

SUMMARY

[0008] Disclosed is a bispecific heterodimeric protein comprising (a) a fusion protein comprising a first protein domain, a second protein domain, and a first Fc domain, wherein the first protein domain is covalently attached to the N-terminus of the second protein domain using a first domain linker, wherein the second protein domain is covalently attached to the N-terminus of said first Fc domain using a second domain linker, and wherein the first protein domain comprises an IL-15R α protein and the second protein domain comprises an IL-15 protein; and (b) an antibody fusion protein comprising an PD-1 antigen binding domain and a second Fc domain, wherein the PD-1 antigen binding domain is covalently attached to the N-terminus of the second Fc domain, and the PD-1 antigen binding domain is a single chain variable fragment (scFv) or a Fab fragment. The first and the second Fc domains can have a set of amino acid substitutions selected from the group consisting of S267K/L368D/K370S : S267K/LS364K/E357Q; S364K/E357Q : L368D/K370S; L368D/K370S : S364K; L368E/K370S : S364K; T411T/E360E/Q362E : D401K; L368D/K370S : S364K/E357L and K370S : S364K/E357Q, according to EU numbering. In some examples, the first and/or the second Fc domains have an additional set of amino acid substitutions comprising Q295E/N384D/Q418E/N421D, according to EU numbering. Optionally, the first and/or the second Fc domains have an additional set of amino acid substitutions consisting of G236R/L328R, E233P/L234V/L235A/G236del/S239K, E233P/L234V/L235A/G236del/S267K, E233P/L234V/L235A/G236del/S239K/A327G, E233P/L234V/L235A/G236del/S267K/A327G and E233P/L234V/L235A/G236del, according to EU numbering. In one aspect, the present disclosure refers to a bispecific heterodimeric protein comprising: a) a fusion protein comprising a first protein domain, a second protein domain, and a first Fc domain, wherein said first protein domain is covalently attached to the N-terminus of said second protein domain using a first domain linker, wherein said second protein domain is covalently attached to the N-terminus of said first Fc domain using a second domain linker, and wherein said first protein domain comprises a human IL-15R α protein and said second protein domain comprises a human IL-15 variant protein comprising one or more amino acid substitutions selected from the group consisting of N1D, N4D, D8N, D30N, D61N, E64Q, N65D, and Q108E; and b) an antibody fusion protein comprising a PD-1 antigen binding domain (ABD) and a second Fc domain, wherein said PD-1 antigen binding domain is covalently attached to the N-terminus of said second Fc domain; wherein said first and said second Fc domains have a set of amino acid substitutions selected from the group consisting of S267K/L368D/K370S : S267K/S364K/E357Q; S364K/E357Q : L368D/K370S; L368D/K370S : S364K; L368E/K370S : S364K; T411E/K360E/Q362E : D401K; L368D/K370S : S364K/E357L and K370S : S364K/E357Q, according to EU numbering.

[0009] Disclosed is a bispecific heterodimeric protein comprising: (a) a fusion protein comprising a first protein domain and a first Fc domain, wherein the first protein domain is covalently attached to the N-terminus of the first Fc domain using a domain linker and the first protein domain comprises an IL-15R α protein; (b) a second protein domain noncovalently attached to the first protein domain, the second protein domain comprises an IL-15 protein; and (c) an antibody fusion protein comprising an PD-1 antigen binding domain and a second Fc domain, wherein the PD-1 antigen binding domain is covalently attached to the N-terminus of the second Fc

domain and said PD-1 antigen binding domain is a single chain variable fragment (scFv) or a Fab fragment. The first and the second Fc domains can have a set of amino acid substitutions selected from the group consisting of S267K/L368D/K370S : S267K/LS364K/E357Q; S364K/E357Q : L368D/K370S; L368D/K370S : S364K; L368E/K370S : S364K; T411T/E360E/Q362E : D401K; L368D/K370S : S364K/E357L and K370S : S364K/E357Q, according to EU numbering. In some examples, the first and/or the second Fc domains have an additional set of amino acid substitutions comprising Q295E/N384D/Q418E/N421D, according to EU numbering. In other instances, the first and/or the second Fc domains have an additional set of amino acid substitutions consisting of G236R/L328R, E233P/L234V/L235A/G236del/S239K, E233P/L234V/L235A/G236del/S267K, E233P/L234V/L235A/G236del/S239K/A327G, E233P/L234V/L235A/G236del/S267K/A327G and E233P/L234V/L235A/G236del, according to EU numbering. In another aspect, the present disclosure refers to a bispecific heterodimeric protein comprising: a) a fusion protein comprising a first protein domain and a first Fc domain, wherein said first protein domain is covalently attached to the N-terminus of said first Fc domain using a domain linker and said first protein domain comprises a human IL-15 α protein; b) a second protein domain noncovalently attached to said first protein domain, said second protein domain comprises a human IL-15 variant protein comprising one or more amino acid substitutions selected from the group consisting of N1D, N4D, D8N, D30N, D61N, E64Q, N65D, and Q108E; and c) an antibody fusion protein comprising an PD-1 antigen binding domain and a second Fc domain, wherein said PD-1 antigen binding domain is covalently attached to the N-terminus of said second Fc domain; wherein said first and said second Fc domains have a set of amino acid substitutions selected from the group consisting of S267K/L368D/K370S : S267K/S364K/E357Q; S364K/E357Q : L368D/K370S; L368D/K370S : S364K; L368E/K370S : S364K; T411E/K360E/Q362E : D401K; L368D/K370S : S364K/E357L and K370S : S364K/E357Q, according to EU numbering.

[0010] Disclosed is a bispecific heterodimeric protein comprising: (a) a first antibody fusion protein comprising a first PD-1 antigen binding domain and a first Fc domain, wherein the first PD-1 antigen binding domain is covalently attached to the N-terminus of the first Fc domain via a first domain linker, and the first PD-1 antigen binding domain is a single chain variable fragment (scFv) or a Fab fragment; (b) a second antibody fusion protein comprising a second PD-1 antigen binding domain, a second Fc domain, and a first protein domain, wherein the second PD-1 antigen binding domain is covalently attached to the N-terminus of the second Fc domain via a second domain linker, the first protein domain is covalently attached to the C-terminus of the second Fc domain via a third domain linker, the second PD-1 antigen binding domain is a single chain variable fragment (scFv) or a Fab fragment, and the first protein domain comprises an IL-15 α protein; and (c) a second protein domain noncovalently attached to the first protein domain of the second antibody fusion protein and comprising an IL-15 protein. In some examples, the first and the second Fc domains can have a set of amino acid substitutions selected from the group consisting of S267K/L368D/K370S : S267K/LS364K/E357Q; S364K/E357Q : L368D/K370S; L368D/K370S : S364K; L368E/K370S : S364K; T411T/E360E/Q362E : D401K; L368D/K370S : S364K/E357L and K370S : S364K/E357Q, according to EU numbering. In some examples, the first and/or the second Fc domains have an additional set of amino acid substitutions comprising Q295E/N384D/Q418E/N421D,

according to EU numbering. In other instances, the first and/or the second Fc domains have an additional set of amino acid substitutions consisting of G236R/L328R, E233P/L234V/L235A/G236del/S239K, E233P/L234V/L235A/G236del/S267K, E233P/L234V/L235A/G236del/S239K/A327G, E233P/L234V/L235A/G236del/S267K/A327G and E233P/L234V/L235A/G236del, according to EU numbering. In another aspect, the present disclosure refers to a bispecific heterodimeric protein comprising: a) a first antibody fusion protein comprising a first PD-1 antigen binding domain and a first Fc domain, wherein said first PD-1 antigen binding domain is covalently attached to the N-terminus of said first Fc domain via a first domain linker; b) a second antibody fusion protein comprising a second PD-1 antigen binding domain, a second Fc domain, and a first protein domain, wherein said second PD-1 antigen binding domain is covalently attached to the N-terminus of said second Fc domain via a second domain linker, said first protein domain is covalently attached to the C-terminus of said second Fc domain via a third domain linker, said second PD-1 antigen binding domain is a single chain variable fragment (scFv) or a Fab fragment, and said first protein domain comprises a human IL-15R α protein; and (c) a second protein domain noncovalently attached to said first protein domain of said second antibody fusion protein and comprising a human IL-15 variant protein comprising one or more amino acid substitutions selected from the group consisting of N1D, N4D, D8N, D30N, D61N, E64Q, N65D, and Q108E, wherein said first and said second Fc domains have a set of amino acid substitutions selected from the group consisting of S267K/L368D/K370S : S267K/S364K/E357Q; S364K/E357Q : L368D/K370S; L368D/K370S : S364K; L368E/K370S : S364K; T411E/K360E/Q362E : D401K; L368D/K370S : S364K/E357L and K370S : S364K/E357Q, according to EU numbering.

[0011] In some examples, the IL15 protein of the bispecific heterodimeric protein described herein has one or more amino acid substitutions selected from the group consisting of N1D, N4D, D8N, D30N, D61N, E64Q, N65D, and Q108E.

[0012] In other examples, provided herein is a bispecific heterodimeric protein selected from the group consisting of XENP25850, XENP25937, XENP21480, XENP22022, XENP22112, XENP22641, XENP22642, and XENP22644. In another example, there is provided a bispecific heterodimeric protein selected from the group consisting of XENP21480, XENP22022, XENP22112, XENP22641, XENP22642, XENP22644, XENP25850, and XENP25937. In another example, there is provided a nucleic acid composition comprising one or more nucleic acids encoding a bispecific heterodimeric protein selected from the group consisting of XENP21480, XENP22022, XENP22112, XENP22641, XENP22642, XENP22644, XENP25850, and XENP25937. In another example, there is provided an expression vector composition comprising one or more expression vectors each comprising a nucleic acid such that the one or more expression vectors encode a bispecific heterodimeric protein selected from the group consisting of XENP21480, XENP22022, XENP22112, XENP22641, XENP22642, XENP22644, XENP25850, and XENP25937.

[0013] Nucleic acids, expression vectors and host cells are all provided as well, in addition to methods of making these proteins and treating patients with them. In another aspect, the present disclosure refers to a nucleic acid composition encoding the fusion protein disclosed herein. In another aspect, the present disclosure refers to a nucleic acid composition encoding the antibody fusion protein disclosed herein. In another aspect, the present disclosure refers to an expression vector comprising the nucleic acid composition disclosed herein. In another aspect, the present disclosure refers to a host cell comprising one or two expression vectors disclosed herein. In another aspect, the present disclosure refers to a host cell comprising one or more expression vectors disclosed herein. In another aspect, the present disclosure refers to a nucleic acid composition encoding the first antibody fusion protein disclosed herein. In another aspect, the present disclosure refers to a nucleic acid composition encoding the second antibody fusion protein disclosed herein. In another aspect, the present disclosure refers to a nucleic acid composition encoding the second protein domain disclosed herein. In another example, there is provided a host cell comprising the nucleic acid composition disclosed herein. In another example, there is provided a host cell comprising the expression vector composition disclosed herein. In another aspect, the present disclosure refers to a method of producing a bispecific heterodimeric protein comprising culturing the host cell disclosed herein under conditions wherein said bispecific heterodimeric protein is expressed, and recovering said protein. In another aspect, the present disclosure refers to use of the bispecific heterodimeric protein disclosed herein in the manufacture of a medicament for treating cancer in a patient in need thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] Figure 1 depicts the structure of IL-15 in complex with its receptors IL-15R α (CD215), IL-15R β (CD122), and the common gamma chain (CD132).

[0015] Figure 2 depicts the sequences for IL-15 and its receptors.

[0016] Figure 3 depicts useful pairs of Fc heterodimerization variant sets (including skew and pI variants). There are variants for which there are no corresponding “monomer 2” variants; these are pI variants which can be used alone on either monomer. In addition, reference is made to Figure 88.

[0017] Figure 4 depict a list of isosteric variant antibody constant regions and their respective substitutions. pI₋(-) indicates lower pI variants, while pI₊(+) indicates higher pI variants. These can be optionally and independently combined with other heterodimerization variants of the inventions (and other variant types as well, as outlined herein.)

WHAT IS CLAIMED IS:

1. A bispecific heterodimeric protein comprising:

a) a fusion protein comprising a first protein domain, a second protein domain, and a first Fc domain, wherein said first protein domain is covalently attached to the N-terminus of said second protein domain using a first domain linker, wherein said second protein domain is covalently attached to the N-terminus of said first Fc domain using a second domain linker, and wherein said first protein domain comprises a human IL-15R α protein and said second protein domain comprises a human IL-15 variant protein comprising one or more amino acid substitutions selected from the group consisting of N1D, N4D, D8N, D30N, D61N, E64Q, N65D, and Q108E; and

b) an antibody fusion protein comprising a PD-1 antigen binding domain (ABD) and a second Fc domain, wherein said PD-1 antigen binding domain is covalently attached to the N-terminus of said second Fc domain;

wherein said first and said second Fc domains have a set of amino acid substitutions selected from the group consisting of S267K/L368D/K370S : S267K/S364K/E357Q; S364K/E357Q : L368D/K370S; L368D/K370S : S364K; L368E/K370S : S364K; T411E/K360E/Q362E : D401K; L368D/K370S : S364K/E357L and K370S : S364K/E357Q, according to EU numbering.

2. The bispecific heterodimeric protein according to claim 1, wherein said first and/or said second Fc domains have an additional set of amino acid substitutions comprising Q295E/N384D/Q418E/N421D, according to EU numbering.

3. The bispecific heterodimeric protein according to claim 1 or 2, wherein said first and/or said second Fc domains have an additional set of amino acid substitutions consisting of G236R/L328R, E233P/L234V/L235A/G236del/S239K, E233P/L234V/L235A/G236del/S267K, E233P/L234V/L235A/G236del/S239K/A327G, E233P/L234V/L235A/G236del/S267K/A327G and E233P/L234V/L235A/G236del, according to EU numbering.

4. The bispecific heterodimeric protein according to any one of claims 1 to 3, wherein said IL-15 variant protein has a polypeptide sequence selected from the group consisting of SEQ ID NO:1 (full-length human IL-15) and SEQ ID NO:2 (truncated human IL-15) and said one or more amino acid substitutions, and said IL-15R α protein has a polypeptide sequence selected from the group consisting of SEQ ID NO:3 (full-length human IL-15R α) and SEQ ID NO:4 (sushi domain of human IL-15R α).

5. The bispecific heterodimeric protein according to any one of claims 1 to 4, wherein said IL-15 protein and said IL-15R α protein have a set of amino acid substitutions selected from the group consisting of E87C : D96/P97/C98; E87C : D96/C97/A98; V49C : S40C; L52C : S40C; E89C : K34C; Q48C : G38C; E53C : L42C; C42S : A37C; and L45C : A37C, respectively.

6. The bispecific heterodimeric protein according to any one of claims 1 to 5, wherein said PD-1 antigen binding domain comprises an anti-PD-1 scFv or an anti-PD-1 Fab.

7. A nucleic acid composition encoding the fusion protein of any one of claims 1 to 6.

8. A nucleic acid composition encoding the antibody fusion protein of any one of claims 1 to 6.

9. An expression vector comprising the nucleic acid composition of claim 7.

10. An expression vector comprising the nucleic acid composition of claim 8.

11. The expression vector of claim 10, further comprising the nucleic acid composition of claim 7.

12. A host cell comprising one or two expression vectors of any one of claims 9 to 11.

13. A bispecific heterodimeric protein comprising:

a) a fusion protein comprising a first protein domain and a first Fc domain, wherein said first protein domain is covalently attached to the N-terminus of said first Fc

domain using a domain linker and said first protein domain comprises a human IL-15R α protein;

b) a second protein domain noncovalently attached to said first protein domain, said second protein domain comprises a human IL-15 variant protein comprising one or more amino acid substitutions selected from the group consisting of N1D, N4D, D8N, D30N, D61N, E64Q, N65D, and Q108E; and

c) an antibody fusion protein comprising an PD-1 antigen binding domain and a second Fc domain, wherein said PD-1 antigen binding domain is covalently attached to the N-terminus of said second Fc domain;

wherein said first and said second Fc domains have a set of amino acid substitutions selected from the group consisting of S267K/L368D/K370S : S267K/S364K/E357Q; S364K/E357Q : L368D/K370S; L368D/K370S : S364K; L368E/K370S : S364K; T411E/K360E/Q362E : D401K; L368D/K370S : S364K/E357L and K370S : S364K/E357Q, according to EU numbering.

14. The bispecific heterodimeric protein according to claim 13, wherein said first and/or said second Fc domains have an additional set of amino acid substitutions comprising Q295E/N384D/Q418E/N421D, according to EU numbering.

15. The bispecific heterodimeric protein according to claim 13 or 14, wherein said first and/or said second Fc domains have an additional set of amino acid substitutions consisting of G236R/L328R, E233P/L234V/L235A/G236del/S239K, E233P/L234V/L235A/G236del/S267K, E233P/L234V/L235A/G236del/S239K/A327G, E233P/L234V/L235A/G236del/S267K/A327G and E233P/L234V/L235A/G236del, according to EU numbering.

16. The bispecific heterodimeric protein according to any one of claims 13 to 15, wherein said IL-15 variant protein has a polypeptide sequence selected from the group consisting of SEQ ID NO:1 (full-length human IL-15) and SEQ ID NO:2 (truncated human IL-15) and said one or more amino acid substitutions, and said IL-15R α protein has a

polypeptide sequence selected from the group consisting of SEQ ID NO:3 (full-length human IL-15R α) and SEQ ID NO:4 (sushi domain of human IL-15R α).

17. The bispecific heterodimeric protein according to any one of claims 13 to 16, wherein said IL-15 protein and said IL-15R α protein have a set of amino acid substitutions selected from the group consisting of E87C : D96/P97/C98; E87C : D96/C97/A98; V49C : S40C; L52C : S40C; E89C : K34C; Q48C : G38C; E53C : L42C; C42S : A37C; and L45C : A37C, respectively.

18. The bispecific heterodimeric protein according to any one of claims 13 to 17, wherein said PD-1 antigen binding domain comprises an anti-PD-1 scFv or an anti-PD-1 Fab.

19. A nucleic acid composition encoding the fusion protein of any one of claims 13 to 18.

20. A nucleic acid composition encoding the antibody fusion protein of any one of claims 13 to 18.

21. An expression vector comprising the nucleic acid composition of claim 19.

22. An expression vector comprising the nucleic acid composition of claim 20.

23. The expression vector of claim 22, further comprising the nucleic acid composition of claim 19.

24. The expression vector of any one of claims 21 to 23, further comprising a nucleic acid composition encoding said second protein domain.

25. A host cell comprising one or more expression vectors of any one of claims 21 to 24.

26. A bispecific heterodimeric protein comprising:

a) a first antibody fusion protein comprising a first PD-1 antigen binding domain and a first Fc domain, wherein said first PD-1 antigen binding domain is covalently attached to the N-terminus of said first Fc domain via a first domain linker;

b) a second antibody fusion protein comprising a second PD-1 antigen binding domain, a second Fc domain, and a first protein domain, wherein said second PD-1 antigen binding domain is covalently attached to the N-terminus of said second Fc domain via a second domain linker, said first protein domain is covalently attached to the C-terminus of said second Fc domain via a third domain linker, said second PD-1 antigen binding domain is a single chain variable fragment (scFv) or a Fab fragment, and said first protein domain comprises a human IL-15R α protein; and

(c) a second protein domain noncovalently attached to said first protein domain of said second antibody fusion protein and comprising a human IL-15 variant protein comprising one or more amino acid substitutions selected from the group consisting of N1D, N4D, D8N, D30N, D61N, E64Q, N65D, and Q108E,

wherein said first and said second Fc domains have a set of amino acid substitutions selected from the group consisting of S267K/L368D/K370S : S267K/S364K/E357Q; S364K/E357Q : L368D/K370S; L368D/K370S : S364K; L368E/K370S : S364K; T411E/K360E/Q362E : D401K; L368D/K370S : S364K/E357L and K370S : S364K/E357Q, according to EU numbering.

27. The bispecific heterodimeric protein according to claim 26, wherein said first and/or said second Fc domains have an additional set of amino acid substitutions comprising Q295E/N384D/Q418E/N421D, according to EU numbering.

28. The bispecific heterodimeric protein according to claim 26 or 27, wherein said first and/or said second Fc domains have an additional set of amino acid substitutions consisting of G236R/L328R, E233P/L234V/L235A/G236del/S239K, E233P/L234V/L235A/G236del/S267K, E233P/L234V/L235A/G236del/S239K/A327G, E233P/L234V/L235A/G236del/S267K/A327G and E233P/L234V/L235A/G236del, according to EU numbering.

29. The bispecific heterodimeric protein according to any one of claims 26 to 28, wherein said IL-15 variant protein has a polypeptide sequence selected from the group consisting of SEQ ID NO:1 (full-length human IL-15) and SEQ ID NO:2 (truncated human IL-15) and said one or more amino acid substitutions, and said IL-15R α protein has a polypeptide sequence selected from the group consisting of SEQ ID NO:3 (full-length human IL-15R α) and SEQ ID NO:4 (sushi domain of human IL-15R α).

30. The bispecific heterodimeric protein according to any one of claims 26 to 29, wherein said IL-15 variant protein and said IL-15R α protein have a set of amino acid substitutions selected from the group consisting of E87C : D96/P97/C98; E87C : D96/C97/A98; V49C : S40C; L52C : S40C; E89C : K34C; Q48C : G38C; E53C : L42C; C42S : A37C; and L45C : A37C, respectively.

31. The bispecific heterodimeric protein according to any one of claims 26 to 30, wherein said first PD-1 antigen binding domain comprises an anti-PD-1 scFv or an anti-PD-1 Fab.

32. The bispecific heterodimeric protein according to any one of claims 26 to 31, wherein said second PD-1 antigen binding domain comprises an anti-PD-1 scFv or an anti-PD-1 Fab.

33. A nucleic acid composition encoding the first antibody fusion protein of any one of claims 26 to 32.

34. A nucleic acid composition encoding the second antibody fusion protein of any one of claims 26 to 32.

35. A nucleic acid composition encoding the second protein domain of any one of claims 26 to 32.

36. An expression vector comprising the nucleic acid composition of claim 33.

37. An expression vector comprising the nucleic acid composition of claim 34.

38. An expression vector comprising the nucleic acid composition of claim 35.

39. The expression vector of claim 36, further comprising the nucleic acid composition of claim 34 and/or 35.

40. A host cell comprising one or more expression vectors of any one of claims 36 to 39.

41. The bispecific heterodimeric protein according to any one of claims 1 to 6, 13 to 18, and 26 to 32, wherein said IL-15 variant protein has one or more amino acid substitutions selected from the group consisting of (i) N4D/N65D, (ii) D30N/N65D, and (iii) D30N/E64Q/N65D.

42. The bispecific heterodimeric protein according to any one of claims 1 to 6, 13 to 18, 26 to 32 and 41, wherein said first and said second Fc domains have an additional set of amino acid substitutions M428L/N434S.

43. A method of producing a bispecific heterodimeric protein comprising culturing the host cell of any one of claims 12, 25, and 40 under conditions wherein said bispecific heterodimeric protein is expressed, and recovering said protein.

44. Use of the bispecific heterodimeric protein of any one of claims 1 to 6, 13 to 18, 26 to 32, and 41 to 42 in the manufacture of a medicament for treating cancer in a patient in need thereof.