

21R). IL-21R is expressed not just on T cells, but also on B cells, NK cells and myeloid cells. Accordingly, care must be taken to limit broad IL-21 activation in leukocytes and avoid the potential for toxicity. Restriction of IL-21 signaling must be balanced and selective. Triggering the effects of IL-21 must be designed to occur at the right time and place.

[0007] Indeed, any success, especially clinical success with IL-21 moieties as a monotherapy or in combination with checkpoint inhibitors, has been muted. Thus, there remains a need for treatment modalities utilizing IL-21, including modalities combining IL-21 moieties with checkpoint inhibitors. There also remains a need for IL-21 therapies combined with immune checkpoint inhibition.

SUMMARY

[0008] In one aspect, the present disclosure refers to a PD-1 antigen-binding protein comprising (a) a heavy chain (HC) complementarity-determining region (CDR) 1 amino acid sequence set forth in Table D or a sequence selected from the group consisting of: SEQ ID NOs: 352, 312, 322, 332, 342, 362, 372, and 382, or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 70% sequence identity; (b) an HC CDR2 amino acid sequence set forth in Table D or a sequence selected from the group consisting of: SEQ ID NOs: 353, 313, 323, 333, 343, 363, 373, and 383, or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 70% sequence identity; (c) an HC CDR3 amino acid sequence set forth in Table D or a sequence selected from the group consisting of: SEQ ID NOs: 354, 314, 324, 334, 344, 364, 374, and 384, or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 70% sequence identity; (d) a light chain (LC) CDR1 amino acid sequence set forth in Table D or a sequence selected from the group consisting of: 355, 315, 325, 335, 345, 365, 375, and 385, or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 70% sequence identity; (e) an LC CDR2 amino acid sequence set forth in Table D or a sequence selected from the group consisting of: 356, 316, 326, 336, 346, 366, 376, and 386, or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 70% sequence identity; (f) an LC CDR3 amino acid sequence set forth in Table D or a sequence selected from the group consisting of: 357, 317, 327, 337, 347, 367, 377, and 387, or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 70% sequence identity; or (g) a combination of any two or more of (a)-(f).

[0008a] In another aspect, the present disclosure refers to a nucleic acid comprising a nucleotide sequence encoding the PD-1 antigen-binding protein as disclosed herein.

[0008b] In another aspect, the present disclosure refers to a vector comprising the nucleic acid as disclosed herein.

[0008c] In another aspect, the present disclosure refers to a host cell comprising the nucleic acid as disclosed herein.

[0008d] In another aspect, the present disclosure refers to a kit comprising any one of a PD-1 antigen-binding protein, the nucleic acid, the vector as disclosed herein, or a combination thereof, and a container.

[0008e] In another aspect, the present disclosure refers to a pharmaceutical composition comprising any one of a PD-1 antigen-binding protein, the nucleic acid, the vector, the host cell as disclosed herein, or a combination thereof, and a pharmaceutically acceptable carrier, excipient, or diluent.

[0008f] In another aspect, the present disclosure refers to a method of making PD-1 antigen-binding protein comprising culturing the host cell as disclosed herein, so as to express the PD-1 antigen-binding protein and harvesting the expressed PD-1 antigen-binding protein.

[0008g] In yet another aspect, the present disclosure refers to the use of a pharmaceutical composition as disclosed herein in the manufacture of a medicament for treating a solid tumor.

[0008h] In yet another aspect, the present disclosure refers to the pharmaceutical composition as disclosed herein for use in therapy.

[0008i] The present disclosure provides IL-21 muteins comprising the amino acid sequence of SEQ ID NO: 2, wherein SEQ ID NO: 2 is

QGQDX HMXXM XXXXX XVDXL KNXVN DLVPE FLPAP EDVET NCEWS AFSCF
QKAQL KSANT GNNEX XIXXX XXXLX XXXXX TNAGR RQKHR LTCPS CDSYE
KKPPK EFLXX FXXLL XXMXX QHXSS RTHGS EDS (SEQ ID NO: 2), and X is any
amino acid, and wherein the IL-21 mutein amino acid sequence differs from the amino acid
sequence of human IL-21 (SEQ ID NO: 1) by at least 1 amino acid.

[0009] Thus, in one example, the present disclosure also provides IL-21 muteins comprising only one amino acid substitution, relative to the wild-type IL-21 amino acid sequence, which is provided herein as SEQ ID NO: 1. In exemplary aspects, the amino acid substitution is located at an amino acid position selected from the group consisting of: 5, 8, 9, 11, 12, 13, 14, 15, 16, 19, 23, 65, 66, 68, 69, 70, 72, 73, 75, 76, 77, 78, 79, 80, 109, 110, 112, 113, 116, 117, 119, 120, or 123, according to the amino acid position numbering of SEQ ID NO: 1.

[0010] The present disclosure further provides IL-21 muteins comprising only two amino acid substitutions, relative to SEQ ID NO: 1. In exemplary aspects, the amino acid substitutions are located at two amino acid

positions selected from the group consisting of: 5, 9, 15, 70, 71, 72, 73, and 76, according to the amino acid position numbering of SEQ ID NO: 1.

[0011] In exemplary disclosures, the IL-21 muteins bind to the IL-21 receptor (IL-21R) with a reduced affinity, relative to the affinity of wild-type IL-21 for the IL-21 receptor. In exemplary aspects, the IL-21 mutein binds to the human IL-21R with a K_D that is greater than or is about 0.04 nM. For example, the IL-21 mutein binds to the cynomolgus monkey IL-21R with a K_D that is greater than or is about 0.055 nM.

[0012] The present disclosure also provides conjugates comprising an IL-21 mutein of the present disclosure linked to a heterologous moiety. For example, the heterologous moiety is a polypeptide, such that the conjugate is a fusion protein. Therefore, the present disclosure provides fusion proteins comprising an IL-21 mutein of the present disclosure. For example, the fusion protein comprises an IL-21 mutein of the present disclosure linked to an antigen-binding protein, such as an antibody, or an antigen binding antibody fragment thereof.

[0013] In particular disclosures, the fusion protein comprises an IL-21 mutein linked to a PD-1 antigen-binding protein (e.g., a PD-1 antigen-binding antibody) of the present disclosure.

[0014] The present disclosure also provides PD-1 antigen-binding proteins and conjugates and fusion proteins comprising a PD-1 antigen-binding protein.

[0015] The present disclosure further provides nucleic acids comprising a nucleotide sequence encoding an IL-21 mutein, a PD-1 antigen-binding protein (e.g., a PD-1 antigen-binding antibody), or a fusion protein comprising an IL-21 mutein and a PD-1 antigen-binding protein (e.g., a PD-1 antigen-binding antibody) of the present disclosure. In one example, the nucleic acid molecule comprises a nucleotide sequence encoding a conjugate or fusion protein of the present disclosure. Vectors comprising the nucleic acids of the present disclosure and host cells comprising the nucleic acids of the present disclosure are furthermore provided herein.

[0016] The present disclosure additionally provides kits comprising an IL-21 mutein, a PD-1 antigen-binding protein (e.g., a PD-1 antigen-binding antibody), a conjugate, fusion protein (e.g., a fusion protein comprising an IL-21 mutein and a PD-1 antigen-binding protein (e.g., a PD-1 antigen-binding antibody)), nucleic acid, vector, or host cell of the present disclosure, or a combination thereof.

[0017] Pharmaceutical compositions comprising an IL-21 mutein, a PD-1 antigen-binding protein (e.g., a PD-1 antigen-binding antibody), a conjugate, fusion protein (e.g., a fusion protein comprising an IL-21 mutein and a PD-1 antigen-binding protein (e.g., a PD-1 antigen-binding antibody)), nucleic acid, vector, or host cell of the present disclosure, or a combination thereof, are provided herein.

[0018] Methods of making an IL-21 mutein, PD-1 antigen-binding protein (e.g., a PD-1 antigen-binding antibody), and a fusion protein comprising an IL-21 mutein and a PD-1 antigen-binding protein (e.g., a PD-1 antigen-binding antibody) are provided herein. The method, in exemplary disclosures, comprises culturing a host cell of the present disclosure to express the IL-21 mutein, PD-1 antigen-binding protein (e.g., a PD-1 antigen-binding antibody), or a fusion protein comprising an IL-21 mutein and a PD-1 antigen-binding protein (e.g., a PD-1 antigen-binding antibody) and harvesting the expressed IL-21 mutein, PD-1 antigen-binding protein (e.g., a PD-1 antigen-binding antibody), or fusion protein comprising an IL-21 mutein and a PD-1 antigen-binding protein (e.g., a PD-1 antigen-binding antibody).

[0019] Methods of treatment are additionally provided by the present disclosure. The method, in exemplary embodiments, is a method of treating a subject in need thereof, comprising administering to the subject in need thereof a pharmaceutical composition of the present disclosure in an amount effective to treat the subject. In an example, the subject has a tumor (e.g., a solid tumor, a hematological malignancy, or a lymphoid malignancy) and the pharmaceutical composition is administered to the subject in an amount effective to treat the tumor in the subject. In another example, the tumor is non-small cell lung cancer (NSCLC) (e.g., Stage III or IV NSCLC), small cell lung cancer (SCLC), head and neck cancer, renal cancer, breast cancer, melanoma, ovarian cancer, liver cancer, pancreatic cancer, colon cancer, prostate cancer, gastric cancer, bladder cancer, hepatocellular carcinoma, cancers with high microsatellite instability (i.e., MSI-high cancers), lymphoma or leukemia.

BRIEF DESCRIPTION OF THE DRAWINGS

[0020] Figure 1A represents a graph of the tumor volume (mm^3) of BALB/c mice implanted with CT26/3E5 colon carcinoma cells as a function of time (days). On Day 12, tumors were measured and the mice were given an intraperitoneal (IP) injection of 300 μg isotype control antibody (mIgG1) on day 12, 15 and 18.

[0021] Figure 1B represents a graph of the tumor volume (mm^3) of BALB/c mice implanted with CT26/3E5 colon carcinoma cells as a function of time (days). On Day 12, tumors were measured and the mice were given an IP injection of 300 μg of an anti-PD-1 antibody on day 12, 15 and 18..

[0022] Figure 1C represents a graph of the tumor volume (mm^3) of BALB/c mice implanted with CT26/3E5 colon carcinoma cells as a function of time (days). On Day 12, tumors were measured and the mice were given 50 μg recombinant murine IL-21 (rmIL-21) three times per week for 3 weeks. Dosing ended on Day 33

[0023] Figure 1D represents a graph of the tumor volume (mm^3) of BALB/c mice implanted with CT26/3E5 colon carcinoma cells as a function of time (days). On Day 12, tumors were measured and the mice were given 300 μg of an anti-PD-1 antibody on day 12, 15 and 18 and 50 μg rmIL-21 three times per week for 3 weeks. Dosing ended on Day 33.

[0024] Figure 2 represents a graph of percent survival of the four groups of BALB/c mice implanted with CT26/3E5 colon carcinoma cells. Group 1 mice were given an intraperitoneal (IP) injection of 300 μg isotype control antibody (mIgG1) on day 12, 15 and 18. Group 2 mice were given an IP injection of

WHAT IS CLAIMED IS:

1. A PD-1 antigen-binding protein comprising (a) a heavy chain (HC) complementarity-determining region (CDR) 1 amino acid sequence set forth in Table D or a sequence selected from the group consisting of: SEQ ID NOs: 352, 312, 322, 332, 342, 362, 372, and 382, or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 70% sequence identity; (b) an HC CDR2 amino acid sequence set forth in Table D or a sequence selected from the group consisting of: SEQ ID NOs: 353, 313, 323, 333, 343, 363, 373, and 383, or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 70% sequence identity; (c) an HC CDR3 amino acid sequence set forth in Table D or a sequence selected from the group consisting of: SEQ ID NOs: 354, 314, 324, 334, 344, 364, 374, and 384, or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 70% sequence identity; (d) a light chain (LC) CDR1 amino acid sequence set forth in Table D or a sequence selected from the group consisting of: 355, 315, 325, 335, 345, 365, 375, and 385, or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 70% sequence identity; (e) an LC CDR2 amino acid sequence set forth in Table D or a sequence selected from the group consisting of: 356, 316, 326, 336, 346, 366, 376, and 386, or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 70% sequence identity; (f) an LC CDR3 amino acid sequence set forth in Table D or a sequence selected from the group consisting of: 357, 317, 327, 337, 347, 367, 377, and 387, or a variant sequence thereof which differs by only one or two amino acids or which has at least or about 70% sequence identity; or (g) a combination of any two or more of (a)-(f).
2. The PD-1 antigen-binding protein of claim 1, comprising six CDR amino acid sequences selected from the group consisting of: (a) SEQ ID NOs: 352-357; (b) SEQ ID NOs: 322-327; (c) SEQ ID NOs: 332-337; (d) SEQ ID NOs: 342-347; (e) SEQ ID NOs: 312-317; (f) SEQ ID NOs: 362-367; (g) SEQ ID NOs: 372-377; and (h) SEQ ID NOs: 382-387.
3. The PD-1 antigen-binding protein of claim 2, comprising a pair of amino acid sequences selected from the group consisting of: (a) SEQ ID NOs: 358 and 359; (b) SEQ ID NOs: 328 and 329; (c) SEQ ID NOs: 338 and 339; (d) SEQ ID NOs: 348 and 349; (e) SEQ ID NOs: 318 and 319; (f) SEQ ID NOs: 368 and 369; (g) SEQ ID NOs: 378 and 379; and (h) SEQ ID NOs: 388 and 389.
4. The PD-1 antigen-binding protein of claim 3, comprising a pair of amino acid sequences selected from the group consisting of: (a) SEQ ID NOs: 360 and 361; (b) SEQ ID NOs: 330 and 331; (c)

SEQ ID NOs: 340 and 341; (d) SEQ ID NOs: 350 and 351; (e) SEQ ID NOs: 320 and 321; (f) SEQ ID NOs: 370 and 371; (g) SEQ ID NOs: 380 and 381; and (h) SEQ ID NOs: 390 and 391.

5. The PD-1 antigen-binding protein of any one of claims 1-4, which is an antibody.
6. The PD-1 antigen-binding protein of any one of claims 1-4, which is an antigen-binding fragment of an antibody.
7. The PD-1 antigen-binding protein of any one of claims 1-4, which is an antibody protein product, optionally, an scFv.
8. A nucleic acid comprising a nucleotide sequence encoding the PD-1 antigen-binding protein of any one of the preceding claims.
9. The nucleic acid of claim 8, comprising the sequence of any one of SEQ ID NOs: 392-471.
10. A vector comprising the nucleic acid of claim 8 or 9.
11. A host cell comprising the nucleic acid of claim 8 or 9 or the vector of claim 10.
12. A kit comprising an PD-1 antigen-binding protein of any one of the preceding claims, the nucleic acid of claim 8 or 9, the vector of claim 10, the host cell of claim 11, or a combination thereof, and a container.
13. A pharmaceutical composition comprising an PD-1 antigen-binding protein of any one of the preceding claims, the nucleic acid of claim 8 or 9, the vector of claim 10, the host cell of claim 11, or a combination thereof, and a pharmaceutically acceptable carrier, excipient, or diluent.
14. A method of making PD-1 antigen-binding protein comprising culturing the host cell of claim 13 so as to express the PD-1 antigen-binding protein and harvesting the expressed PD-1 antigen-binding protein.
15. Use of a pharmaceutical composition of claim 13 in the manufacture of a medicament for treating a solid tumor.
16. The pharmaceutical composition of claim 13 for use in therapy.