

[005] PD-1 has been shown to play a role in cancer. In humans, expression of PD-1 and/or PD-L1 has been found in a number of primary tumor biopsies assessed by immunohistochemistry. Such tissues include cancers of the lung, liver, ovary, cervix, skin, colon, glioma, bladder, breast, kidney, esophagus, stomach, oral squamous cell, urothelial cell, and pancreas as well as tumors of the head and neck. Furthermore, PD-ligand expression on tumor cells has been correlated to poor prognosis of cancer patients across multiple tumor types.

[006] There is an ongoing need for new therapeutics, including antibodies and other antigen-binding proteins that target PD-1 and function either as agonists of PD-1 or antagonists thereof.

Summary

[007] The present invention describes antigen-binding proteins such as antibodies and chimeric antigen receptors that are able to specifically bind a protein receptor associated with programmed cell death, PD-1, on T cells, thereby modulating immune response by the T cells. By inhibiting the binding of PD-1 to its ligand, PD-L1, blockade of the PD-1 signaling pathway inhibits apoptosis of the T cells.

The present invention relates to the following:

In one aspect, the present invention refers to a recombinant antigen-binding protein or antigen-binding fragment thereof comprising one of:

(A) an antigen binding region comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 10, SEQ ID NO: 21, SEQ ID NO: 32, SEQ ID NO: 43, SEQ ID NO: 53, SEQ ID NO: 61, SEQ ID NO: 72, SEQ ID NO: 83, SEQ ID NO: 94, SEQ ID NO:

103, SEQ ID NO: 114, SEQ ID NO: 125, SEQ ID NO: 133, SEQ ID NO: 142; a fragment thereof and a homologous sequence thereof;

(B) an antigen binding region comprising a variable light chain (VL) and variable heavy chain (VH), respectively, with amino acid sequences selected from SEQ ID NOS: 6 and 8; SEQ ID NOS: 17 and 19; SEQ ID NOS: 28 and 30; SEQ ID NOS: 39 and 41; SEQ ID NOS: 49 and 51; SEQ ID NOS: 57 and 59; SEQ ID NOS: 68 and 70; SEQ ID NOS: 79 and 81; SEQ ID NOS: 90 and 92; SEQ ID NOS: 99 and 101; SEQ ID NOS: 110 and 112; SEQ ID NOS: 121 and 123; SEQ ID NOS: 129 and 131; SEQ ID NOS: 138 and 140; fragments thereof and homologous sequences thereof;

(C) an antigen binding region comprising:

(i) a light chain (LC) comprising light chain complementarity determining regions (LCCDR) LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence QSISSY (SEQ ID NO: 1), AAS and QQSYSTPLT (SEQ ID NO: 2) and a heavy chain (HC) comprising heavy chain complementarity determining regions (HCCDR) HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GFTSSSYW (SEQ ID NO: 4), IKQDGSEK (SEQ ID NO: 5) and ARGGWSYDM (SEQ ID NO: 6); fragments thereof and homologous sequences thereof;

(ii) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence SSNIGAGYA (SEQ ID NO: 12), TNN and QSYDSSLSGVI (SEQ ID NO: 13) and a heavy chain (HC) comprising HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GYTLTELS (SEQ ID NO: 14), FDPEDGET (SEQ ID NO: 15) and ARAYYGFDQ (SEQ ID NO: 16); fragments thereof and homologous sequences thereof;

(iii) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence SSNIGNNA (SEQ ID NO: 23), YND and AAWDDSVNGYV (SEQ ID NO: 24) and a heavy chain (HC) comprising HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GYTFTRFG (SEQ ID NO: 25), ISVNNGNT (SEQ ID NO. 26) and ARYMYGRRDS (SEQ ID NO: 27); fragments thereof and homologous sequences thereof;

(iv) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence NIGSKS (SEQ ID NO: 34), YDS and QWWDNHSDVV (SEQ ID NO: 35) and a heavy chain (HC) comprising HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences RNKFSSYA (SEQ ID NO: 36), ISGSGGTT (SEQ ID NO. 37) and ARWYSSYYDV (SEQ ID NO: 38); fragments thereof and homologous sequences thereof;

(v) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence NIGSKS (SEQ ID NO: 34), YDS and QWWDSSSDYV (SEQ ID NO: 45) and a heavy chain (HC) comprising HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GFTFSSYA (SEQ ID NO: 46), ISGSGGST (SEQ ID NO. 47) and ARNYISMFDS (SEQ ID NO: 48); fragments thereof and homologous sequences thereof;

(vi) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence NIGSKS (SEQ ID NO: 34), YDS and QWWDSSSDHV (SEQ ID NO: 55) and a heavy chain (HC) comprising HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GFTFSSYA (SEQ ID NO: 46), ISGSGGST (SEQ ID NO. 47) and ARGYSSYYDA (SEQ ID NO: 56); fragments thereof and homologous sequences thereof;

(vii) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence RSNIGENT (SEQ ID NO: 63), SNN and AAWDDRLNGYV (SEQ ID NO: 64) and a heavy chain (HC) comprising HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GYTFTNYG (SEQ ID NO: 65), IGAQKGDT (SEQ ID NO. 66) and ARSQGVPFDS (SEQ ID NO: 67); fragments thereof and homologous sequences thereof;

(viii) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence RSNIGSNT (SEQ ID NO: 74), NNN and ATWDDSLNEYV (SEQ ID NO: 75) and a heavy chain (HC) comprising HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GYTFTTRYG (SEQ ID NO: 76), ISGYNGNT (SEQ ID NO. 77) and ARHGYGYHGD (SEQ ID NO: 78); fragments thereof and homologous sequences thereof;

(ix) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence SSNIGAGYV (SEQ ID NO: 85), HNN and QSYDSSLGWW (SEQ ID NO: 86) and a heavy chain (HC) comprising HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GFTFKDYY (SEQ ID NO: 87), ISTSGNSV (SEQ ID NO. 88) and ARSPGHSDYDS (SEQ ID NO: 89); fragments thereof and homologous sequences thereof;

(x) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence NIGDKS (SEQ ID NO: 96), YDS and QVWASGTDHPYVI (SEQ ID NO: 97) and a heavy chain (HC) comprising HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GFTFSSYA (SEQ ID NO: 46), ISGSGGST (SEQ ID NO. 47) and ARMYGSYTDM (SEQ ID NO: 98); fragments thereof and homologous sequences thereof;

(xi) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence SSNIGYNY (SEQ ID NO: 105), RNN and TSWDDSLSGYV (SEQ ID NO: 106) and a heavy chain (HC) comprising HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GNAFTNFY (SEQ ID NO: 107), INPSGTDLT (SEQ ID NO. 108) and ARQYAYGYSGFDM (SEQ ID NO: 109); fragments thereof and homologous sequences thereof;

(xii) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence QSVSNW (SEQ ID NO: 116), AAS and QQSYSTPIT (SEQ ID NO: 117) and a heavy chain (HC) comprising HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GYTFTSY (SEQ ID NO: 118), INPNTGGS (SEQ ID NO. 119) and ARGDVITYDE (SEQ ID NO: 120); fragments thereof and homologous sequences thereof;

(xiii) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence NIGSKS (SEQ ID NO: 34), YDD and QVWDINDHYV (SEQ ID NO: 127) and a heavy chain (HC) comprising HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GFTFSSYA (SEQ ID NO: 46), ISGSGGST (SEQ ID NO. 47) and ARSQASFMDI (SEQ ID NO: 128); fragments thereof and homologous sequences thereof; or

(xiv) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence NIGSKS (SEQ ID NO: 34), DDS and QVWDSSSDQGV (SEQ ID NO: 135) and a heavy chain (HC) comprising HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GFTFSSYA (SEQ ID NO: 46), IGTGGGT (SEQ ID NO. 136) and ARGTYDGDQ (SEQ ID NO: 137); fragments thereof and homologous sequences thereof;

wherein said antigen-binding protein specifically binds to programmed cell death (PD-1).

In another aspect, the present invention refers to a nucleic acid encoding an antigen-binding protein of the present invention.

In yet another aspect, the present invention refers to a vector comprising a nucleic acid of the present invention.

In yet another aspect, the present invention refers to a cell comprising an antigen-binding protein of the present invention, a nucleic acid of the present invention or a vector comprising a nucleic acid of the present invention.

In another aspect, the present invention refers to a pharmaceutical composition comprising an antigen-binding protein of the present invention, a nucleic acid of the present invention, a vector of the present invention, a cell of the present invention.

In yet another aspect, the present invention refers to the use of an antigen-binding protein or an antigen binding fragment thereof of the present invention, a nucleic acid of the present invention, a vector of the present invention, a cell of the present invention, or a pharmaceutical composition the present invention in the manufacture of a medicament for treating cancer.

In another aspect, the present invention refers to a vector comprising a nucleic acid encoding a recombinant anti-PD-1 antigen-binding protein and a nucleic acid encoding a chimeric antigen receptor, wherein said recombinant anti-PD-1 antigen-binding protein is not identical to said chimeric antigen receptor.

In yet another aspect, the present invention refers to a cell comprising the vector of the present invention.

In yet another aspect, the present invention refers to a cell comprising a nucleic acid encoding a recombinant anti-PD-1 antigen-binding protein and a nucleic acid encoding a chimeric antigen receptor, wherein said recombinant anti-PD-1 antigen-binding protein is not identical to said chimeric antigen receptor.

In yet another aspect, the present invention refers to a cell comprising a recombinant anti-PD-1 antigen-binding protein and a chimeric antigen receptor, wherein said recombinant anti-PD-1 antigen-binding protein is not identical to said chimeric antigen receptor.

In yet another aspect, the present invention refers to a pharmaceutical composition comprising a vector or a cell of the present invention.

In yet another invention, the present invention refers to the use of a vector or cell of the present invention, or a pharmaceutical composition of the present invention, in the manufacture of a medicament for treating cancer, wherein the recombinant anti-PD-1 antigen-binding protein is a PD-1 antagonist.

Also disclosed herein is:

[008] In one example, the disclosure relates to recombinant antigen-binding proteins, antibodies and chimeric antigen receptors or antigen-binding portions thereof that bind specifically to PD-1 and prevent binding of its ligand.

[009] In one example, the disclosure relates to a recombinant antigen-binding protein or antigen-binding fragment thereof comprising one of:

(A) an antigen binding region having an amino acid sequence selected from the group consisting of SEQ ID NO: 10, SEQ ID NO: 21, SEQ ID NO: 32, SEQ ID NO: 43, SEQ ID NO: 53, SEQ ID NO: 61, SEQ ID NO: 72, SEQ ID NO: 83, SEQ ID NO: 94, SEQ ID NO: 103, SEQ ID NO: 114, SEQ ID NO: 125, SEQ ID NO: 133, SEQ ID NO: 142; a fragment thereof, and a homologous sequence thereof;

(B) an antigen binding region comprising a variable light chain (VL) and variable heavy chain (VH), respectively, with amino acid sequences selected from SEQ ID NOS: 6 and 8; SEQ ID NOS: 17 and 19; SEQ ID NOS: 28 and 30; SEQ ID NOS: 39 and 41; SEQ ID NOS: 49 and 51; SEQ ID NOS: 57 and 59; SEQ ID NOS: 68 and 70; SEQ ID NOS: 79 and 81; SEQ ID NOS: 90 and 92; SEQ ID NOS: 99 and 101; SEQ ID NOS: 110 and 112; SEQ ID NOS: 121 and 123; SEQ ID NOS: 129 and 131; SEQ ID NOS: 138 and 140; fragments thereof, and homologous sequences thereof; or

(C) an antigen binding region comprising:

(i) a light chain (LC) comprising light chain complementarity determining regions (LCCDR) LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence QSISSY (SEQ ID NO: 1), AAS and QQSYSTPLT (SEQ ID NO: 2) and a heavy chain (HC) comprising heavy chain complementarity determining regions (HCCDR) HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GFTSSSYW (SEQ ID NO: 4), IKQDGSEK (SEQ ID NO: 5) and ARGGWSYDM (SEQ ID NO: 6); fragments thereof or homologous sequences thereof;

(ii) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence SSNIGAGYA (SEQ ID NO: 12), TNN and QSYDSSLGVI (SEQ ID NO: 13) and a heavy chain (HC) comprising

HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GYTLTELS (SEQ ID NO: 14), FDPEDGET (SEQ ID NO. 15) and ARAYYGFDQ (SEQ ID NO: 16); fragments thereof or homologous sequences thereof;

(iii) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence SSNIGNNA (SEQ ID NO: 23), YND and AAWDDSVNGYV (SEQ ID NO: 24) and a heavy chain (HC) comprising HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GYTFTRFG (SEQ ID NO: 25), ISVNNGNT (SEQ ID NO. 26) and ARYMYGRRDS (SEQ ID NO: 27); fragments thereof or homologous sequences thereof;

(iv) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence NIGSKS (SEQ ID NO: 34), YDS and QVWDNHSDVV (SEQ ID NO: 35) and a heavy chain (HC) comprising HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences RNKFSSYA (SEQ ID NO: 36), ISGSGGTT (SEQ ID NO. 37) and ARWYSSYYDV (SEQ ID NO: 38); fragments thereof or homologous sequences thereof;

(v) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence NIGSKS (SEQ ID NO: 34), YDS and QVWDSSSDYV (SEQ ID NO: 45) and a heavy chain (HC) comprising HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GFTFSSYA (SEQ ID NO: 46), ISGSGGST (SEQ ID NO. 47) and ARNYISMFDS (SEQ ID NO: 48); fragments thereof or homologous sequences thereof;

(vi) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence NIGSKS (SEQ ID NO: 34), YDS and QVWDSSSDHV (SEQ ID NO: 55) and a heavy chain (HC) comprising HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GFTFSSYA (SEQ ID NO: 46), ISGSGGST (SEQ ID NO. 47) and ARGYSSYYDA (SEQ ID NO: 56); fragments thereof or homologous sequences thereof;

(vii) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence RSNIGENT (SEQ ID NO: 63), SNN and AAWDDRLNGYV (SEQ ID NO: 64) and a heavy chain (HC) comprising HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GYTFTNYG (SEQ ID NO: 65), IGAQKGDT (SEQ ID NO. 66) and ARSQGVPFDS (SEQ ID NO: 67); fragments thereof or homologous sequences thereof;

(viii) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence RSNIGSNT (SEQ ID NO: 74), NNN and ATWDDSLNEYV (SEQ ID NO: 75) and a heavy chain (HC) comprising HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GYTFTRYG (SEQ ID NO: 76), ISGYNGNT (SEQ ID NO. 77) and ARHGYGYHGD (SEQ ID NO: 78); fragments thereof or homologous sequences thereof;

(ix) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence SSNIGAGYV (SEQ ID NO: 85), HNN and QSYDSSLGWW (SEQ ID NO: 86) and a heavy chain (HC) comprising HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GFTFKDYY (SEQ ID NO: 87), ISTSGNSV (SEQ ID NO. 88) and

ARSPGHSDYDS (SEQ ID NO: 89); fragments thereof or homologous sequences thereof;

(x) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence NIGDKS (SEQ ID NO: 96), YDS and QWWASGTDHPYVI (SEQ ID NO: 97) and a heavy chain (HC) comprising HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GFTFSSYA (SEQ ID NO: 46), ISGSGGST (SEQ ID NO. 47) and ARMYGSYTDM (SEQ ID NO: 98); and a fragment or homologous sequence thereof;

(xi) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence SSNIGYNY (SEQ ID NO: 105), RNN and TSWDDSLSGYV (SEQ ID NO: 106) and a heavy chain (HC) comprising HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GNAFTNFY (SEQ ID NO: 107), INPSGTDLT (SEQ ID NO. 108) and ARQYAYGYSGFDM (SEQ ID NO: 109); fragments thereof or homologous sequences thereof;

(xii) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence QSVSNW (SEQ ID NO: 116), AAS and QQSYSTPIT (SEQ ID NO: 117) and a heavy chain (HC) comprising HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GYTFTSYY (SEQ ID NO: 118), INPNTGGS (SEQ ID NO. 119) and ARGDVITYDE (SEQ ID NO: 120); fragments thereof or homologous sequences thereof;

(xiii) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence NIGSKS (SEQ ID NO: 34), YDD and QVWDINDHYV (SEQ ID NO: 127) and a heavy chain (HC) comprising

HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GFTFSSYA (SEQ ID NO: 46), ISGSGGST (SEQ ID NO. 47) and ARSQASFMDI (SEQ ID NO: 128); fragments thereof or homologous sequences thereof; or

(xiv) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence NIGSKS (SEQ ID NO: 34), DDS and QVWDSSSDQGV (SEQ ID NO: 135) and a heavy chain (HC) comprising HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GFTFSSYA (SEQ ID NO: 46), IGTGGGT (SEQ ID NO. 136) and ARGTYDGDQ (SEQ ID NO: 137) and fragments thereof or homologous sequences thereof.

[0010] In some examples, the recombinant antigen-binding protein or antigen-binding fragment thereof comprises a fragment of at least one of the recited SEQ ID NOS that is at least 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% of the entire length of the at least one recited SEQ ID NO. In some examples, the recombinant antigen-binding protein or antigen-binding fragment thereof comprises a sequence homologous to at least one of the recited SEQ ID NOS that has at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 99.9% identity to the at least one recited SEQ ID NO.

[0011] In another example, the disclosure relates to recombinant antigen-binding proteins or antigen-binding fragments thereof, wherein the recombinant antigen-binding protein is an antibody, chimeric antigen receptor (CAR), fusion protein or conjugate thereof. In an example, the recombinant antigen-binding protein or antigen-binding fragment thereof is conjugated to a

therapeutic agent, for example, a drug, toxin or cytotoxic moiety, radioisotope, protein or peptide.

[0012] In another example, an antibody of the disclosure is a full-length antibody, an intact antibody, fragments and homologous sequences thereof, including but not limited to, an Fab fragment, an F(ab')₂ fragment or a single chain variable fragment (scFv).

[0013] In another example, in the recombinant antigen-binding protein, the antigen-binding region specifically binds to an epitope of human PD-1 and blocks binding of PD-1 to its ligand(s).

[0014] In an example, the disclosure relates to nucleic acids encoding an antigen-binding protein of the disclosure as well as vectors and cells comprising such nucleic acids or antigen-binding proteins.

[0015] In yet another example, the disclosure relates to a method of increasing a T cell response in a subject comprising administering a therapeutically effective amount of an antigen-binding protein or an antigen binding fragment thereof. The administration of a therapeutically effective amount of the antigen-binding protein or antigen binding fragment thereof inhibits, reduces, modulates or abolishes signal transduction mediated by PD-1.

[0016] In yet another example, the disclosure relates to a method for treatment of a subject having a PD1-positive disease comprising administering to the subject a therapeutically effective amount of an antigen-binding protein or antigen binding fragment thereof. In an example, a pharmaceutical composition comprising the antigen-binding protein or antigen binding fragment thereof is administered.

[0017] In another example, the disclosure relates to a vector comprising a nucleic acid encoding a recombinant anti-PD-1 antigen-binding protein and a nucleic acid encoding a chimeric antigen receptor, wherein said recombinant anti-PD-1 antigen-binding protein is not identical to said chimeric antigen receptor.

[0018] In yet another example, the disclosure relates to a cell comprising the vector described herein. In a related aspect, the disclosure relates to a cell comprising a nucleic acid encoding a recombinant anti-PD-1 antigen-binding protein and a nucleic acid encoding a chimeric antigen receptor, wherein said recombinant anti-PD-1 antigen-binding protein is not identical to said chimeric antigen receptor. In another related aspect, the disclosure relates to a cell comprising a recombinant anti-PD-1 antigen-binding protein and a chimeric antigen receptor, wherein said recombinant anti-PD-1 antigen-binding protein is not identical to said chimeric antigen receptor.

[0019] In some examples of the vectors or the cells described herein, the chimeric antigen receptor does not specifically bind to PD-1.

[0020] In another example, the disclosure provides a method of increasing a T cell response in a subject comprising administering to the subject a therapeutically effective amount of a recombinant anti-PD-1 antigen-binding protein, a vector, a cell, or a pharmaceutical composition described herein, wherein the recombinant anti-PD-1 antigen-binding protein is a PD-1 antagonist.

[0021] In yet another example, the disclosure provides a method of decreasing a T cell response in a subject comprising administering to the subject a therapeutically effective amount of a recombinant anti-PD-1 antigen-binding

protein, a vector, a cell, or a pharmaceutical composition described herein, wherein the recombinant anti-PD-1 antigen-binding protein is a PD-1 agonist.

[0022] In another example, the disclosure provides a method for treatment of a subject having a PD1-positive disease, comprising administering to the subject a therapeutically effective amount of a recombinant anti-PD-1 antigen-binding protein, a vector, a cell, or a pharmaceutical composition described herein.

[0023] In another example, the disclosure provides a method for treatment of a subject having a PD1-positive disease, comprising transducing at least one T cell of the subject with a nucleic acid encoding a recombinant anti-PD-1 antigen-binding protein and a nucleic acid encoding a chimeric antigen receptor, wherein said recombinant anti-PD-1 antigen-binding protein is not identical to said chimeric antigen receptor.

[0024] In some examples of the methods described herein, the chimeric antigen receptor does not specifically bind to PD-1. In some examples, the PD1-positive disease is a cancer. In some examples, the PD1-positive disease is an autoimmune disease.

Brief Description of the Drawings

[0025] **Figure 1** shows relative binding affinity of scFv-Fc clones to PD-1 monomer. The ability of scFv-Fc clones to bind PD-1 was determined by detecting level of binding to PD-1 monomer. Binding affinity of the scFv-Fc clones was ranked where clone 31 bound weakly and clones 26 and 27 bound the most (31 < 23 < 40 < 18 < 16 = 27 < 26.)

CLAIMS

We claim:

1. A recombinant antigen-binding protein or antigen-binding fragment thereof comprising one of:

(A) an antigen binding region comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 10, SEQ ID NO: 21, SEQ ID NO: 32, SEQ ID NO: 43, SEQ ID NO: 53, SEQ ID NO: 61, SEQ ID NO: 72, SEQ ID NO: 83, SEQ ID NO: 94, SEQ ID NO: 103, SEQ ID NO: 114, SEQ ID NO: 125, SEQ ID NO: 133, SEQ ID NO: 142; a fragment thereof and a homologous sequence thereof;

(B) an antigen binding region comprising a variable light chain (VL) and variable heavy chain (VH), respectively, with amino acid sequences selected from SEQ ID NOS: 6 and 8; SEQ ID NOS: 17 and 19; SEQ ID NOS: 28 and 30; SEQ ID NOS: 39 and 41; SEQ ID NOS: 49 and 51; SEQ ID NOS: 57 and 59; SEQ ID NOS: 68 and 70; SEQ ID NOS: 79 and 81; SEQ ID NOS: 90 and 92; SEQ ID NOS: 99 and 101; SEQ ID NOS: 110 and 112; SEQ ID NOS: 121 and 123; SEQ ID NOS: 129 and 131; SEQ ID NOS: 138 and 140; fragments thereof and homologous sequences thereof;

(C) an antigen binding region comprising:
(i) a light chain (LC) comprising light chain complementarity determining regions (LCCDR) LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence QSISSY (SEQ ID NO: 1), AAS and QQSYSTPLT (SEQ ID NO: 2) and a heavy chain (HC) comprising heavy chain complementarity determining regions (HCCDR) HCCDR1, HCCDR2 and HCCDR3 respectively,

having amino acid sequences GFTSSSYW (SEQ ID NO: 4), IKQDGSEK (SEQ ID NO: 5) and ARGGWSYDM (SEQ ID NO: 6); fragments thereof and homologous sequences thereof;

(ii) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence SSNIGAGYA (SEQ ID NO: 12), TNN and QSYDSSLSGVI (SEQ ID NO: 13) and a heavy chain (HC) comprising HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GYTLTELS (SEQ ID NO: 14), FDPEDGET (SEQ ID NO: 15) and ARAYYGFDQ (SEQ ID NO: 16); fragments thereof and homologous sequences thereof;

(iii) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence SSNIGNNA (SEQ ID NO: 23), YND and AAWDDSVNGYV (SEQ ID NO: 24) and a heavy chain (HC) comprising HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GYTFTRFG (SEQ ID NO: 25), ISVNNGNT (SEQ ID NO: 26) and ARYMYGRRDS (SEQ ID NO: 27); fragments thereof and homologous sequences thereof;

(iv) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence NIGSKS (SEQ ID NO: 34), YDS and QVWDNHSDVV (SEQ ID NO: 35) and a heavy chain (HC) comprising HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences RNKFSSYA (SEQ ID NO: 36), ISGSGGTT (SEQ ID NO: 37) and ARWYSSYYDV (SEQ ID NO: 38); fragments thereof and homologous sequences thereof;

(v) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence NIGSKS (SEQ ID NO: 34), YDS

and QVWDSSSDYV (SEQ ID NO: 45) and a heavy chain (HC) comprising HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GFTFSSYA (SEQ ID NO: 46), ISGSGGST (SEQ ID NO. 47) and ARNYISMFDS (SEQ ID NO: 48); fragments thereof and homologous sequences thereof;

(vi) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence NIGSKS (SEQ ID NO: 34), YDS and QVWDSSSDHV (SEQ ID NO: 55) and a heavy chain (HC) comprising HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GFTFSSYA (SEQ ID NO: 46), ISGSGGST (SEQ ID NO. 47) and ARGYSSYYDA (SEQ ID NO: 56); fragments thereof and homologous sequences thereof;

(vii) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence RSNIGENT (SEQ ID NO: 63), SNN and AAWDDRLNGYV (SEQ ID NO: 64) and a heavy chain (HC) comprising HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GYTFTNYG (SEQ ID NO: 65), IGAQKGDT (SEQ ID NO. 66) and ARSQGVPFDS (SEQ ID NO: 67); fragments thereof and homologous sequences thereof;

(viii) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence RSNIGSNT (SEQ ID NO: 74), NNN and ATWDDSLNEYV (SEQ ID NO: 75) and a heavy chain (HC) comprising HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GYTFTRYG (SEQ ID NO: 76), ISGYNGNT (SEQ ID NO. 77) and ARHGYGYHGD (SEQ ID NO: 78); fragments thereof and homologous sequences thereof;

(ix) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence SSNIGAGYV (SEQ ID NO: 85), HNN and QSYDSSLGWW (SEQ ID NO: 86) and a heavy chain (HC) comprising HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GFTFKDYY (SEQ ID NO: 87), ISTSGNSV (SEQ ID NO. 88) and ARSPGHSDYDS (SEQ ID NO: 89); fragments thereof and homologous sequences thereof;

(x) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence NIGDKS (SEQ ID NO: 96), YDS and QVWASGTDHPYVI (SEQ ID NO: 97) and a heavy chain (HC) comprising HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GFTFSSYA (SEQ ID NO: 46), ISGSGGST (SEQ ID NO. 47) and ARMYGSYTDM (SEQ ID NO: 98); fragments thereof and homologous sequences thereof;

(xi) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence SSNIGYNY (SEQ ID NO: 105), RNN and TSWDDSLSGYV (SEQ ID NO: 106) and a heavy chain (HC) comprising HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GNAFTNFY (SEQ ID NO: 107), INPSGTDLT (SEQ ID NO. 108) and ARQYAYGYSGFDM (SEQ ID NO: 109); fragments thereof and homologous sequences thereof;

(xii) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence QSVSNW (SEQ ID NO: 116), AAS and QQSYSTPIT (SEQ ID NO: 117) and a heavy chain (HC) comprising HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GYTFTSY (SEQ ID NO: 118), INPNTGGS (SEQ ID NO. 119) and

ARGDVTYDE (SEQ ID NO: 120); fragments thereof and homologous sequences thereof;

(xiii) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence NIGSKS (SEQ ID NO: 34), YDD and QVWDINDHYV (SEQ ID NO: 127) and a heavy chain (HC) comprising HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GFTFSSYA (SEQ ID NO: 46), ISGSGGST (SEQ ID NO. 47) and ARSQASFMDI (SEQ ID NO: 128); fragments thereof and homologous sequences thereof; or

(xiv) a light chain (LC) comprising LCCDR1, LCCDR2 and LCCDR3 respectively, having the amino acid sequence NIGSKS (SEQ ID NO: 34), DDS and QVWDSSSDQGV (SEQ ID NO: 135) and a heavy chain (HC) comprising HCCDR1, HCCDR2 and HCCDR3 respectively, having amino acid sequences GFTFSSYA (SEQ ID NO: 46), IGTGGGT (SEQ ID NO. 136) and ARGTTYDGDQ (SEQ ID NO: 137); fragments thereof and homologous sequences thereof;

wherein said antigen-binding protein specifically binds to programmed cell death (PD-1).

2. The recombinant antigen-binding protein of claim 1, wherein said protein is an antibody.

3. The recombinant antigen-binding protein of claim 2, wherein the antibody is a human antibody.

4. The recombinant antigen-binding protein of claim 2, wherein said antibody or antigen-binding fragment thereof is intact Ig, Fab, F(ab')₂, Fv, or scFv.

5. The antigen-binding protein of claim 1, wherein said antigen-binding protein is a PD-1 agonist.

6. The antigen-binding protein of claim 1, wherein said antigen-binding protein is a PD-1 antagonist.

7. The antigen-binding protein of claim 1, wherein said antigen-binding protein is a chimeric antigen receptor (CAR).

8. A nucleic acid encoding an antigen-binding protein of any one of claims 1-7.

9. A vector comprising a nucleic acid of claim 8.

10. A cell comprising an antigen-binding protein of any one of claims 1-7, a nucleic acid of claim 8 or a vector of claim 9.

11. A pharmaceutical composition comprising an antigen-binding protein of any one of claims 1-7, a nucleic acid of claim 8, a vector of claim 9, a cell of claim 10.

12. Use of an antigen-binding protein or an antigen binding fragment thereof of any one of claims 1-7, a nucleic acid of claim 8, a vector of claim 9, a cell of claim 10, or a pharmaceutical composition of claim 11 in the manufacture of a medicament for treating cancer.

13. The use of claim 12, wherein the antigen-binding protein or antigen binding fragment thereof inhibits, reduces, modulates or abolishes signal transduction mediated by PD-1.

14. A vector comprising a nucleic acid encoding a recombinant anti-PD-1 antigen-binding protein and a nucleic acid encoding a chimeric antigen receptor, wherein said recombinant anti-PD-1 antigen-binding protein is not identical to said chimeric antigen receptor.

15. A cell comprising the vector of claim 14.

16. A cell comprising a nucleic acid encoding a recombinant anti-PD-1 antigen-binding protein and a nucleic acid encoding a chimeric antigen receptor, wherein said recombinant anti-PD-1 antigen-binding protein is not identical to said chimeric antigen receptor.

17. A cell comprising a recombinant anti-PD-1 antigen-binding protein and a chimeric antigen receptor, wherein said recombinant anti-PD-1 antigen-binding protein is not identical to said chimeric antigen receptor.

18. The vector or the cell of any one of claims 14-17, wherein the chimeric antigen receptor does not specifically bind to PD-1.

19. The vector or the cell of any one of claims 14-18, wherein the recombinant anti-PD-1 antigen-binding protein is an antibody.

20. The vector or the cell of any one of claims 14-19, wherein the recombinant anti-PD-1 antigen-binding protein is a human antibody.

21. The vector or the cell of any one of claims 14-20, wherein the recombinant anti-PD-1 antigen-binding protein is an intact Ig, Fab, F(ab')₂, Fv, or scFv.

22. The vector or the cell of any one of claims 14-21, wherein the recombinant anti-PD-1 antigen-binding protein is a PD-1 agonist.

23. The vector or the cell of any one of claims 14-21, wherein the recombinant anti-PD-1 antigen-binding protein is a PD-1 antagonist.

24. The vector or the cell of any one of claims 14-23, wherein the recombinant anti-PD-1 antigen-binding protein is a secretable protein.

25. The vector or the cell of any one of claims 14-24, wherein the recombinant anti-PD-1 antigen-binding protein comprises an antigen binding region recited in claim 1.

26. The vector or the cell of any one of claims 14-25, wherein the chimeric antigen receptor specifically binds to CD-19.

27. The vector or the cell of any one of claims 14-26, wherein the chimeric antigen receptor can be inserted in a human T cell membrane.

28. The cell of any one of claims 14-27, wherein the cell is a T cell.

29. A pharmaceutical composition comprising a vector or a cell of any one of claims 14-28.

30. Use of a vector or cell of any one of claims 17-31, or a pharmaceutical composition of claim 32, in the manufacture of a medicament for treating cancer, wherein the recombinant anti-PD-1 antigen-binding protein is a PD-1 antagonist.

31. The use of claim 30, wherein the recombinant anti-PD-1 antigen-binding protein inhibits, reduces, modulates or abolishes signal transduction mediated by PD-1.

32. Use of a combination of a nucleic acid encoding a recombinant anti-PD-1 antigen-binding protein and a nucleic acid encoding a chimeric antigen receptor in the manufacture of a pharmaceutical for treating cancer, wherein the use comprises that at least one T cell of the subject is to be transduced with the nucleic acid encoding a recombinant anti-PD-1 antigen-binding protein and the nucleic acid encoding a chimeric antigen receptor, wherein said recombinant anti-PD-1 antigen-binding protein is not identical to said chimeric antigen receptor.

33. The use of claim 32, wherein the chimeric antigen receptor does not specifically bind to PD-1.

34. The antigen-binding protein or an antigen binding fragment thereof of any one of claims 1-7, a nucleic acid of claim 8, a vector of claim 9, a cell of claim 10, a pharmaceutical composition of claim 11 or 32 or a vector or cell of any one of claims 17-31 for use in therapy.