

the IL-15/IL-15R α complex to NK cells and CD8+ T cells expressing IL-2R β and the common gamma chain.

[0005] As potential drugs, both cytokines suffer from a very fast clearance, with half-lives measured in minutes. In addition, IL-15 by itself is less stable due to its preference for the IL-15R α -associated complex. It has also been shown that recombinantly produced IL15/IL15R α heterodimer can potently activate T cells. Nevertheless, a short half-life hinders favorable dosing. The present invention solves this problem by providing novel IL15/IL15R α heterodimer Fc fusion proteins.

SUMMARY

[0006] Accordingly, in one aspect the present invention provides a heterodimeric protein comprising a) a first 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 first domain linker; b) a second fusion protein comprising a second protein domain and a second Fc domain, wherein the second protein domain is covalently attached to the N-terminus of the Fc domain using a second domain linker; wherein the first and the second Fc domains 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 and wherein the first protein domain comprises an IL15 protein and the second protein domain comprises an IL15R α protein. In some embodiments, the first protein domain is covalently attached to the N-terminus of the first Fc domain directly and without using the first domain linker and/or the second protein domain is covalently attached to the N-terminus of the second Fc domain directly and without using the second domain linker.

[0007] In some embodiments, the heterodimeric protein comprises: (i) the first fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP15902) and the second fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP5908), (ii) the first fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP15902) and the second fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP15909), (iii)

[0015] In an additional aspect, the invention provides a heterodimeric protein comprising: a) a first fusion protein comprising a first Fc domain and a first protein domain, wherein the first Fc domain is covalently attached to the N-terminus of the first protein domain using a domain linker; b) a second Fc domain, and c) a second protein domain noncovalently attached to the first protein domain of the first fusion protein; wherein the first and the second Fc domains have a set of amino acid substitutions selected from the group consisting of S267K/L368D/K370S : S267K/L364K/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 and wherein the first protein domain comprises an IL15R α protein and the second protein domain comprises an IL15 protein.

[0016] In some embodiments, the heterodimer protein comprises (i) the first fusion protein having a polypeptide sequence of SEQ ID NO: XX (17603), the second Fc domain having a polypeptide sequence of SEQ ID NO: XX (8927), and the second protein domain having a polypeptide sequence of SEQ ID NO: XX (16484); or ii) the first fusion protein having a polypeptide sequence of SEQ ID NO: XX (17605), the second Fc domain having a polypeptide sequence of SEQ ID NO: XX (8927), and the second protein domain having a polypeptide sequence of SEQ ID NO: XX (17074).

[0017] In any of the embodiments of the present disclosure, the first and/or the second Fc domains can have an additional set of amino acid substitutions comprising Q295E/N384D/Q418E/N421D, according to EU numbering. In some cases, 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.

[0018] In any of the embodiments of the present disclosure, the IL15 protein has a polypeptide sequence selected from the group consisting of SEQ ID NO:1 (full-length human IL15) and SEQ ID NO:2 (truncated human IL15), and the IL15R α protein has a polypeptide sequence selected from the group consisting of SEQ ID NO:3 (full-length human IL15R α) and SEQ ID NO:4 (sushi domain of human IL15R α). In some cases, the IL15

protein and the IL15R α protein have a set of amino acid substitutions or additions 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.

[0019] Disclosed is a heterodimeric protein selected from the group consisting of XENP20818, XENP20819, XENP21471, XENP21472, XENP21473, XENP21474, XENP21475, XENP21476, XENP21477, XENP21478, XENP21479, XENP21978, XENP22013, XENP22015, XENP22017, XENP22354, XENP22355, XENP22356, XENP22357, XENP22358, XENP22359, XENP22360, XENP22361, XENP22362, XENP22363, XENP22364, XENP22365, XENP22366, XENP22637, and XENP22639. Disclosed is a heterodimeric protein selected from the group consisting of XENP20818, XENP20819, XENP21471, XENP21472, XENP21473, XENP21474, XENP21475, XENP21476, XENP21477, XENP22013, XENP22815, XENP22816, XENP22817, XENP22818, XENP22819, XENP22820, XENP22821, XENP22822, XENP22823, XENP22824, XENP22825, XENP22826, XENP22827, XENP22828, XENP22829, XENP22830, XENP22831, XENP22832, XENP22833, XENP22834, XENP23343, XENP23554, XENP23555, XENP23557, XENP23559, XENP24019, and XENP24020. In an additional aspect, there is provided a heterodimeric protein selected from the group consisting of XENP20818, XENP20819, XENP21471, XENP21472, XENP21473, XENP21474, XENP21475, XENP21476, XENP21477, XENP22013, XENP22815, XENP22816, XENP22817, XENP22818, XENP22819, XENP22820, XENP22821, XENP22822, XENP22823, XENP22824, XENP22825, XENP22826, XENP22827, XENP22828, XENP22829, XENP22830, XENP22831, XENP22832, XENP22833, XENP22834, XENP23343, XENP23504, XENP23554, XENP23555, XENP23557, XENP23559, XENP24019, XENP24020, XENP24045, XENP24051, XENP24052, XENP24113, XENP24301, XENP24306, XENP24341, XENP21478, XENP21479, XENP22357, XENP22354, XENP22355, XENP22356, XENP22357, XENP22358, XENP22359, XENP22360, XENP22361, XENP22362, XENP22363, XENP22364, XENP22365, XENP22366, XENP22637, XENP24349, XENP24383, XENP21978, XENP22634, XENP24342, XENP24306, XENP22637, and XENP22639. In an additional aspect, there is provided a nucleic acid composition comprising one or more nucleic acids encoding a heterodimeric protein selected from the group consisting of XENP20818, XENP20819, XENP21471, XENP21472, XENP21473,

XENP21474, XENP21475, XENP21476, XENP21477, XENP22013, XENP22815, XENP22816, XENP22817, XENP22818, XENP22819, XENP22820, XENP22821, XENP22822, XENP22823, XENP22824, XENP22825, XENP22826, XENP22827, XENP22828, XENP22829, XENP22830, XENP22831, XENP22832, XENP22833, XENP22834, XENP23343, XENP23504, XENP23554, XENP23555, XENP23557, XENP23559, XENP24019, XENP24020, XENP24045, XENP24051, XENP24052, XENP24113, XENP24301, XENP24306, XENP24341, XENP21478, XENP21479, XENP22357, XENP22354, XENP22355, XENP22356, XENP22357, XENP22358, XENP22359, XENP22360, XENP22361, XENP22362, XENP22363, XENP22364, XENP22365, XENP22366, XENP22637, XENP24349, XENP24383, XENP21978, XENP22634, XENP24342, XENP24306, XENP22637, and XENP22639. In an additional aspect, 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 heterodimeric protein selected from the group consisting of XENP20818, XENP20819, XENP21471, XENP21472, XENP21473, XENP21474, XENP21475, XENP21476, XENP21477, XENP22013, XENP22815, XENP22816, XENP22817, XENP22818, XENP22819, XENP22820, XENP22821, XENP22822, XENP22823, XENP22824, XENP22825, XENP22826, XENP22827, XENP22828, XENP22829, XENP22830, XENP22831, XENP22832, XENP22833, XENP22834, XENP23343, XENP23504, XENP23554, XENP23555, XENP23557, XENP23559, XENP24019, XENP24020, XENP24045, XENP24051, XENP24052, XENP24113, XENP24301, XENP24306, XENP24341, XENP21478, XENP21479, XENP22357, XENP22354, XENP22355, XENP22356, XENP22357, XENP22358, XENP22359, XENP22360, XENP22361, XENP22362, XENP22363, XENP22364, XENP22365, XENP22366, XENP22637, XENP24349, XENP24383, XENP21978, XENP22634, XENP24342, XENP24306, XENP22637, and XENP22639. 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.

[0019a] In an additional aspect, there is provided a nucleic acid composition encoding the first fusion protein disclosed herein. In an additional aspect, there is provided a nucleic acid composition encoding the second fusion protein disclosed herein. In an additional aspect, there is provided an expression vector comprising the nucleic acid composition disclosed herein. In an additional aspect, there is provided a host cell comprising one or

more expression vectors disclosed herein. In an additional aspect, there is provided a nucleic acid composition encoding the fusion protein disclosed herein. In an additional aspect, there is provided a nucleic acid composition encoding the second Fc domain disclosed herein. In an additional aspect, there is provided a host cell comprising one or two expression vectors disclosed herein. In another aspect, there is provided a host cell comprising the nucleic acid composition disclosed herein. In another aspect, there is provided a host cell comprising the expression vector composition disclosed herein. In an additional aspect, there is provided a method of producing the heterodimeric protein disclosed herein comprising culturing the host cell disclosed herein under suitable conditions wherein said heterodimeric protein is expressed, and recovering said protein. In an additional aspect, there is provided use of a therapeutically effective amount of the heterodimeric protein disclosed herein in the manufacture of a medicament for treating cancer in a patient in need thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

[0020] 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).

[0021] Figures 2A-2B depict the sequences for IL-15 and its receptors. Figure 2A shows the sequences for human IL-15, human IL-15R α and human IL-15R β . Figure 2B shows the sequences for the human common gamma receptor.

[0022] Figures 3A-3E depict useful pairs of Fc heterodimerization variant sets (including skew and pI variants). On Figures 3D and 3E, there are variants for which there are no corresponding "monomer 2" variants; these are pI variants which can be used alone on either monomer.

XENP23343, XENP23504, XENP23554, XENP23555, XENP23557, XENP23559, XENP24019, XENP24020, XENP24045, XENP24051, XENP24052, XENP24113, XENP24301, XENP24306, and XENP24341.

10. A nucleic acid composition encoding the first fusion protein of any one of claims 1 to 9.

11. A nucleic acid composition encoding the second fusion protein of any one of claims 1 to 9.

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

13. An expression vector comprising the nucleic acid composition of claim 11.

14. The expression vector of claim 13 further comprising the nucleic acid composition of claim 10.

15. A host cell comprising one or more expression vectors of any one of claims 12 to 14.

16. A 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, and wherein said second protein domain is covalently attached to the N-terminus of said first Fc domain using a second domain linker;

b) a 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/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 and wherein said first protein domain comprises an IL15R α protein and said second protein domain comprises an IL15 protein.

53. A nucleic acid composition encoding the first fusion protein of any one of claims 46 to 52.

54. A nucleic acid composition encoding the second fusion protein of any one of claims 46 to 52.

55. An expression vector comprising the nucleic acid composition of claim 39.

56. An expression vector comprising the nucleic acid composition of claim 54.

57. The expression vector of claim 56, further comprising the nucleic acid composition of claim 53.

58. The expression vector of any one of claims 55 to 57, further comprising a nucleic acid composition encoding said third protein domain.

59. The expression vector of any one of claims 55 to 58, further comprising a nucleic acid composition encoding said fourth protein domain.

60. A host cell comprising one or more expression vectors of any one of claims 56 to 59.

61. A heterodimeric protein comprising:

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

b) a second Fc domain, and

c) a second protein domain noncovalently attached to said first protein domain of said first fusion protein;

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/LS364K/E357Q; S364K/E357Q : L368D/K370S; L368D/K370S : S364K; L368E/K370S : S364K; T411T/E360E/Q362E : D401K; L368D/K370S : S364K/E357L and K370S : S364K/E357Q,

80. A method of producing the heterodimeric protein of claim 75 comprising culturing the host cell claim 78 or 79 under suitable conditions wherein said heterodimeric protein is expressed, and recovering said protein.

81. Use of a therapeutically effective amount of the heterodimeric protein of claim 75 in the manufacture of a medicament for treating cancer in a patient in need thereof.