

pembrolizumab, many patients still fail to achieve sufficient response to checkpoint blockade alone.

[0005] Therefore, there remains an unmet need in oncology treatment for therapeutic strategies with cytokines which do not require high doses and are targeted to tumors to avoid systemic toxicity. Further, there is a need to identify additional therapeutic modalities to stack with checkpoint blockade that could increase patient response rate. This can be especially complex as the additional therapeutic modality should not compete with the checkpoint blockade. The present invention addresses these needs and caveats by providing PD-1-targeted IL-15 fusion proteins with enhanced half-life and more selective targeting of TILs to improve safety profile, and which do not compete with checkpoint blockade antibodies with which they may be combined.

SUMMARY

[0006] The disclosure is directed to fusion proteins that include the combination of IL-15 complexes generally comprising an IL-15 domain, an IL-15R α domain, and an anti-PD-1 antigen binding domain (ABD) that does not compete for binding with nivolumab or pembrolizumab.

[0007] Accordingly, in some aspects, the present disclosure provides targeted IL-15/R α heterodimeric Fc fusion proteins comprising: a) a first monomer comprising, from N- to C-terminal: i) a IL-15 R α sushi domain protein; ii) a first domain linker; iii) An IL-15 protein; and iv) a first variant Fc domain; and b) a second monomer comprising a heavy chain comprising VH-CH1-hinge-CH2-CH3, wherein said CH2-CH3 is a second variant Fc domain; c) a third monomer comprising a light chain comprising VL-CL; wherein said VH and VL domains form an antigen binding domain that binds to human PD-1 and does not compete for the human PD-1 with nivolumab and/or pembrolizumab.

[0008] In additional examples, the targeted IL-15/R α heterodimeric Fc fusion proteins has a VH domain which is a variant of SEQ ID NO:5 comprising the amino acid substitution F32L and the VL domain is a variant of SEQ ID NO:6 comprising N27dH, and optionally further comprising the amino acid substitutions K30Y/S93T.

[0009] In further examples, the targeted IL-15/R α heterodimeric Fc fusion protein has the H1 VH and the L1.1 VL.

[0010] In additional examples, the targeted IL-15/R α heterodimeric Fc fusion proteins have a variant IL-15 protein comprising amino acid substitution(s) selected from the group of D30N/E64Q/N65D; D30N/N65D; N1D; N4D; D8N; D30N; D61N; E64Q; N65D; Q108E; N1D/N4D/D8N; N1D/N4D/N65D; N1D/D30N; N1D/D61N; N1D/D61N/E64Q/Q108E; N1D/E64Q; N1D/N65D; N1D/Q108E; N4D; N4D/D30N; N4D/D61N; N4D/D61N/N65D; N4D/D61N/E64Q/Q108E; N4D/E64Q; N4D/N65D; D8N/D61N; D8N/E64Q; D30N/E64Q; D30N/Q180E; D61N/E64Q/N65D; E64Q; E64Q/N65D; E64Q/Q108E; and N65D/Q108E (as compared to SEQ ID NO:2).

[0011] In further examples, the targeted IL-15/R α heterodimeric Fc fusion proteins have a first variant Fc domain and a second variant Fc domains comprise amino acid substitutions L368D/K370S : LS364K/E357Q, according to EU numbering.

[0012] In additional examples, the targeted IL-15/R α heterodimeric Fc fusion proteins of the disclosure have a first variant Fc domain and a second variant Fc domain that each comprise amino acid substitutions M428L/N343S, according to EU numbering.

[0013] In further examples, the targeted IL-15/R α heterodimeric Fc fusion protein comprise:
a) a first monomer comprising, from N- to C-terminal: i) a IL-15 R α sushi domain protein;
ii) a first domain linker; iii) a variant IL-15 protein; iv) a second domain linker; and v) a first variant Fc domain comprising CH2-CH3; and b) a second monomer comprising from N- to C-terminal: i) an scFv domain; ii) a third domain linker; iii) a second variant Fc domain comprising CH2-CH3; wherein said scFv domain comprises a first variable heavy domain, an scFv linker, and a first variable light domain, wherein said scFv domain binds human PD-1 and does not compete for said human PD-1 with nivolumab and/or pembrolizumab.

[0014] In additional examples, the targeted IL-15/R α heterodimeric Fc fusion proteins comprise: a) a first monomer comprising, from N- to C-terminal: i) an IL-15 R α sushi domain protein; ii) a first domain linker; iii) a first variant Fc domain comprising CH2-CH3; b) a second monomer comprising, from N- to C-terminal: i) an scFv domain; ii) a third domain linker; iii) a second variant Fc domain comprising CH2-CH3; wherein said scFv domain comprises a first variable heavy domain, an scFv linker, and a first variable light domain, and said scFv domain binds human PD-1 and does not compete for said human PD-1 with nivolumab and/or pembrolizumab; and c) a third monomer comprising a variant IL-15 domain protein.

[0015] In further examples, the targeted IL-15/R α heterodimeric Fc fusion proteins comprise: a) a first monomer comprising a heavy chain comprising VH-CH1-hinge-CH2-CH3, wherein said CH2-CH3 is a first variant Fc domain; b) a second monomer comprising, from N- to C-terminal: i) an IL-15 R α sushi domain protein; ii) a second domain linker; iii) a second variant Fc domain comprising CH2-CH3; b) a third monomer comprising a variant IL-15 domain protein; c) a fourth monomer comprising a light chain comprising VL-CL; wherein said VH and VL domains form an antigen binding domain that binds to human PD-1 and does not compete for said human PD-1 with nivolumab and/or pembrolizumab.

[0016] In additional examples, the targeted IL-15/R α heterodimeric Fc fusion proteins comprises: a) a first monomer comprising a heavy chain comprising VH-CH1-hinge-CH2-CH3, wherein said CH2-CH3 is a first variant Fc domain; b) a second monomer comprising VH-CH1-hinge-CH2-CH3-domain linker-IL-15R α sushi domain protein-domain linker-variant IL-15 domain protein, wherein said CH2-CH3 is a second variant Fc domain; c) a third monomer comprising a light chain comprising VL-CL; wherein said VH and VL domains form an antigen binding domain that binds to human PD-1 and does not compete for said human PD-1 with nivolumab and/or pembrolizumab.

[0017] In further examples, the targeted IL-15/R α heterodimeric Fc fusion proteins comprise: a) a first monomer comprising a heavy chain comprising VH-CH1-hinge-CH2-CH3, wherein said CH2-CH3 is a first variant Fc domain; b) a second monomer comprising VH-CH1-hinge-CH2-CH3-domain linker-IL-15R α sushi domain protein, wherein said CH2-CH3 is a second variant Fc domain; c) a third monomer comprising a variant IL-15 domain protein; d) a fourth monomer comprising a light chain comprising VL-CL; wherein said VH and VL domains bind to human PD-1 and does not compete for said human PD-1 with nivolumab and/or pembrolizumab.

[0018] In additional examples, the targeted IL-15/R α heterodimeric Fc fusion proteins comprise: a) a first monomer comprising, from N- to C-terminal: a VH-CH1-domain linker-variant IL-15 domain protein-domain linker-CH2-CH3, wherein said CH2-CH3 is a first variant Fc domain; b) a second monomer comprising, from N- to C-terminal: a VH-CH1-domain linker-IL-15 R α sushi domain protein-domain linker-CH2-CH3, wherein said CH2-CH3 is a second variant Fc domain; c) a third monomer comprising a light chain comprising VL-CL; wherein said VH and VL domains form an antigen binding domain that binds to

human PD-1 and does not compete for said human PD-1 with nivolumab and/or pembrolizumab.

[0019] In further examples, the targeted IL-15/R α heterodimeric Fc fusion protein comprising: a) a first monomer comprising, from N- to C-terminal: a VH-CH1-domain linker-variant IL-15 R α sushi domain protein-domain linker-variant IL-15 domain protein-domain linker-CH2-CH3, wherein said CH2-CH3 is a first variant Fc domain; b) a second monomer comprising a heavy chain comprising VH-CH1-hinge CH2-CH3, wherein said CH2-CH3 is a second variant Fc domain; c) a third monomer comprising a light chain comprising VL-CL; wherein said VH and VL domains form an antigen binding domain that binds to human PD-1 and does not compete for said human PD-1 with nivolumab and/or pembrolizumab.

[0020] In additional aspects, the disclosure provides nucleic acid compositions comprising: a) a first nucleic acid encoding the first monomer of the embodiments; b) a second nucleic acid encoding the second monomer of the embodiments; and c) a third nucleic acid encoding the third monomer of the embodiments.

[0021] In further aspects, the disclosure provides expression vector compositions comprising: a) an expression vector comprising the first nucleic acids; b) an expression vector comprising the second nucleic acids; and c) an expression vector comprising the third nucleic acids.

[0022] In an additional aspect, the disclosure provides host cells comprising the expression vector compositions.

[0023] In a further aspect, the disclosure provides methods of producing the targeted IL-15/R α heterodimeric Fc fusion proteins of the disclosure the method comprising: culturing the host cells under conditions where said targeted IL-15/R α heterodimeric Fc fusion protein is expressed; and recovering said protein.

[0024] In an additional aspect, the disclosure provides the use of a pharmaceutical composition comprising the targeted IL-15/R α heterodimeric Fc fusion protein as disclosed herein in the manufacture of a medicament for treating a cancer, wherein the pharmaceutical composition is to be administered to a patient in need thereof. In an additional example, the disclosure provides methods of treating a cancer comprising administering to a patient in need thereof a pharmaceutical composition comprising the targeted IL-15/R α heterodimeric Fc fusion proteins of the disclosure.

[0025] In a further example, the disclosure provides methods of treating a cancer comprising administering to a patient in need thereof a pharmaceutical composition comprising the targeted IL-15/R α heterodimeric Fc fusion proteins of the disclosure in combination with an anti-PD-1 antibody including nivolumab or pembrolizumab.

WHAT IS CLAIMED IS:

1. A targeted IL-15/R α heterodimeric Fc fusion protein comprising:
 - a) a first monomer comprising, from N- to C-terminal:
 - i) a IL-15 R α sushi domain protein;
 - ii) a first domain linker;
 - iii) An IL-15 protein; and
 - iv) a first variant Fc domain; and
 - b) a second monomer comprising a heavy chain comprising VH-CH1-hinge-CH2-CH3, wherein said CH2-CH3 is a second variant Fc domain;
 - c) a third monomer comprising a light chain comprising VL-CL; wherein said VH and VL domains form an antigen binding domain that binds to human PD-1 and does not compete for said human PD-1 with nivolumab and/or pembrolizumab.
2. The targeted IL-15/R α heterodimeric Fc fusion protein according to claim 1 wherein said VH domain is a variant of SEQ ID NO:5 comprising the amino acid substitution F32L and said VL domain is a variant of SEQ ID NO:6 comprising N27dH.
3. The targeted IL-15/R α heterodimeric Fc fusion protein according to claim 2 wherein said VL domain further comprises the amino acid substitutions K30Y/S93T.
4. The targeted IL-15/R α heterodimeric Fc fusion protein according to any previous claim wherein said VH is H1 and said VL is L1.1.
5. The targeted IL-15/R α heterodimeric Fc fusion protein according to any previous claim wherein said IL-15 protein is a variant IL-15 protein comprising amino acid substitution(s) selected from the group of D30N/E64Q/N65D; D30N/N65D; N1D; N4D; D8N; D30N; D61N; E64Q; N65D; Q108E; N1D/N4D/D8N; N1D/N4D/N65D; N1D/D30N; N1D/D61N; N1D/D61N/E64Q/Q108E; N1D/E64Q; N1D/N65D; N1D/Q108E; N4D; N4D/D30N; N4D/D61N; N4D/D61N/N65D; N4D/D61N/E64Q/Q108E; N4D/E64Q; N4D/N65D; D8N/D61N; D8N/E64Q; D30N/E64Q; D30N/Q180E; D61N/E64Q/N65D; E64Q; E64Q/N65D; E64Q/Q108E; and N65D/Q108E.

6. The targeted IL-15/R α heterodimeric Fc fusion protein according to any previous claim, wherein said variant IL-15 protein comprises amino acid substitution(s) selected from the group of N4D/N65D; D30N; D30N/E64Q; D30N/N65D; and D30N/E64Q/N65D.
7. The targeted IL-15/R α heterodimeric Fc fusion protein according to any previous claim, wherein said first variant Fc domain and said second variant Fc domains comprise amino acid substitutions L368D/K370S : LS364K/E357Q, according to EU numbering.
8. The targeted IL-15/R α heterodimeric Fc fusion protein of according to any previous claim, wherein said first variant Fc domain and said second variant Fc domain each comprise amino acid substitutions M428L/N343S, according to EU numbering.
9. A nucleic acid composition comprising:
- a) a first nucleic acid encoding the first monomer according to any one of claims 1 to 8;
 - b) a second nucleic acid encoding the second monomer according to any one of claims 1 to 8; and
 - c) a third nucleic acid encoding the third monomer according to any one of claims 1 to 8; respectively.
10. An expression vector composition comprising:
- a) an expression vector comprising the first nucleic acid of claim 9;
 - b) an expression vector comprising the second nucleic acid of claim 9; and
 - c) an expression vector comprising the third nucleic acid of claim 9.
11. A host cell comprising the expression vector composition according to claim 10.
12. A method of producing the targeted IL-15/R α heterodimeric Fc fusion protein according to any one of claims 1 to 8, the method comprising: culturing the host cell of claim 11 under conditions where said targeted IL-15/R α heterodimeric Fc fusion protein is expressed; and recovering said protein.
13. Use of a pharmaceutical composition comprising the targeted IL-15/R α heterodimeric Fc fusion protein according to any one of claims 1 to 8 in the manufacture of a medicament for

treating a cancer, wherein the pharmaceutical composition is to be administered to a patient in need thereof.

14. The use according to claim 13, wherein an anti-PD-1 antibody is to be further administered.

15. The use according to claim 14, wherein said anti-PD-1 antibody is nivolumab or pembrolizumab.