

CLAIMS

1. An antibody or antigen-binding fragment thereof that
 - (a) binds to an epitope of human PD-1 recognized by an antibody comprising a light chain variable region having an amino acid sequence of SEQ ID NO:8 and a heavy chain variable region having an amino acid sequence of SEQ ID NO:13; or
 - (b) competes for the binding to human PD-1 with an antibody comprising a light chain variable region having an amino acid sequence of SEQ ID NO:8 and a heavy chain variable region having an amino acid sequence of SEQ ID NO:13.
2. An antibody or antigen-binding fragment thereof that binds to PD-1, wherein the antibody or antigen-binding fragment thereof comprises:
 - (a) a light chain variable region (VL) comprising VL complementarity determining region 1 (CDR1), VL CDR2, and VL CDR3 of any one of antibodies PD1AB-1, PD1AB-2, PD1AB-3, PD1AB-4, PD1AB-5, or PD1AB-6 as set forth in Table 1; and/or
 - (b) a heavy chain variable region (VH) comprising VH complementarity determining region 1 (CDR1), VH CDR2, and VH CDR3 of any one of antibodies PD1AB-1, PD1AB-2, PD1AB-3, PD1AB-4, PD1AB-5, or PD1AB-6 as set forth in Table 2.
3. The antibody or antigen-binding fragment thereof of claim 2, wherein the antibody or antigen-binding fragment thereof comprises:
 - (a) a VL further comprising VL framework 1 (FR1), VL FR2, VL FR3, and VL FR4 of any one of antibodies PD1AB-1, PD1AB-2, PD1AB-3, PD1AB-4, PD1AB-5, or PD1AB-6 as set forth in Table 3; and/or
 - (b) a VH further comprising VH framework 1 (FR1), VH FR2, VH FR3, and VH FR4 of any one of antibodies PD1AB-1, PD1AB-2, PD1AB-3, PD1AB-4, PD1AB-5, or PD1AB-6 as set forth in Table 4.

4. The antibody or antigen-binding fragment thereof of claim 2, wherein
 - (a) the VL CDR1, VL CDR2, and VL CDR3 comprise amino acid sequences of SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3, respectively, and the VH CDR1, VH CDR2, and VH CDR3 comprise amino acid sequences of SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6, respectively; or
 - (b) the VL CDR1, VL CDR2, and VL CDR3 comprise amino acid sequences of SEQ ID NO:7, SEQ ID NO:2, and SEQ ID NO:3, respectively, and the VH CDR1, VH CDR2, and VH CDR3 comprise amino acid sequences of SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6, respectively.
5. The antibody or antigen-binding fragment thereof of claim 2, wherein the antibody or antigen-binding fragment thereof comprises (i) a VL comprising an amino acid sequence of SEQ ID NO:8, SEQ ID NO:9, or SEQ ID NO:10; or (ii) a VH comprising an amino acid sequence of SEQ ID NO:11 or SEQ ID NO:12, or SEQ ID NO:13.
6. The antibody or antigen-binding fragment thereof of claim 2, wherein the antibody or antigen-binding fragment thereof comprises:
 - (a) a VL comprising an amino acid sequence of SEQ ID NO:8; and a VH comprising an amino acid sequence of SEQ ID NO:11;
 - (b) a VL comprising an amino acid sequence of SEQ ID NO:9; and a VH comprising an amino acid sequence of SEQ ID NO:11;
 - (c) a VL comprising an amino acid sequence of SEQ ID NO:10; and a VH comprising an amino acid sequence of SEQ ID NO:11;
 - (d) a VL comprising an amino acid sequence of SEQ ID NO:8; and a VH comprising an amino acid sequence of SEQ ID NO:12;
 - (e) a VL comprising an amino acid sequence of SEQ ID NO:9; and a VH comprising an amino acid sequence of SEQ ID NO:12;

- (f) a VL comprising an amino acid sequence of SEQ ID NO:10; and a VH comprising an amino acid sequence of SEQ ID NO:12;
 - (g) a VL comprising an amino acid sequence of SEQ ID NO:8; and a VH comprising an amino acid sequence of SEQ ID NO:13;
 - (h) a VL comprising an amino acid sequence of SEQ ID NO:9; and a VH comprising an amino acid sequence of SEQ ID NO:13; or
 - (i) a VL comprising an amino acid sequence of SEQ ID NO:10; and a VH comprising an amino acid sequence of SEQ ID NO:13.
- 7. The antibody or antigen-binding fragment thereof of any one of claims 1 to 6, wherein the antibody or antigen-binding fragment thereof comprises a human IgG1 Fc region, a human IgG4 Fc region, a human IgG4P Fc region, a human IgG4PE Fc region, or a mutant thereof.
 - 8. The antibody or antigen-binding fragment thereof of any one of claims 1 to 6, wherein the antibody or antigen-binding fragment thereof comprises a human IgG1-K322A Fc region.
 - 9. The antibody or antigen-binding fragment thereof of any one of claims 1 to 6, wherein the antibody or antigen-binding fragment thereof comprises a heavy chain Fc region comprising an amino acid sequence selected from the group consisting of SEQ ID NOS:36-40.
 - 10. The antibody or antigen-binding fragment thereof of claim 9, wherein the antibody or antigen-binding fragment thereof further comprises a light chain constant region comprising an amino acid sequence of SEQ ID NO:41.
 - 11. The antibody or antigen-binding fragment thereof of any one of claims 1 to 10, wherein the antibody or antigen-binding fragment thereof comprises a light chain comprising an amino acid sequence of SEQ ID NO:31.

12. The antibody or antigen-binding fragment thereof of claim any one of claims 1 to 10, wherein the antibody or antigen-binding fragment thereof comprises a heavy chain comprising an amino acid sequence of SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34 or SEQ ID NO:35.
13. The antibody or antigen-binding fragment thereof of any one of claims 1 to 10, wherein the antibody or antigen-binding fragment thereof comprises:
 - (a) a light chain comprising an amino acid sequence of SEQ ID NO:31; and a heavy chain comprising an amino acid sequence of SEQ ID NO:32;
 - (b) a light chain comprising an amino acid sequence of SEQ ID NO:31; and a heavy chain comprising an amino acid sequence of SEQ ID NO:33;
 - (c) a light chain comprising an amino acid sequence of SEQ ID NO:31; and a heavy chain comprising an amino acid sequence of SEQ ID NO:34; or
 - (d) a light chain comprising an amino acid sequence of SEQ ID NO:31; and a heavy chain comprising an amino acid sequence of SEQ ID NO:35.
14. The antibody or antigen-binding fragment thereof of any one of claims 1 to 13, wherein, when bound to PD-1, the antibody or antigen-binding fragment binds to at least one of residues 100-109 within an amino acid sequence of SEQ ID NO:42; wherein optionally the antibody or antigen-binding fragment binds to at least one of residues 100-105 within an amino acid sequence of SEQ ID NO:42.
15. The antibody or antigen-binding fragment thereof of any one of claims 1 to 13, wherein, when bound to PD-1, the antibody or antigen-binding fragment binds to at least one residue selected from the group consisting of N33, T51, S57, L100, N102, G103, R104, D105, H107, and S109 within an amino acid sequence of SEQ ID NO:42; wherein optionally the antibody or antigen-binding fragment binds to G103 and R104.
16. The antibody or antigen-binding fragment thereof of any one of claims 1 to 15, wherein the antibody or antigen-binding fragment thereof:

- (a) (i) attenuates T cell activity;

wherein optionally the attenuation of T cell activity occurs in human PBMC or whole blood samples; and/or the attenuation of T cell activity is measured by inhibition of cytokine production; wherein optionally the cytokine comprises IL-1, IL-2, IL-6, IL-12, IL-17, IL-22, IL-23, GM-CSF, TNF- α , and/or IFN- γ ;

wherein optionally the maximal percent attenuation of T cell activity is at least about 10%, 20%, 30%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%;

wherein optionally the EC₅₀ for attenuating T cell activity is from about 1 pM to about 10 pM, from about 10 pM to about 100 pM, from about 100 pM to about 1 nM, from about 1 nM to about 10 nM, or from about 10 nM to about 100 nM; and/or

- (ii) downregulates PD-1 expression on the surface of T cells

wherein optionally the downregulation of PD-1 expression on the surface of T cells occurs as early as 4 hours after the treatment with the antibody or antigen-binding fragment thereof; and/or is concurrent with or precedes cytokine inhibition;

wherein optionally the maximal percent downregulation of PD-1 expression is at least about 10%, 20%, 30%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%;

- (b) specifically binds to human PD-1 and/or monkey PD-1, but not rodent PD-1;

wherein optionally the K_D for binding to purified human PD-1 is from about 100 pM to about 10 nM, and the K_D for binding to human PD-1 expressed on cell surface and monkey PD-1 expressed on cell surface is from about 100 pM to about 10 nM; and/or

- (c) has attenuated ADCC activity and/or attenuated CDC activity.
17. The antibody or antigen-binding fragment thereof of any one of claims 1-16, wherein
- (a) the antibody is a monoclonal antibody;
 - (b) the antibody is a humanized, human, or chimeric antibody; wherein optionally the humanized antibody is a deimmunized antibody or a composite human antibody;
 - (c) the antibody or antigen-binding fragment thereof is a Fab, a Fab', a F(ab')₂, a Fv, a scFv, a dsFv, a diabody, a triabody, a tetrabody, or a multispecific antibody formed from antibody fragments; and/or
 - (d) the antibody or antigen-binding fragment thereof is conjugated to an agent, wherein optionally the agent selected from the group consisting of a radioisotope, a metal chelator, an enzyme, a fluorescent compound, a bioluminescent compound, and a chemiluminescent compound.
18. A composition comprising the antibody or antigen-binding fragment thereof of any one of claims 1-17, and a pharmaceutically acceptable carrier.
19. A polynucleotide comprising nucleotide sequences encoding (a) a VH, a VL, or both a VH and a VL; or (b) a heavy chain, a light chain, or both a heavy chain and a light chain of the antibody of any one of claims 1-18; wherein optionally the polynucleotide is operably linked to a promoter.
20. A vector comprising the polynucleotide of claim 19.
21. A cell comprising the polynucleotide of claim 19, or the vector of claim 20.
22. An isolated cell producing the antibody or antigen-binding fragment thereof of any one of claims 1 to 17.
23. A kit comprising the antibody or antigen-binding fragment thereof of any one of claims 1 to 17.

24. A method of making an antibody or antigen-binding fragment thereof which specifically binds to an epitope of human PD-1, comprising: (a) culturing the cell of claim 21 or 22 to express the antibody or antigen-binding fragment thereof; or (b) expressing the polynucleotide of claim 19.
25. An antibody or antigen-binding fragment thereof as defined in any one of claims 1 to 17, for use in a method of attenuating activity of a T cell, wherein optionally
- (a) the maximal percent attenuation of T cell activity is at least about 10%, 20%, 30%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%; and/or
 - (b) the attenuation of T cell activity is measured by inhibition of cytokine production; wherein optionally the cytokine is IL-1, IL-2, IL-6, IL-12, IL-17, IL-22, IL-23, GM-CSF, TNF- α , IFN- γ , or any combination thereof; wherein optionally the inhibition of cytokine production is concurrent with or follows downregulation of PD-1 expression on the surface of the T cell; wherein optionally the downregulation of PD-1 expression on the surface of the T cell occurs as early as 4 hours after contact with an effective amount of an antibody or antigen-binding fragment thereof.
26. An antibody or antigen-binding fragment thereof as defined in any one of claims 1 to 17, for use in a method of downregulating PD-1 expression on the surface of a T cell, wherein optionally
- (a) the maximal percent downregulation of PD-1 expression by the antibody or antigen-binding fragment thereof is at least about 10%, 20%, 30%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%;
 - (b) the downregulation of PD-1 expression on the surface of the T cell occurs as early as 4 hours after contact with the antibody or antigen-binding fragment thereof; and/or

(c) the downregulation of PD-1 expression on the surface of the T cell precedes cytokine inhibition;

wherein optionally the cytokine is IL-1, IL-2, IL-6, IL-12, IL-17, IL-22, IL-23, GM-CSF, TNF- α , IFN- γ , or any combination thereof.

27. An antibody or antigen-binding fragment thereof as defined in any one of claims 1 to 17, for use in a method of inhibiting cytokine production by a T cell, wherein optionally the cytokine is IL-1, IL-2, IL-6, IL-12, IL-17, IL-22, IL-23, GM-CSF, TNF- α , IFN- γ , or any combination thereof.