

CLAIMS

We claim:

1. ~~An anti-PD-1 antibody, or an antigen-binding fragment thereof, wherein the antibody or antigen-binding fragment thereof comprises a heavy chain variable region and a light chain variable region, wherein each of the heavy chain and the light chain variable regions comprise a CDR1, CDR2, and CDR3, and wherein:~~

~~the sequence of CDR1 of the heavy chain variable region (CDR1H) comprises the sequence of SEQ ID NO: 21;~~

~~the sequence of CDR2 of the heavy chain variable region (CDR2H) comprises the sequence of SEQ ID NO: 22;~~

~~the sequence of CDR2 of the heavy chain variable region (CDR3H) comprises the sequence of SEQ ID NO: 23;~~

~~the sequence of CDR1 of the light chain variable region (CDR1L) comprises the sequence RX₁₃X₁₄X₁₅X₁₆IX₁₇X₁₈WX₁₉X₂₀ (SEQ ID NO: 135);~~

~~wherein X₁₃ is A or V;~~

~~wherein X₁₄ is S or G;~~

~~wherein X₁₅ is Q, E, or R;~~

~~wherein X₁₆ is G, S, D or N;~~

~~wherein X₁₇ is G, S, or N;~~

~~wherein X₁₈ is S, I, R, T, K, P, N, H, or V;~~

~~wherein X₁₉ is L or V; and~~

~~wherein X₂₀ is G or A; and~~

~~the sequence of CDR2 of the light chain variable region (CDR2L) comprises the sequence X₂₁AX₂₂X₂₃X₂₄X₂₅X₂₆ (SEQ ID NO: 136); and~~

~~—— wherein X₂₁ is S, D, E, or A;~~

~~—— wherein X₂₂ is S, or K;~~

~~wherein X₂₃ is S, N, T, R, or D;~~

~~wherein X₂₄ is L or V;~~

~~wherein X₂₅ is Q, E, or H; and~~

~~—— wherein X₂₆ is S, N, A, R, P, or T; and~~

~~the sequence of CDR3 of the light chain variable region (CDR3L) comprises the sequence QQX₂₇X₂₈SFPX₂₉X₃₀ (SEQ ID NO: 137);~~

~~wherein X₂₇ is A, or G;~~
~~wherein X₂₈ is N, D, or Y;~~
~~wherein X₂₉ is F or L; and~~
~~wherein X₃₀ is A, or T.~~

~~2.1. The anti PD-1 antibody, or an antigen binding fragment thereof, of claim 1, wherein:~~
~~the sequence of CDR1H comprises the sequence of SEQ ID NO: 21;~~
~~the sequence of CDR2H comprises the sequence of SEQ ID NO: 22;~~
~~the sequence of CDR3H comprises the sequence of SEQ ID NO: 23~~
~~the sequence of CDR1L comprises the sequence of SEQ ID NO: 96;~~
~~the sequence of CDR2L comprises the sequence of SEQ ID NO: 97; and~~
~~the sequence of CDR3L comprises the sequence of SEQ ID NO: 82.~~

~~3.1. The anti PD-1 antibody, or an antigen binding fragment thereof, of claim 2, wherein:~~
~~a. the heavy chain variable region comprises SEQ ID NO: 24, or a sequence that is at~~
~~least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%~~
~~identical to SEQ ID NO: 24; and~~
~~b. the light chain variable region comprises SEQ ID NO: 98, or a sequence that is at~~
~~least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%~~
~~identical to SEQ ID NO: 98.~~

~~4.1. The An anti-PD-1 antibody, or an antigen-binding fragment thereof, of claim 1, wherein:~~
~~a. the sequences of CDR1H, CDR2H and CDR3H comprise SEQ ID NOS: 21, 22,~~
~~and 23; and~~
~~b. the sequences of CDR1L, CDR2L and CDR3L comprise:~~
~~i. SEQ ID NOS: 96, 97 and 82~~
~~ii. SEQ ID NOS: 45, 46, and 47;~~
~~iii. SEQ ID NOS: 66, 67, and 68;~~
~~iv. SEQ ID NOS: 70, 67, and 71;~~
~~v. SEQ ID NOS: 73, 74, and 75;~~
~~vi. SEQ ID NOS: 77, 78, and 47;~~
~~vii. SEQ ID NOS: 80, 81, and 82;~~
~~viii. SEQ ID NOS: 77, 78, and 84;~~
~~ix. SEQ ID NOS: 77, 86, and 47;~~

~~i-x~~.x. SEQ ID NOS: 88, 89, and 47;
~~x~~.xi. SEQ ID NOS: 66, 67, and 47;
~~x~~.xi. SEQ ID NOS: 80, 92, and 75;
~~xi~~.xiii. SEQ ID NOS: 80, 94, and 71;
~~xiii~~.xiv. SEQ ID NOS: 99, 100, and 47;
~~xiv~~.xv. SEQ ID NOS: 102, 103, and 104;
~~xv~~.xvi. SEQ ID NOS: 106, 103, and 47;
~~xvi~~.xvii. SEQ ID NOS: 108, 103, and 47;
~~xvii~~.xviii. SEQ ID NOS: 110, 111, and 75;
~~xviii~~.xix. SEQ ID NOS: 77, 103, and 113;
~~xix~~.xx. SEQ ID NOS: 77, 111, and 47;
~~xx~~.xxi. SEQ ID NOS: 116, 67, and 47;
~~xxi~~.xxii. SEQ ID NOS: 118, 119, and 47;
~~xxii~~.xxiii. SEQ ID NOS: 80, 78, and 47;
~~xxiii~~.xxiv. SEQ ID NOS: 122, 103 and 47;
~~xxiv~~.xxv. SEQ ID NOS: 124, 125, and 75;
~~xxv~~.xxvi. SEQ ID NOS: 127, 38, and 68;
~~xxvi~~.xxvii. SEQ ID NOS: 129, 130, and 47; or
~~xxvii~~.xxviii. SEQ ID NOS: 132, 133, and 75.

5.2. The anti-PD-1 antibody, or an antigen-binding fragment thereof, of claim 41, wherein:

- a. the heavy chain variable region comprises:
 - i. SEQ ID NO: 24, or
 - ii. a sequence that is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 24; and
- b. the light chain variable region comprises:
 - i. SEQ ID NO: 48, 69, 72, 76, 79, 83, 85, 87, 90, 91, 93, 95, 98, 101, 105, 107, 109, 112, 114, 115, 117, 120, 121, 123, 126, 128, 131, or 134, or
 - ii. a sequence that is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to any of SEQ ID NO: 48, 69, 72, 76, 79, 83, 85, 87, 90, 91, 93, 95, 98, 101, 105, 107, 109, 112, 114, 115, 117, 120, 121, 123, 126, 128, 131, or 134.

3. The anti-PD-1 antibody, or an antigen-binding fragment thereof, of claim 1 or 2, wherein:

the sequence of CDR1H comprises the sequence of SEQ ID NO: 21;

the sequence of CDR2H comprises the sequence of SEQ ID NO: 22;

the sequence of CDR3H comprises the sequence of SEQ ID NO: 23

the sequence of CDR1L comprises the sequence of SEQ ID NO: 96;

the sequence of CDR2L comprises the sequence of SEQ ID NO: 97; and

the sequence of CDR3L comprises the sequence of SEQ ID NO: 82.

4. The anti-PD-1 antibody, or an antigen-binding fragment thereof, of claim 23, wherein:

a. the heavy chain variable region comprises SEQ ID NO: 24, or a sequence that is at

least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%

identical to SEQ ID NO: 24; and

b. the light chain variable region comprises SEQ ID NO: 98, or a sequence that is at

least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%

identical to SEQ ID NO: 98.

~~6. An anti-PD-1 antibody, or an antigen-binding fragment thereof, wherein the antibody or antigen-binding fragment thereof comprises a heavy chain variable region and a light chain variable region, wherein each of the heavy chain and the light chain variable regions comprise a CDR1, CDR2, and CDR3, and wherein:~~

~~the sequence of CDR1H comprises the sequence of SEQ ID NO: 17;~~

~~the sequence of CDR2H comprises the sequence of SEQ ID NO: 18;~~

~~the sequence of CDR3H comprises the sequence of SEQ ID NO: 19;~~

~~the sequence of CDR1L comprises the sequence RSSX₁SLLX₂SNGX₃X₄YLD (SEQ ID NO:62),~~

~~wherein X₁ is Q or E;~~

~~wherein X₂ is H or Y;~~

~~wherein X₃ is Y or N; and~~

~~wherein X₄ is T or N;~~

~~the sequence of CDR2L comprises the sequence X₅X₆SX₇X₈X₉X₁₀ (SEQ ID NO: 63),~~

~~wherein X₅ is L, Q or E;~~

~~wherein X₆ is S, A, or V;~~

~~wherein X₇ is H, N, T, or S;~~

~~wherein X₈ is R or L;~~

wherein X_9 is G, A, or H; and
— wherein X_{10} is S or T; and
the sequence of CDR3L comprises the sequence MQGX₁₁X₁₂WPYT (SEQ ID NO:
64);
— wherein X_{11} is A, T, or S; and
— wherein X_{12} is H or R.

7. The anti-PD-1 antibody, or an antigen-binding fragment thereof, of claim 6, wherein:
the sequence of CDR1H comprise the sequence of SEQ ID NO: 17;
the sequence of CDR2H comprises the sequence of SEQ ID NO: 18;
the sequence of CDR3H comprises the sequence of SEQ ID NO: 19;
the sequence of CDR1L comprises the sequence of SEQ ID NO: 41;
the sequence of CDR2L comprises the sequence of SEQ ID NO: 49; and
the sequence of CDR3L comprises the sequence of SEQ ID NO: 50.

8. The anti-PD-1 antibody, or an antigen-binding fragment thereof, of claim 7, wherein:
a. the heavy chain variable region comprises SEQ ID NO: 20, or a sequence that is at
least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%
identical to SEQ ID NO: 20; and
b. the light chain variable region comprises SEQ ID NO: 51, or a sequence that is at
least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%
identical to SEQ ID NO: 51.

9.5. The An anti-PD-1 antibody, or an antigen-binding fragment thereof, of claim 6, wherein:
a. the sequence of CDR1H, CDR2H and CDR3H comprise SEQ ID NOS: 17, 18,
and 19; and
b. the sequence of CDR1L, CDR2L and CDR3L comprise:
i. SEQ ID NOS: 41, 49, and 50;
ii. SEQ ID NOS: 41, 42, and 43;
iii. SEQ ID NOS: 41, 52, and 53;
iv. SEQ ID NOS: 41, 55, and 56; or
v. SEQ ID NOS: 58, 59, and 60.

10.6. The anti-PD-1 antibody, or an antigen-binding fragment thereof, of claim 95, wherein:

- a. the heavy chain variable region comprises:
 - i. SEQ ID NO: 20, or
 - ii. a sequence that is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 20; and
- b. the light chain variable region comprises:
 - i. SEQ ID NO: 44, 51, 54, 57, or 61, or
 - ii. a sequence that is at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to any one of SEQ ID NO: 44, 51, 54, 57, or 61.

~~An anti-PD-1 antibody, or an antigen-binding fragment thereof that binds to the same epitope on PD-1 as the anti-PD-1 antibody, or an antigen-binding fragment thereof of any one of the preceding claims.~~

~~The anti-PD-1 antibody, or an antigen-binding fragment thereof of any one of the preceding claims, wherein the antibody or antigen-binding fragment is a chimeric antibody, a CDR-grafted antibody, or a humanized antibody or antigen-binding fragment thereof.~~

7. The anti-PD-1 antibody, or an antigen-binding fragment thereof of any one of the preceding claims, wherein the antibody or antigen-binding fragment is a multispecific or a bispecific antibody or antigen-binding fragment thereof.

~~The anti-PD-1 antibody, or an antigen-binding fragment thereof of claim 13, wherein the antibody or antigen-binding fragment is a bispecific antibody comprising a complementary region that binds binds to PD-L1 or PD-L2.~~

8. The antibody or antigen-binding fragment according to any of the preceding claims, wherein the antibody or antigen-binding fragment is an scFv, Fv, Fab', Fab, F(ab')₂, or diabody.

9. The anti-PD-1 antibody, or an antigen-binding fragment thereof of any one of the preceding claims, wherein the antibody is an IgG class immunoglobulin, optionally having isotype IgG1 or IgG4.

~~The anti-PD-1 antibody, or an antigen-binding fragment thereof of claim 16, wherein the antibody or antigen-binding fragment has isotype IgG4.~~

~~The anti-PD-1 antibody, or an antigen-binding fragment thereof of any one of the preceding claims, wherein the antibody or antigen-binding fragment thereof contains a S241P substitution in the constant region of the heavy chain.~~

~~The anti-PD-1 antibody, or an antigen-binding fragment thereof of any one of the preceding claims, wherein the antibody or antigen-binding fragment is deglycosylated.~~

~~The anti-PD-1 antibody, or an antigen-binding fragment thereof of any one of the preceding claims, wherein the antibody or antigen-binding fragment is lacking a C-terminal lysine in the heavy chain.~~

27.—~~10.~~ The anti-PD-1 antibody, or an antigen-binding fragment thereof of any one of the preceding claims, wherein the antibody or antigen-binding fragment comprises a first and a second heavy chain constant region, and wherein the antibody or antigen-binding fragment thereof comprises at least one modification in the CH3 domains of the first and the second heavy chain causing heterodimerization, optionally wherein

~~The anti-PD-1 antibody, or an antigen-binding fragment thereof of claim 21, wherein the modification in the CH3 domain of first heavy chain is different from the modification in the CH3 domain of the second heavy chain.~~

28.—~~The anti-PD-1 antibody, or an antigen-binding fragment thereof of claim 21 or 22, wherein the first heavy chain comprises an amino acid substitution selected from the group consisting of T350V, L351Y, S354C, S364H, T366Y, T366W, F405A, Y407V (Kabat EU index numbering).~~

29.—~~The anti-PD-1 antibody, or an antigen-binding fragment thereof of any one of claims 21-23, wherein the hole modification comprises an amino acid substitution selected from the~~

~~group consisting of Y349C, T350V, T366L, T366S, L368A, K392L, T394W, Y407V, Y407T (Kabat EU index numbering).~~

~~11. The anti-PD-1 antibody, or an antigen-binding fragment thereof of any one of claims 22-24~~
claim 10, wherein:

- a. the CH3 domain of the first heavy chain comprises one or more of amino acid substitutions S354C and T366W and the CH3 domain of the second heavy chain comprises one or more of amino acid substitutions Y349C, T366S, L368A, and Y407V;
- b. the CH3 domain of the first heavy chain comprises one or more of amino acid substitutions T350V, L351Y, F405A, and Y407V and the CH3 domain of the second heavy chain comprises one or more of amino acid substitutions T350V, T366L, K392L, and T394W; or
- c. the CH3 domain of the first heavy chain comprises one or more of amino acid substitutions L351Y, F405A, and Y407V and the CH3 domain of the second heavy chain comprises one or more of amino acid substitutions T366L, K392L, and T394W (all Kabat EU index numbering).

~~35. 12. The anti-PD-1 antibody, or an antigen-binding fragment thereof of any one of the preceding claims, wherein the Fc domain, if present, comprises one or more amino acid substitution that reduces binding to an Fc receptor or reduces effector function, optionally -~~

~~36. The anti-PD-1 antibody, or an antigen-binding fragment thereof of claim 26, wherein the one or more amino acid substitution is at one or more position selected from the group of L234, L235, and P329 (Kabat EU index numbering).~~

~~The anti-PD-1 antibody, or an antigen-binding fragment thereof of claim 27, wherein the one or more amino acid substitution is selected from L234A, L235A, and P329G (Kabat EU index numbering).~~

~~37. 13. A fusion protein comprising:~~

- a. an anti-PD-1 antibody, or an antigen-binding fragment thereof, wherein the antibody or antigen-binding fragment thereof comprises a heavy chain variable region and a light chain variable region, wherein each of the heavy chain and the light chain variable regions comprise a CDR1, CDR2, and CDR3;

- b. an IL-15R α sushi domain polypeptide comprising the amino acid sequence of SEQ ID NO: 214, or an amino acid sequence that is at least 95% identical to SEQ ID NO: 214; and
- c. an IL-15 polypeptide comprising the amino acid sequence of SEQ ID NO: 212, or an amino acid sequence that is at least 90% or at least 95% identical to SEQ ID NO: 212;
- d. a first linker polypeptide joining the IL-15R α sushi domain polypeptide and the IL-15 polypeptide, optionally wherein the IL-15R α sushi domain polypeptide is fused to the N-terminus of the IL-15 polypeptide.

14. The fusion protein of claim 13, wherein the anti-PD-1 antibody, or an antigen-binding fragment thereof, is according to any one of claims 1 to 12.

~~The fusion protein of claim 29, wherein the IL-15R α sushi domain polypeptide is fused to the N-terminus of the IL-15 polypeptide.~~

~~38. The fusion protein of claim 29 or 30, wherein the anti-PD-1 antibody or antigen-binding fragment thereof comprises a constant region, and wherein the IL-15R α sushi domain polypeptide and the IL-15 polypeptide are fused to the C-terminus of the constant region.~~

39. 15. The fusion protein of claim 29-13 or 3014, wherein (i) the IL-15R α sushi domain polypeptide and the IL-15 polypeptide are fused to (ii) the N-terminus of the heavy chain variable region, optionally by a second linker.

~~40. The fusion protein of claim 32, wherein a second linker joins (i) the IL-15R α sushi domain polypeptide and the IL-15 polypeptide and (ii) the N-terminus of the heavy chain variable region.~~

~~41. 16. The fusion protein of any one of claims 29-3313-15, wherein the first linker (i) is between 25-35-5 and 40 amino acids long, optionally :~~

~~The fusion protein of claim 34, wherein the first linker consists predominantly of Gly (G), Asn (N), Ser (S), Thr (T), Ala (A), Leu (L), and Gln (Q);:~~

~~The fusion protein of any one of claims 29-33, and/or (ii) wherein the first linker comprises a sequence selected from the group consisting of SEQ ID NOS:215-218 and 244, optionally, -~~

~~The fusion protein of claim 36, wherein the first linker comprises the sequence of SEQ ID NO:215.~~

~~42. 17. ____ -The fusion protein of any one of claims 33-37 claim 15 or 16, wherein the second linker (i) is between 25-355 and 40 amino acids long, optionally, -~~

~~The fusion protein of claim 38, wherein the first linker consists predominantly of Gly (G), Asn (N), Ser (S), Thr (T), Ala (A), Leu (L), and Gln (Q); -~~

~~The fusion protein of any one of claims 33-37, wherein the second linker and/or (ii) comprises a sequence selected from the group consisting of SEQ ID NOS:215-218 and 244, optionally, -~~

~~The fusion protein of claim 40, wherein the second linker comprises the sequence SEQ ID NO:216.~~

~~43. 18. ____ The fusion protein of any one of claims 29-41 15-17, wherein the fusion protein comprises not more than one IL-15R α sushi domain polypeptide and not more than one IL-15 polypeptide.~~

~~44. The fusion protein of any one of claims 29-42, wherein the anti-PD-1 antibody, or an antigen-binding fragment thereof is the anti-PD-1 antibody, or an antigen-binding fragment thereof of any one of claims 1-28.~~

~~45. 19. ____ The fusion protein of any one of claims 29-43 15-18, wherein the IL-15 polypeptide is a mutant IL-15 polypeptide comprising one or more amino acid substitutions at positions 45, 65, or 108 as numbered according to the mature human IL-15 sequence of SEQ ID NO:212, optionally, -~~

~~The fusion protein of claim 44, wherein the one or more amino acid substitutions in the IL-15 polypeptide are selected from L45A, N65A, N65D, N65S, N65K, and Q108S as numbered~~

according to the mature human IL-15 sequence of SEQ ID NO:212, optionally wherein the mutation is N65S as numbered according to the mature human IL-15 sequence of SEQ ID NO:212.

~~46. The fusion protein of any one of claims 29-45, wherein the IL-15R α sushi domain polypeptide comprises an amino acid substitution at position 60 as numbered according to the mature human IL-15R α sushi domain sequence of of SEQ ID NO:213.~~

~~47.~~

~~48. The fusion protein of claim 46, wherein the amino acid substitution at position 60 is an N60A substitution.~~

~~49. The fusion protein of claim 29, comprising a light chain sequence comprising SEQ ID NO:219 or SEQ ID NO:98 and:~~

~~a. a first heavy chain sequence comprising SEQ ID NO:220 and a second heavy chain sequence comprising SEQ ID NO:224; or a first heavy chain sequence comprising SEQ ID NO:221 and a second heavy chain sequence comprising SEQ ID NO:225; or~~

~~b. a first heavy chain sequence comprising SEQ ID NO:222 and a second heavy chain sequence comprising SEQ ID NO:226; or a first heavy chain sequence comprising SEQ ID NO:223 and a second heavy chain sequence comprising SEQ ID NO:227.~~

~~50. — 20. — The A fusion protein of claim 29, comprising a light chain sequence comprising SEQ ID NO:219 or SEQ ID NO:98 and:~~

~~a. a first heavy chain sequence comprising SEQ ID NO:220 and a second heavy chain sequence comprising SEQ ID NO:228; or a first heavy chain sequence comprising SEQ ID NO:221 and a second heavy chain sequence comprising SEQ ID NO:229;~~

~~b. — a first heavy chain sequence comprising SEQ ID NO:222 and a second heavy chain sequence comprising SEQ ID NO:230; or a first heavy chain sequence comprising SEQ ID NO:223 and a second heavy chain sequence comprising SEQ ID NO:231;~~

~~c. — a first heavy chain sequence comprising SEQ ID NO:220 and a second heavy chain sequence comprising SEQ ID NO:236; or a first heavy chain sequence comprising SEQ ID NO:221 and a second heavy chain sequence comprising SEQ ID NO:237;~~

~~d. — a first heavy chain sequence comprising SEQ ID NO:222 and a second heavy chain sequence comprising SEQ ID NO:238; or a first heavy chain sequence comprising SEQ ID NO:223 and a second heavy chain sequence comprising SEQ ID NO:239;~~

a first heavy chain sequence comprising SEQ ID NO:240 and a second heavy chain sequence comprising SEQ ID NO:241; or

e. ~~a first heavy chain sequence comprising SEQ ID NO:240 and a second heavy chain sequence comprising SEQ ID NO:242.~~

~~51. The fusion protein of claim 29, comprising a light chain sequence comprising SEQ ID NO:219 or SEQ ID NO:98 and:~~

a. ~~a first heavy chain sequence comprising SEQ ID NO:220 and a second heavy chain sequence comprising SEQ ID NO:232; or a first heavy chain sequence comprising SEQ ID NO:221 and a second heavy chain sequence comprising SEQ ID NO:233;~~

b. ~~a first heavy chain sequence comprising SEQ ID NO:222 and a second heavy chain sequence comprising SEQ ID NO:234; or a first heavy chain sequence comprising SEQ ID NO:223 and a second heavy chain sequence comprising SEQ ID NO:235; or~~

e. ~~a first heavy chain sequence comprising SEQ ID NO:240 and a second heavy chain sequence comprising SEQ ID NO:243.~~

~~52. 21. A nucleic acid sequence encoding the anti-PD-1 antibody, or an antigen-binding fragment thereof of any one of claims 1-28-12 or the fusion protein of any one of claims 29-13-50-20.~~

~~53. 22. A vector comprising the nucleic acid of claim 51.~~

A cell comprising the nucleic acid of claim 51-21, or the a vector of comprising the nucleic acid of claim 52-21, or nucleic acids encoding the light and heavy chains encoding the anti-PD-1 antibody, or an antigen-binding fragment thereof of any one of claims 1-12 or the fusion protein of any one of claims 13-20.

~~54. A cell expressing the anti-PD-1 antibody, or an antigen-binding fragment thereof of any one of claims 1-28 or the fusion protein of any one of claims 29-47.~~

~~55. A T cell expressing a PD-1 binding protein comprising the CDRs of the anti-PD-1 antibody, or an antigen-binding fragment thereof claims 1-10.~~

~~56. A T cell expressing a PD-1 binding protein comprising the heavy and light variable chains of any one of claims 1-10.~~

~~57. The anti-PD-1 antibody, or an antigen-binding fragment thereof of any one of claims 1-28 or the fusion protein of any one of claims 29-50, wherein the antibody or antigen-binding fragment or the fusion protein is conjugated to one or more of a cytotoxin, a fluorescent label, and an imaging agent.~~

58. 23. A pharmaceutical composition comprising (i) the anti-PD-1 antibody, or an antigen-binding fragment thereof of any one of claims 1-28-12 or the fusion protein of any one of claims ~~29-50~~13-20 and (ii) a ~~pharamaceutically~~pharmaceutically acceptable carrier.

~~59~~ 24. ~~A method of inhibiting binding of PD-1 to a ligand of PD-1, optionally PD-L1 or PD-L2, in a subject in need thereof, the method comprising administering to the subject an effective amount of the~~ Use of an anti-PD-1 antibody, or an antigen-binding fragment thereof of any one of claims 1-28-12 or the fusion protein of any one of claims ~~29-50~~13-20 for the manufacture of a medicament for inhibiting binding of PD-1 to a ligand of PD-1 in a subject, optionally wherein the ligand of PD-1 is PD-L1 or PD-L2.

~~60~~ ~~The method of claim 59, wherein the ligand of PD-1 is PD-L1 or PD-L2.~~

~~61~~ 25. ~~A method for increasing T cell activation in a subject in need thereof, the method comprising administering to the subject an effective amount of the~~ Use of an anti-PD-1 antibody, or an antigen-binding fragment thereof of any one of claims 1-28-12 or the fusion protein of any one of claims ~~29-50~~13-20 for the manufacture of a medicament for increasing T cell activation in a subject.

~~62~~ 26. ~~A method of stimulating the immune system in a subject in need thereof, the method comprising administering to the subject an effective amount of the~~ Use of an anti-PD-1 antibody, or an antigen-binding fragment thereof of any one of claims 1-28-12 or the fusion protein of any one of claims ~~29-50~~13-20; for the manufacture of a medicament for stimulating the immune system in a subject, optionally wherein the subject has upregulated expression of PD-L1, or the subject has been identified as positive for expression of PD-L1.

- ~~63 The method of claim 62, wherein the subject has upregulated expression of PD-L1, or the subject has been identified as positive for expression of PD-L1.~~
- ~~64 A method of reducing replication of a virus in a subject in need thereof, the method comprising administering to the subject an effective amount of the anti-PD-1 antibody, or an antigen-binding fragment thereof of any one of claims 1-28 or the fusion protein of any one of claims 29-50.~~
- ~~65 27. A method of treating cancer in a subject in need thereof, the method comprising administering to the subject an effective amount of the Use of an anti-PD-1 antibody, or an antigen-binding fragment thereof of any one of claims 1-28-12 or the fusion protein of any one of claims 29-5013-20 for the manufacture of a medicament for treating cancer in a subject, optionally, -.~~
- ~~66 The method of claim 65, wherein the cancer is melanoma, pancreatic cancer, thyroid cancer, lung cancer, colorectal cancer, squamous cancer, prostate cancer, breast cancer, bladder cancer, or gastric cancer.~~
- ~~67 28. A method of reducing tumor growth or tumor metastasis in a subject in need thereof, the method comprising administering to the subject an effective amount of the Use of an anti-PD-1 antibody, or an antigen-binding fragment thereof of any one of claims 1-28-12 or the fusion protein of any one of claims 29-5013-20 for the manufacture of a medicament for reducing tumor growth or tumor metastasis in a subject.~~
- ~~68 A method of reducing tumor metastasis in a subject in need thereof, the method comprising administering to the subject an effective amount of the anti-PD-1 antibody, or an antigen-binding fragment thereof of any one of claims 1-28 or the fusion protein of any one of claims 29-50.~~
- ~~69 The method of any one of claims 59-68, wherein the does not respond to therapy with a checkpoint inhibitor or initially responds to checkpoint inhibitor treatment, but has later become resistant to checkpoint inhibitor blockade.~~

- ~~70 The method of any one of claims 59-68, the method further comprising administering an additional therapeutic agent or therapy.~~
- ~~71 The method of claim 70, wherein the additional therapeutic agent is selected from the group consisting of a cancer vaccine, a checkpoint inhibitor, an antibody to a tumor-specific antigen, Bacillus Calmette Guerin vaccine, a cytotoxin, an interleukin 6 receptor (IL-6R) inhibitor, an interleukin 4 receptor (IL-4R) inhibitor, an IL-10 inhibitor, IL-2, IL-7, IL-21, IL-15, an antibody-drug conjugate, an anti-inflammatory drug, and a dietary supplement.~~
- ~~72 The method according to claim 71, wherein the checkpoint inhibitor is a CTLA-4, a PD-1, a PD-L1, or a PD-L2 inhibitor.~~
- ~~73 The method of claim 70, wherein the additional therapeutic agent is an inhibitor of LAG3, TIGIT, LAP, Podoplanin, Protein C receptor, ICOS, GITR, CD226 or CD160.~~
- ~~74 The method of claim 70, wherein the therapy is chemotherapy, radiotherapy, or surgery.~~
- ~~75 The method according to any of claims 70-74, wherein the additional therapeutic agent or therapy is administered concurrently or consecutively with the anti-PD-1 antibody, or an antigen-binding fragment thereof or the fusion protein.~~
- ~~76 The method according to any of claims 70-73, wherein the additional therapeutic agent is administered separately or as a mixture with the anti-PD-1 antibody, or an antigen-binding fragment thereof or the fusion protein.~~