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

[0008] 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 ~~aspect~~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 ~~aspect~~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 ~~aspect~~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 ~~embodiments~~example, 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 ~~aspect~~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 ~~aspect~~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 ~~aspect~~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 ~~aspect~~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 ~~embodiments~~example, 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 ~~aspect~~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 ~~embodiments~~example, 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~~example, 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 ~~aspect~~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 ~~aspect~~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.

[0019a] In one aspect, the present disclosure provides 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, wherein the difference(s) occur(s) at amino acids 5, 9, 73, and/or 76 of SEQ ID NO: 2.

[0019b] In another aspect, the present disclosure provides an IL-21 mutein comprising an amino acid sequence of SEQ ID NO: 244.

[0019c] In another aspect, the present disclosure provides a conjugate comprising: an IL-21 mutein of the disclosure and a heterologous moiety.

[0019d] In another aspect, the present disclosure provides a fusion polypeptide or fusion protein comprising an IL-21 mutein of the disclosure and a heterologous polypeptide or peptide; optionally wherein the heterologous polypeptide or peptide comprises an immunoglobulin or an antigen binding antibody fragment thereof.

[0019e] In another aspect, the present disclosure provides a nucleic acid encoding an IL-21 mutein of the disclosure; or a vector comprising said nucleic acid; or a host cell comprising said nucleic acid or said vector.

[0019f] In another aspect, the present disclosure provides a kit comprising an IL-21 mutein, a nucleic acid, vector, host cell, conjugate, fusion polypeptide or fusion protein, or a combination thereof, of the disclosure, and a container.

[0019g] In another aspect, the present disclosure provides a pharmaceutical composition comprising an IL-21 mutein, a nucleic acid, vector, host cell, conjugate, fusion polypeptide or fusion protein, or a combination thereof, of the disclosure, and a pharmaceutically acceptable carrier, excipient, or diluent.

[0019h] In another aspect, the present disclosure provides a method of making an IL-21 mutein comprising culturing the host cell of the disclosure so as to express the IL-21 mutein and harvesting the expressed IL-21 mutein.

[0019i] In another aspect, the present disclosure provides a use of a pharmaceutical composition of the disclosure in the manufacture of a medicament for treating a solid tumor.

[0019j] In another aspect, the present disclosure provides a composition of the disclosure for use as a medicament.

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. 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, wherein the difference(s) occur(s) at amino acids 5, 9, 73, and/or 76 of SEQ ID NO: 2.

~~2. The IL-21 mutein of claim 1, comprising 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.~~

~~3. The IL-21 mutein of claim 2, comprising an amino acid sequence which differs from the amino acid sequence of human IL-21 (SEQ ID NO: 1) by 3, 4, 5, or 6 amino acids.~~

~~4.~~2. The IL-21 mutein of claim 3, comprising an amino acid sequence which differs from the amino acid sequence of human IL-21 (SEQ ID NO: 1) by 1 or 2 amino acids.

~~5. The IL-21 mutein of any one of claims 1-4, 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 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.~~

~~6. The IL-21 mutein of any one of claims 1-4, 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 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.~~

~~7.~~3. The IL-21 mutein of ~~any one of~~ claims ~~4~~ 1 ~~to~~ or ~~6~~2, comprising an amino acid sequence with one amino acid substitution relative to the amino acid sequence of human IL-21 (SEQ ID NO: 1).

~~8. The IL-21 mutein of claim 7, 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.~~

~~9. The IL-21 mutein of claim 8, wherein the amino acid substitution occurs 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.~~

~~10.~~4. The IL-21 mutein of claim ~~9~~3, comprising an amino acid substitution:

a. at position 5, ~~8,~~ 9, ~~12, 14, 15, 65, 66, 69, 70, 72,~~ 73, ~~75,~~or 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,~~or 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, or 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, or 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, or 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, or ~~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,~~, or 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.

~~11. The IL-21 mutein of claim 10, wherein the substitute amino acid is a naturally occurring amino acid.~~

~~12.~~5. The IL-21 mutein of claim ~~7~~3, comprising an amino acid substitution with an amino acid at the position according to ~~TABLE A~~the following table:

~~TABLE A~~

~~Amino Acid position of SEQ ID NO: 1~~	~~Amino Acid (in single letter code)~~		~~Amino Acid position of SEQ ID NO: 1~~	~~Amino Acid (in single letter code)~~
~~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~~

Amino Acid position of SEQ ID NO: 1	Amino Acid (in single letter code)
5	A, D, E, G, H, I, K, L, M, N, Q, S, T, V, or Y
9	A, D, E, G, H, I, K, L, M, N, Q, S, T, V, or Y
73	A, D, E, G, H, I, N, P, Q, S, or V

76	A, D, E, G, H, I, K, L, M, N, P, Q, S, T, V, or Y

~~13.~~6. ~~An~~ The IL-21 mutein of claim 1, herein the IL-21 mutein comprises an~~comprising an~~ amino acid sequence of any one of SEQ ID NOs: 73-87, 94-108, 145-155, and 159-174~~3-21, 23-56, 58-112, 114-198, 249-254, and 283~~.

~~14.~~7. The IL-21 mutein of ~~any one of claims 4-6~~claim 1 or 2, comprising an amino acid sequence with two amino acid substitutions relative to the amino acid sequence of human IL-21 (SEQ ID NO: 1); ~~.~~

~~15.~~ wherein ~~The IL-21 mutein of claim 14, 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,~~and 76~~, 77, 78, 79, 80, 109, 110, 112, 113, 116, 117, 119, 120, or 123~~ of SEQ ID NO: 1.

~~16. The IL-21 mutein of claim 15, wherein the amino acid substitutions occur at two of positions 5, 9, 15, 70, 71, 72, 73, and 76 of SEQ ID NO: 1.~~

~~17. The IL-21 mutein of claim 16, wherein the amino acid substitutions occur at two of positions 5, 9, 73, and 76 or SEQ ID NO: 1.~~

~~18.~~8. The IL-21 mutein of claim ~~17~~7, wherein one of the substitutions occurs at position 76 of SEQ ID NO: 1~~.~~;~~.~~

~~19.~~ ~~The IL-21 mutein of claim 18,~~ optionally wherein the substitute amino acid at position 76 of SEQ ID NO: 1 is an aliphatic amino acid or an acidic amino acid.

~~20.~~9. The IL-21 mutein of ~~any one of claims 15 to 19~~claim 7 or 8, comprising (a) 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; or ~~.~~

~~21.~~ ~~The IL-21 mutein of any one of claims 15 to 19, comprising~~ (b) an amino acid substitution at position 5 of SEQ ID NO: 1, wherein the substitute amino acid is an amino acid with a side chain amide; optionally wherein:~~.~~

~~22.~~ ~~The IL-21 mutein of claim 19 or 20, wherein~~ (i) the aliphatic amino acid is alanine; ~~.~~

~~23.~~ ~~The IL-21 mutein of claim 19 or 20, wherein~~ (ii) the acidic amino acid is glutamic acid; and/or~~.~~

~~24.~~ ~~The IL-21 mutein of claim 21, wherein~~ (iii) the amino acid with a side chain amide is glutamine.

~~25.~~10. ~~An~~ The IL-21 mutein of claim 7, wherein the IL-21 mutein comprises ~~comprising~~ an amino acid sequence selected from the group consisting of SEQ ID NOs: ~~199 to~~ 208, 210, 211, 216, ~~to~~ 222, 224, 225, and 233-245~~to 248, and 255~~.

11. An IL-21 mutein comprising an amino acid sequence of SEQ ID NO: 244.

~~26.~~12. The IL-21 mutein of any one of the ~~previous~~ preceding claims~~:~~, wherein (a) the IL-21 mutein binds to the IL-21 receptor with a reduced affinity, relative to the affinity of wild-type IL-21 for the IL-21 receptor; and/or~~.~~

~~27.~~ ~~The IL-21 mutein of any one of the previous claims,~~(b) which binds to an IL-21 receptor alpha chain, wherein the IL-21 receptor alpha chain comprises the~~having an~~ amino acid sequence of SEQ ID NO: 256~~.~~ ; and/or

~~28.~~ ~~The IL-21 mutein of any one of the previous claims,~~ (c) which binds to an IL-21 receptor gamma chain, wherein the IL-21 receptor gamma chain comprises the ~~having an~~ amino acid sequence of SEQ ID NO: 257; and/or~~.~~

~~29.~~ ~~The IL-21 mutein of any one of the preceding claims,~~ (d) which binds to the human IL-21 receptor with a Kd that is greater than or is about 0.04 nM.

~~30.~~13. A conjugate comprising:

an IL-21 mutein of any one of the preceding claims and
a heterologous moiety.

~~31.~~14. The conjugate of claim ~~30~~13, wherein the IL-21 mutein is: (a) directly attached to the heterologous moiety; ~~.~~or

~~32.~~ ~~The conjugate of claim 30, wherein the IL-21 mutein is~~ (b) attached to the heterologous moiety via a linker;~~.~~

33. The conjugate of claim 32, optionally wherein the linker comprises a peptide; .

34. The conjugate of claim 33, optionally wherein the peptide comprises an amino acid sequence of Gly-Gly-Gly-Gly-Ser (SEQ ID NO: 262).

35.15. The conjugate of any one of claimsclaim 30-3413 or 14, wherein the heterologous moiety is (a) a polypeptide;.

36. The conjugate of claim 35, wherein the polypeptide is (b) an antigen-binding protein; or .

37. The conjugate of claim 36, wherein the antigen-binding protein is (c) an antibody or an antigen binding antibody fragment thereof.

38. The conjugate of claim 37, wherein the antibody is an anti-PD-1 antibody.

39. The conjugate of any one of claims 30-38, wherein the anti-PD-1 antibody comprises a constant region comprising an amino acid sequence of any one of SEQ ID NOs: 265-267, 282, 284-311.

40.16. The conjugate of any one of claims 13-15claim 30-39, wherein: (a) the IL-21 mutein is directly attached to the Fc of the antibody.

41. The conjugate of claim 40 , wherein the IL-21 mutein or is attached to the Fc of the antibody via a linker to the Fc of the antibody; and/or.

42. The conjugate of claim 40 or 41, 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.

43. The conjugate of claim 40 or 41, 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.

44. The conjugate of claim 43, wherein said first IL-21 mutein has the same amino acid sequence as the second IL-21 mutein.

45. The conjugate of claim 43, wherein said first IL-21 mutein has a different amino acid sequence than the second IL-21 mutein.

~~46.~~ ~~The conjugate of any one of claims 30-45, wherein~~ (b) the antibody heavy chains comprise charge pair mutations.

17. The conjugate of claim 15 or 16, wherein said conjugate:

(a) 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; or

(b) 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; wherein said first IL-21 mutein:

(i) has the same amino acid sequence as the second IL-21 mutein; or

(ii) has a different amino acid sequence than the second IL-21 mutein.

~~47. The conjugate of any one of claims 30-46, wherein the IL-21 mutein comprises amino acid substitutions at two of positions 5, 9, 73, and 76 of SEQ ID NO: 1.~~

~~48. 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.~~

~~49. The conjugate of claim 48, wherein the anti-PD-1 antibody comprises a constant region comprising an amino acid sequence of any one of SEQ ID NOs: 265-267, 282, 284-311.~~

~~50.~~18. A fusion polypeptide or fusion protein comprising an IL-21 mutein of any one of ~~the preceding claims~~claims 1-12 and a heterologous polypeptide or peptide; optionally~~.~~

~~51.~~ ~~The fusion polypeptide or fusion protein of claim 50, comprising~~ wherein the heterologous polypeptide or peptide comprises an immunoglobulin or an antigen binding antibody fragment thereof.

~~52.~~19. A nucleic acid ~~comprising~~ encoding ~~a nucleotide sequence encoding~~ an IL-21 mutein of any one of ~~the preceding claims~~claims 1-12;~~.~~

~~53.~~ ~~A~~ or a vector comprising ~~the~~ said nucleic acid ~~of claim 52.~~;

~~54.~~ ~~A~~ or a host cell comprising ~~the~~ said nucleic acid ~~of claim 52~~ or ~~the~~ said vector ~~of claim 53~~.

~~55.~~20. A kit comprising an IL-21 mutein, a nucleic acid, vector, host cell, conjugate, fusion polypeptide or fusion protein, or a combination thereof, of any one of the ~~previous~~ preceding claims, and a container.

~~56.~~21. A pharmaceutical composition comprising an IL-21 mutein, a nucleic acid, vector, host cell, conjugate, fusion polypeptide or fusion protein, or a combination thereof, of any one of the previous claims, and a pharmaceutically acceptable carrier, excipient, or diluent.

~~57.~~22. A method of making an IL-21 mutein comprising culturing the host cell of claim ~~54~~ 19 so as to express the IL-21 mutein and harvesting the expressed IL-21 mutein.

~~58.~~23. Use of ~~A method of treating a subject in need thereof, comprising administering to the subject in need thereof~~ a pharmaceutical composition of claim ~~56~~ 21 in the manufacture of a medicament for ~~in an amount effective to treat the subject.~~

~~The method of claim 58, wherein the subject has~~ treating a solid tumor ~~and the pharmaceutical composition is administered to the subject in an amount effective to treat the solid tumor in the subject~~.

24. The composition of claim 21 for use as a medicament.