heavy chain variable region comprises:

a) heavy chain CDR1 (HCDR1), heavy chain CDR2 (HCDR2), and heavy chain CDR3 (HCDR3), having an amino acid sequence as set forth in SEQ ID NO: 22, SEQ ID NO: 23 and SEQ ID NO: 24, respectively;

b) heavy chain CDR1 (HCDR1), heavy chain CDR2 (HCDR2), and heavy chain CDR3 (HCDR3), having an amino acid sequence as set forth in SEQ ID NO: 25, SEQ ID NO: 26, and SEQ ID NO: 27, respectively;

c) heavy chain CDR1 (HCDR1), heavy chain CDR2 (HCDR2), and heavy chain CDR3 (HCDR3), having an amino acid sequence as set forth in SEQ ID NO: 28, SEQ ID NO: 29, and SEQ ID NO: 30, respectively;

d) heavy chain CDR1 (HCDR1), heavy chain CDR2 (HCDR2), and heavy chain CDR3 (HCDR3), having an amino acid sequence as set forth in SEQ ID NO: 31, SEQ ID NO: 32, and SEQ ID NO: 33, respectively;

e) heavy chain CDR1 (HCDR1), heavy chain CDR2 (HCDR2), and heavy chain CDR3 (HCDR3), having an amino acid sequence as set forth in SEQ ID NO: 34, SEQ ID NO: 35, and SEQ ID NO: 36, respectively;

f) heavy chain CDR1 (HCDR1), heavy chain CDR2 (HCDR2), and heavy chain CDR3 (HCDR3), having an amino acid sequence as set forth in SEQ ID NO: 37, SEQ ID NO: 38, and SEQ ID NO: 39, respectively;

g) heavy chain CDR1 (HCDR1), heavy chain CDR2 (HCDR2), and heavy chain CDR3 (HCDR3), having an amino acid sequence as set forth in SEQ ID NO: 40, SEQ ID NO: 41, and SEQ ID NO: 42, respectively;

h) heavy chain CDR1 (HCDR1), heavy chain CDR2 (HCDR2), and heavy chain CDR3 (HCDR3), having an amino acid sequence as set forth in SEQ ID NO: 43, SEQ ID NO: 44, and SEQ ID NO: 45, respectively; or

i) heavy chain CDR1 (HCDR1), heavy chain CDR2 (HCDR2), and heavy chain CDR3 (HCDR3), having an amino acid sequence as set forth in SEQ ID NO: 46, SEQ ID NO: 47, and SEQ ID NO: 48, respectively;

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

~~13~~⁹. The anti-human PD-1 binding domain according to any one of claims 1-~~12~~⁸, comprising a heavy chain variable region having an amino acid sequence as set forth in any one of SEQ ID NO: 1-9, or having at least 80%, preferably 85%, more preferably 90%, or most preferably 95% sequence identity thereto.

~~14. The anti-human PD-1 binding domain according to any one of claims 3-13, further comprising a CH1 and CL region.~~

~~15~~¹⁰. An anti-human PD-1 binding domain comprising a heavy chain variable region, wherein the heavy chain variable region comprises a heavy chain CDR1 (HCDR1) from a heavy chain variable region having an amino acid sequence from the group consisting of SEQ ID NO: 1-9, a heavy chain CDR2 (HCDR2) from a heavy chain variable region having an amino acid sequence from the group consisting of SEQ ID NO: 1-9, and a heavy chain CDR3 (HCDR3) from a heavy chain variable regions having an amino acid sequence from the group consisting of SEQ ID NO: 1-9.

~~16~~¹¹. The anti-human PD-1 binding domain according to claim ~~15~~¹⁰, wherein the heavy chain variable region comprises:

a) heavy chain CDR1 (HCDR1), heavy chain CDR2 (HCDR2), and heavy chain CDR3 (HCDR3), having an amino acid sequence as set forth in SEQ ID NO: 22, SEQ ID NO: 23 and SEQ ID NO: 24, respectively;

b) heavy chain CDR1 (HCDR1), heavy chain CDR2 (HCDR2), and heavy chain CDR3 (HCDR3), having an amino acid sequence as set forth in SEQ ID NO: 25, SEQ ID NO: 26, and SEQ ID NO: 27, respectively;

c) heavy chain CDR1 (HCDR1), heavy chain CDR2 (HCDR2), and heavy chain CDR3 (HCDR3), having an amino acid sequence as set forth in SEQ ID NO: 28, SEQ ID NO: 29, and SEQ ID NO: 30, respectively;

d) heavy chain CDR1 (HCDR1), heavy chain CDR2 (HCDR2), and heavy chain CDR3 (HCDR3), having an amino acid sequence as set forth in SEQ ID NO: 31, SEQ ID NO: 32, and SEQ ID NO: 33, respectively;

e) heavy chain CDR1 (HCDR1), heavy chain CDR2 (HCDR2), and heavy chain CDR3 (HCDR3), having an amino acid sequence as set forth in SEQ ID NO: 34, SEQ ID NO: 35, and SEQ ID NO: 36, respectively;

f) heavy chain CDR1 (HCDR1), heavy chain CDR2 (HCDR2), and heavy chain CDR3 (HCDR3), having an amino acid sequence as set forth in SEQ ID NO: 37, SEQ ID NO: 38, and SEQ ID NO: 39, respectively;

g) heavy chain CDR1 (HCDR1), heavy chain CDR2 (HCDR2), and heavy chain CDR3 (HCDR3), having an amino acid sequence as set forth in SEQ ID NO: 40, SEQ ID NO: 41, and SEQ ID NO: 42, respectively;

h) heavy chain CDR1 (HCDR1), heavy chain CDR2 (HCDR2), and heavy chain CDR3 (HCDR3), having an amino acid sequence as set forth in SEQ ID NO: 43, SEQ ID NO: 44, and SEQ ID NO: 45, respectively; or

i) heavy chain CDR1 (HCDR1), heavy chain CDR2 (HCDR2), and heavy chain CDR3 (HCDR3), having an amino acid sequence as set forth in SEQ ID NO: 46, SEQ ID NO: 47, and SEQ ID NO: 48, respectively;

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

~~17~~12. The anti-human PD-1 binding domain according to claim ~~15~~10 or ~~16~~11, comprising a heavy chain variable region having an amino acid sequence as set forth in any one of SEQ ID NO: 1-9 or having at least 80%, preferably 85%, more preferably 90%, or most preferably 95% sequence identity thereto.

~~18~~13. The anti-human PD-1 binding domain according to any one of claims ~~15-17~~1-12, further comprising a CH1 and CL region.

~~19~~14. A binding moiety comprising an anti-PD-1 binding domain as claimed in any one of claims 1-~~18~~13, in particular wherein the binding moiety is a monospecific binding moiety, preferably a bivalent monospecific antibody.

~~20. The binding moiety according to claim 19, wherein the binding moiety is a monospecific binding moiety, preferably a bivalent monospecific antibody.~~

~~21~~15. A pharmaceutical composition comprising an effective amount of the anti-human PD-1 binding domain according to any one of claims 1-~~18~~13, or the binding moiety according to claim ~~19 or 20~~14, and a pharmaceutically acceptable carrier.

~~22~~16. The anti-human PD-1 binding domain according to any one of claims 1-~~18~~13, the binding moiety according to claim ~~19 or 20~~14, or the pharmaceutical composition according to claim ~~21~~15, for use in therapy.

~~23~~17. Use of ~~T~~the anti-human PD-1 binding domain according to any one of claims 1-~~18~~13, the binding moiety according to claim ~~19 or 20~~14, or the pharmaceutical composition according to claim ~~24~~15, ~~for use in~~ the manufacture of a medicament for the treatment of a disease associated with a suppressed immune system.

~~24~~18. Use of ~~T~~the anti-human PD-1 binding domain according to any one of claims 1-~~18~~13, the binding moiety according to claim ~~19 or 20~~14, or the pharmaceutical composition according to claim ~~24~~15, ~~for use in~~ the manufacture of a medicament for the treatment of cancer.

~~25. A method for treating a disease, comprising administering an effective amount of the anti-human PD-1 binding domain as claimed in any one of claims 1-18, the binding moiety as claimed in claim 19 or 20, or the pharmaceutical composition as claimed in claim 21, to an individual in need thereof.~~

~~26. A method for treating a disease associated with a suppressed immune system, comprising administering an effective amount of the anti-human PD-1 binding domain as claimed in any one of claims 1-18, the binding moiety as claimed in claim 19 or 20, or the pharmaceutical composition as claimed in claim 21, to an individual in need thereof.~~

~~27. A method for treating cancer, comprising administering an effective amount of the anti-human PD-1 binding domain as claimed in any one of claims 1-18, the binding moiety as claimed in claim 19 or 20, or the pharmaceutical composition as claimed in claim 21, to an individual in need thereof.~~

~~28~~19. A nucleic acid sequence encoding the heavy chain variable region of the anti-human PD-1 binding domain as claimed in any one of claims 1-~~18~~13.

~~29~~20. A vector comprising a nucleic acid sequence as claimed in claim ~~28~~19.

~~30~~21. The vector according to claim ~~29~~20, wherein the vector further comprises a nucleic acid sequence encoding a CH1 region and preferably a hinge, CH2 and CH3 region, in particular wherein the vector further comprises at least one nucleic acid sequence encoding a light chain variable region, and preferably a CL region.

~~31. The vector according to claim 29 or 30, wherein the vector further comprises at least one nucleic acid sequence encoding a light chain variable region, and preferably a CL region.~~

~~32~~22. The vector according to claim ~~31~~21, 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.

~~33~~23. A cell comprising a nucleic acid sequence encoding the heavy chain variable region of the anti-human PD-1 binding domain as claimed in any one of claims 1-~~18~~13.

~~34~~24. The cell according to claim ~~33~~23, wherein the cell further comprises a nucleic acid sequence encoding a CH1 region and preferably a hinge, CH2 and CH3 region, in particular wherein the cell further comprises at least one nucleic acid sequence encoding a light chain variable region, and preferably a CL region.

~~35. The cell according to claim 33 or 34, wherein the cell further comprises at least one nucleic acid sequence encoding a light chain variable region, and preferably a CL region.~~

~~36~~25. A cell producing the anti-human PD-1 binding domain as claimed in any one of claims 1-~~18~~13, or a binding moiety as claimed in claim ~~19 or 20~~14, in particular wherein the cell is a recombinant cell, which has been transformed with the vector as claimed in any one of claims 20-22.

~~37. The cell according to claim 36, wherein the cell is a recombinant cell, which has been transformed with the vector as claimed in any one of claims 29-32.~~