

therapeutic effect. Additional challenges exist due to the broad expression of the receptor for IL-21 (IL-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

[0007a] In one aspect, the present disclosure refers to a conjugate of comprising an IL-21 mutein an anti-PD-1 antibody, wherein the IL-21 mutein comprises the amino acid sequence of SEQ ID NO: 2, 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), wherein “X” represents any amino acid, and wherein said 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 optionally, wherein the IL-21 mutein comprises an amino acid sequence which differs from the amino acid sequence of human IL-21 (SEQ ID NO: 1) by no more than 7 amino acids, or by 3, 4, 5, or 6 amino acids, optionally, by 1 or 2 amino acids.

[0007b] In another aspect, the present disclosure refers to a conjugate comprising

an IL-21 mutein comprising the amino acid sequence of SEQ ID NO: 1, except that said IL-21 mutein comprises amino acid substitutions at any two of positions 5, 9, 73, and 76 of SEQ ID NO: 1, and

an anti-PD-1 antibody,

wherein the IL-21 mutein is linked to the C-terminus of the anti-PD-1 antibody.

[0007c] In another aspect, the present disclosure refers to a kit comprising the conjugate as disclosed herein, and a container.

[0007d] In another aspect, the present disclosure refers to a pharmaceutical composition comprising the conjugate as disclosed herein, and a pharmaceutically acceptable carrier, excipient, or diluent.

[0007e] In another aspect, the present disclosure refers to a use of the pharmaceutical composition as disclosed herein for use in the manufacture of a medicament for treating a subject having a solid tumor

[0007f] In another aspect, the present disclosure refers to an IL-21 mutein comprising an amino acid sequence with two amino acid substitutions relative to the amino acid sequence of human IL-21 (SEQ ID NO: 1) wherein the amino acid substitutions occur at two of positions 5, 9, 73, and 76 or SEQ ID NO: 1.

[0008] In yet another aspect, 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 examples, 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 examples, 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 examples, 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 examples, the IL-21 mutein binds to the human IL-21R with a K_D that is greater than or is about 0.04 nM. In

exemplary examples, the IL-21 mutein bind 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. In exemplary examples, 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. In exemplary examples, 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 examples, 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 exemplary examples, 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 examples, 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 examples, 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 exemplary examples, 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 other exemplary examples, 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 conjugate of comprising an IL-21 mutein an anti-PD-1 antibody, wherein the IL-21 mutein comprises the amino acid sequence of SEQ ID NO: 2,

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),

wherein "X" represents any amino acid, and wherein said 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

optionally, wherein the IL-21 mutein comprises an amino acid sequence which differs from the amino acid sequence of human IL-21 (SEQ ID NO: 1) by no more than 7 amino acids, or by 3, 4, 5, or 6 amino acids, optionally, by 1 or 2 amino acids.

2. The conjugate of claim 1, wherein the difference(s) between the amino acid sequence of the IL-21 mutein and the amino acid sequence of SEQ ID NO: 1 occur(s) within (A) amino acids 10-15, inclusive, or amino acids 105-123, inclusive, of SEQ ID NO: 2, optionally, wherein the difference(s) occur(s) at amino acids 11, 14, 15, 109, 110, 112, 113, 116, 119, 120, and/or 123 of SEQ ID NO: 2 or (B) amino acids 5-25, inclusive, or amino acids 65-80, inclusive, of SEQ ID NO: 2, optionally, wherein the difference(s) occur(s) at amino acids 5, 8, 9, 12, 13, 16, 19, 23, 65, 66, 69, 70, 72, 73, 75, 76, 77, 78, 79, and/or 80 of SEQ ID NO: 2.
3. The conjugate of claim 2, wherein the IL-21 mutein comprises an amino acid sequence with one amino acid substitution relative to the amino acid sequence of human IL-21 (SEQ ID NO: 1), optionally, wherein the amino acid substitution occurs at position 5, 8, 9, 11, 12, 13, 14, 15, 16, 19, 23, 65, 66, 68, 69, 70, 71, 72, 73, 75, 76, 77, 78, 79, 80, 109, 110, 112, 113, 116, 117, 119, 120, or 123 of SEQ ID NO: 1 or at position 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 of SEQ ID NO: 1.
4. The conjugate of any one of the preceding claims, wherein the IL-21 mutein comprises an amino acid substitution:

- a. at position 5, 8, 9, 12, 14, 15, 65, 66, 69, 70, 72, 73, 75, 76, 77, 80, 116, or 119 of SEQ ID NO: 1, wherein the substitute amino acid is an aliphatic amino acid
 - b. at position 5, 8, 9, 11, 12, 13, 14, 15, 16, 19, 23, 65, 66, 69, 70, 72, 73, 75, 76, 77, 78, 79, 110, 112, 116, 117, 119, 120, or 123 of SEQ ID NO: 1, wherein the substitute amino acid is an acidic amino acid;
 - c. at position 5, 9, 73, 76, 109, 113, or 116 of SEQ ID NO: 1, wherein the substitute amino acid is a basic amino acid;
 - d. at position 5, 8, 9, 70, or 76 of SEQ ID NO: 1, wherein the substitute amino acid is an aromatic amino acid;
 - e. at position 5, 8, 9, 12, 15, 73, 76, 116, or 119 of SEQ ID NO: 1, wherein the substitute amino acid is an amino acid comprising a side chain amide;
 - f. at position 5, 8, 9, 11, 12, 14, 15, 73, 76, 116, or 119 of SEQ ID NO: 1, wherein the substitute amino acid is a non-aromatic amino acid comprising a side chain hydroxyl;
 - g. at position 65, 66, 69, 70, 72, 73, 75, 76, 77, or 80 of SEQ ID NO: 1, wherein the substitute amino acid is an imino acid;
 - h. at position 5, 9, 15, 76, 116, or 119 of SEQ ID NO: 1, wherein the substitute amino acid is an amino acid comprising a sulfur-containing side chain; or
 - i. or a combination thereof.
5. The conjugate of any one of the preceding claims, wherein the IL-21 mutein comprises an amino acid substitution with an amino acid at the position according to TABLE A:

TABLE A

Amino Acid position	Amino Acid (in single letter code)		Amino Acid position	Amino Acid (in single letter code)
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of SEQ ID NO: 1		of SEQ ID NO: 1	
5	A, D, E, G, H, I, K, L, M, N, Q, S, T, V, or Y	72	D, G, or P
8	A, D, E, N, S, T, V, or Y	73	A, D, E, G, H, I, N, P, Q, S, or V
9	A, D, E, G, H, I, K, L, M, N, Q, S, T, V, or Y	75	D, G, or P
11	D or S	76	A, D, E, G, H, I, K, L, M, N, P, Q, S, T, V, or Y
12	A, D, E, N, S, T, or V	77	D, G, or P
13	D	78	D
14	A, D, or S	79	D
15	A, E, I, M, N, Q, S, T, or V	80	G, or P
16	D or E	109	K
19	D	110	D
23	D	112	D
65	D, G, or P	113	K
66	D, G, or P	116	A, D, E, I, K, L, M, N, S, T, or V
68	Q	117	D
69	D, G, or P	119	A, D, E, M, N, Q, S, or T
70	E, G, P, or Y	120	D
71	L	123	D

6. The conjugate of any one of the preceding claims, wherein the IL-21 mutein comprises an amino acid sequence of any one of SEQ ID NOs: 3-21, 23-56, 58-112, 114-198, 249-254, and 283 or an amino acid sequence of any one of SEQ ID NOs: 199 to 208, 210 to 222, 224 to 248, and 255.
7. The conjugate of any one of the preceding claims, wherein the IL-21 mutein comprises an amino acid sequence with two amino acid substitutions relative to the amino acid sequence of human IL-21 (SEQ ID NO: 1), optionally, wherein the amino acid substitution occurs at two of positions 5, 8, 9, 11, 12, 13, 14, 15, 16, 19, 23, 65, 66, 68, 69, 70, 71, 72, 73, 75, 76, 77, 78, 79, 80, 109, 110, 112, 113, 116, 117, 119, 120, or 123 of SEQ ID NO: 1, or at two of positions 5, 9, 73, and 76 or SEQ ID NO: 1.
8. The conjugate of any one of the preceding claims, wherein the IL-21 mutein comprises any two of the following:

an amino acid at position 5 of SEQ ID NO: 1, wherein said amino acid is selected from the group consisting of: A, D, E, G, H, I, K, L, M, N, Q, S, T, V, or Y;

an amino acid at position 9 of SEQ ID NO: 1, wherein said amino acid is selected from the group consisting of: A, D, E, G, H, I, K, L, M, N, Q, S, T, V, or Y;

an amino acid at position 73 of SEQ ID NO: 1, wherein said amino acid is selected from the group consisting of: A, D, E, G, H, I, N, P, Q, S, or V; and

an amino acid at position 76 of SEQ ID NO: 1, wherein said amino acid is selected from the group consisting of: A, D, E, G, H, I, K, L, M, N, P, Q, S, T, V, or Y.

9. The conjugate of any one of the preceding claims, wherein the IL-21 mutein comprises an amino acid substitution at position 5, 9, or 73 of SEQ ID NO: 1, wherein the substitute amino acid is an aliphatic amino acid or acidic amino acid, optionally, wherein the aliphatic amino acid is alanine or wherein the acidic amino acid is glutamic acid.
10. The conjugate of any one of the preceding claims, wherein the IL-21 mutein comprises an amino acid substitution at position 76 of SEQ ID NO: 1 and the substitute amino acid at position 76 is an aliphatic amino acid or an acidic amino acid, optionally, wherein the aliphatic amino acid is alanine or wherein the acidic amino acid is glutamic acid.
11. The conjugate of any one of the preceding claims, wherein the IL-21 mutein is directly attached to the Fc of the antibody.
12. The conjugate of any one of the preceding claims, wherein the IL-21 mutein is attached to the Fc of the antibody via a linker.
13. The conjugate of claim 11 or 12, wherein said conjugate comprises a single IL-21 mutein, wherein said single IL-21 mutein is linked to the C-terminus of one of the two antibody heavy chains.
14. The conjugate of claim 11 or 12, wherein said conjugate comprises two IL-21 muteins, wherein the first IL-21 mutein is linked to the C-terminus of the first antibody heavy chain, and the second IL-21 mutein is linked to the C-terminus of the second antibody heavy chain, optionally, wherein said first IL-21 mutein has the same amino acid sequence than the second IL-21 mutein.

15. The conjugate of claim 14, wherein said first IL-21 mutein has the same amino acid sequence as the second IL-21 mutein.
16. The conjugate of claim 14, wherein said first IL-21 mutein has a different amino acid sequence than the second IL-21 mutein
17. The conjugate of any one of the preceding claims, wherein the IL-21 mutein comprises an amino acid sequence of any one of SEQ ID NOs: 233-245.
18. The conjugate of claim 17, wherein the IL-21 mutein comprises an amino acid sequence of SEQ ID NO: 242.
19. The conjugate of claim 17, wherein the IL-21 mutein comprises an amino acid sequence of SEQ ID NO: 244.
20. The conjugate of claim 17, wherein the IL-21 mutein comprises an amino acid sequence of SEQ ID NO: 245.
21. The conjugate of any one of claims 30-45, wherein the PD-1 antibody comprises a constant region comprising an amino acid sequence of any one of SEQ ID NOs: 265-267, 282, 284-311 and/or heavy chains comprising charge pair mutations.
22. A conjugate comprising
 - an IL-21 mutein comprising the amino acid sequence of SEQ ID NO: 1, except that said IL-21 mutein comprises amino acid substitutions at any two of positions 5, 9, 73, and 76 of SEQ ID NO: 1, and
 - an anti-PD-1 antibody,wherein the IL-21 mutein is linked to the C-terminus of the anti-PD-1 antibody.
23. A kit comprising the conjugate of any one of the previous claims, and a container.
24. A pharmaceutical composition comprising the conjugate of any one of the previous claims, and a pharmaceutically acceptable carrier, excipient, or diluent.
25. The pharmaceutical composition of claim 24 for use in therapy for treating a subject in need thereof.

26. Use of the pharmaceutical composition of claim 24 for use in the manufacture of a medicament for treating a subject having a solid tumor.
27. An IL-21 mutein comprising an amino acid sequence with two amino acid substitutions relative to the amino acid sequence of human IL-21 (SEQ ID NO: 1) wherein the amino acid substitutions occur at two of positions 5, 9, 73, and 76 or SEQ ID NO: 1.
28. An IL-21 mutein comprising the amino acid sequence of SEQ ID NO: 2,

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),

wherein "X" represents any amino acid, and

wherein said 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.