

CLAIMS

We claim:

1. An anti-PD-1 antibody, or an antigen-binding fragment thereof, 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;
 - x. SEQ ID NOS: 88, 89, and 47;
 - xi. SEQ ID NOS: 66, 67, and 47;
 - xii. SEQ ID NOS: 80, 92, and 75;
 - xiii. SEQ ID NOS: 80, 94, and 71;
 - xiv. SEQ ID NOS: 99, 100, and 47;
 - xv. SEQ ID NOS: 102, 103, and 104;
 - xvi. SEQ ID NOS: 106, 103, and 47;
 - xvii. SEQ ID NOS: 108, 103, and 47;
 - xviii. SEQ ID NOS: 110, 111, and 75;
 - xix. SEQ ID NOS: 77, 103, and 113;
 - xx. SEQ ID NOS: 77, 111, and 47;
 - xxi. SEQ ID NOS: 116, 67, and 47;
 - xxii. SEQ ID NOS: 118, 119, and 47;
 - xxiii. SEQ ID NOS: 80, 78, and 47;
 - xxiv. SEQ ID NOS: 122, 103 and 47;
 - xxv. SEQ ID NOS: 124, 125, and 75;
 - xxvi. SEQ ID NOS: 127, 38, and 68;

- xxvii. SEQ ID NOS: 129, 130, and 47; or
 - xxviii. SEQ ID NOS: 132, 133, and 75.
2. The anti-PD-1 antibody, or an antigen-binding fragment thereof, of claim 1, 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 **Error!**
Reference source not found. 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 3, 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.
5. An anti-PD-1 antibody, or an antigen-binding fragment thereof, 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.
6. The anti-PD-1 antibody, or an antigen-binding fragment thereof, of claim 5, 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.
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.
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.

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 modification in the CH3 domain of first heavy chain is different from the modification in the CH3 domain of the second heavy chain.
11. The anti-PD-1 antibody, or an antigen-binding fragment thereof of 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).
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 wherein the one or more amino acid substitution is selected from L234A, L235A, and P329G (Kabat EU index numbering).
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.

15. The fusion protein of claim 13 or 14, 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.

16. The fusion protein of any one of claims 13-15, wherein the first linker (i) is between 5 and 40 amino acids long, optionally consists predominantly of Gly (G), Asn (N), Ser (S), Thr (T), Ala (A), Leu (L), and Gln (Q); and/or (ii) comprises a sequence selected from the group consisting of SEQ ID NOS:215-218 and 244, optionally comprises the sequence of SEQ ID NO:215.

17. The fusion protein of claim 15 or 16, wherein the second linker (i) is between 5 and 40 amino acids long, optionally consists predominantly of Gly (G), Asn (N), Ser (S), Thr (T), Ala (A), Leu (L), and Gln (Q); and/or (ii) comprises a sequence selected from the group consisting of SEQ ID NOS:215-218 and 244, optionally comprises the sequence SEQ ID NO:216.

18. The fusion protein of any one of claims 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.

19. The fusion protein of any one of claims 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, 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.
20. A fusion protein comprising a light chain sequence comprising SEQ ID NO:219 or SEQ ID NO:98 and a first heavy chain sequence comprising SEQ ID NO:240 and a second heavy chain sequence comprising SEQ ID NO:241.
21. A nucleic acid sequence 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.
22. A cell comprising the nucleic acid of claim 21, a vector comprising the nucleic acid of claim 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.
23. A pharmaceutical composition comprising (i) 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 and (ii) a pharmaceutically acceptable carrier.
24. Use of an 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 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.
25. Use of an 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 for the manufacture of a medicament for increasing T cell activation in a subject.

26. Use of an 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 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.

27. Use of an 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 for the manufacture of a medicament for treating cancer in a subject, optionally wherein the cancer is melanoma, pancreatic cancer, thyroid cancer, lung cancer, colorectal cancer, squamous cancer, prostate cancer, breast cancer, bladder cancer, or gastric cancer.

28. Use of an 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 for the manufacture of a medicament for reducing tumor growth or tumor metastasis in a subject.