IL15/IL15Rα HETERODIMERIC FC-FUSION PROTEINS

PRIORITY CLAIM

[0001] This application claims priority to U.S. Ser. No. 62/408,655, filed Oct. 14, 2016, U.S. Ser. No. 62/416,087, filed Nov. 1, 2016, U.S. Ser. No. 62/443,465, filed Jan. 6, 2017, and U.S. Ser. No. 62/477,926, filed Mar. 28, 2017, which are expressly incorporated herein by reference in their entirety, with particular reference to the figures, legends and claims therein.

BACKGROUND OF THE INVENTION

[0002] IL-2 and IL-15 function in aiding the proliferation and differentiation of B cells, T cells, and NK cells. IL-2 is also essential for regulatory T cell (Treg) function and survival. Both cytokines exert their cell signaling function through binding to a trimeric complex consisting of two shared receptors, the common gamma chain (γc; CD132) and IL-2 receptor B-chain (IL-2Rβ; CD122), as well as an alpha chain receptor unique to each cytokine: IL-2 receptor alpha (IL-2Rα; CD25) or IL-15 receptor alpha (IL-15Rα; CD215). Both cytokines are considered as potentially valuable therapeutics in oncology and IL-2 has been approved for use in patients with metastatic renal-cell carcinoma and malignant melanoma. Currently there are no approved uses of recombinant IL-15, although several clinical trials are ongoing.

[0003] IL-2 presents several challenges as a therapeutic agent. First, it preferentially activates T cells that express the high affinity receptor complex, which depends on CD25 expression. Because Treg cells constitutively express CD25, they compete for IL-2 supplies with effector T cells, whose activation is preferred for oncology treatment. This imbalance has led to the concept of high dose IL-2. However, this approach creates additional problems because of IL-2-mediated toxicities such as vascular leak syndrome.

[0004] IL-2 is secreted primarily by activated T cells, while its receptors are located on activated T cells, Tregs, NK cells, and B cells. In contrast, IL-15 is produced on monocytes and dendritic cells and is primarily presented as a membrane-bound heterodimeric complex with IL-15Rα present on the same cells. Its effects are realized through trans-presentation of

the IL-15/IL-15Rα complex to NK cells and CD8+ T cells expressing IL-2Rβ 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-15Rα-associated complex. It has also been shown that recombinantly produced IL15/IL15Rα heterodimer can potently activate T cells. Nevertheless, a short half-life hinders favorable dosing. The present invention solves this problem by providing novel IL15/IL15Rα heterodimer Fc fusion proteins.

BRIEF SUMMARY OF THE INVENTION

[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 seconddomain 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 IL15Rα 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)

the first fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP16479) and the second fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP15908), (iv) 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 (XENP16481), (v) 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 (XENP16483), (vi) the first fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP16479) and the second fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP15909), (vii) the first fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP16479) and the second fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP16481), (viii) the first fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP16480) and the second fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP16482), (ix) the first fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP16480) and the second fusion protein h having as a polypeptide sequence of SEQ ID NO:XX (XENP15909), (x) the first fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP17064) and the second fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP17038), (xi) the first fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP17064) and the second fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP17040), (xii) the first fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP17062) and the second fusion protein having a polypeptide sequence of SEQ ID NO:XX (V17044), (xiii) the first fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP17686) and the second fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP15908), (xiv) the first fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP17687) and the second fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP15908), (xv) the first fusion protein having a polypeptide sequence of SEQ ID NO:XX (17688) and the second fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP15908), (xvi) the first fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP17689) and the second fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP15908), (xvii) the first fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP17690) and

the second fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP15908), (xviii) the first fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP17691) and the second fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP15908), (xix) the first fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP17692) and the second fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP15908), (xx) the first fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP17693) and the second fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP15908), (xxi) the first fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP17694) and the second fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP15908), (xxii) the first fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP17695) and the second fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP15908), (xxiii) the first fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP17696) and the second fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP15908), (xxiv) the first fusion protein having a polypeptide sequence of SEQ ID NO:XX (17697) and the second fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP15908), (xxv) the first fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP17698) and the second fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP15908), (xxvi) the first fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP17699) and the second fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP15908), (xxvii) the first fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP17701) and the second fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP15908), (xxviii) the first fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP17691) and the second fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP15908), (xxix) the first fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP17702) and the second fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP15908), (xxx) the first fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP17703) and the second fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP15908), (xxxi) the first fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP17704) and the second fusion protein having a

polypeptide sequence of SEQ ID NO:XX (XENP15908), (xxxii) the first fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP17705) and the second fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP15908), (xxxiii) said first fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP18295) and said second fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP17761), (xxxiv) said first fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP18783) and said second fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP15908), (xxxv) said first fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP18784) and said second fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP15908), (xxxvi) said first fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP18786) and said second fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP15908), (xxxvii) said first fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP18788) and said second fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP15908), (xxxviii) said first fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP19242) and said second fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP16481), or (xxxix) said first fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP19243) and said second fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP16481).

[0008] In some instances, the heterodimeric protein is 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.

[0009] In a further aspect, the invention provides a 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, and wherein the second protein domain is covalently attached to the N-terminus of the first Fc domain using a second domain linker;

b) a second Fc domain; 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 IL15Rα protein and the second protein domain comprises an IL15 protein.

[0010] In some embodiments, the first fusion protein has a polypeptide sequence of SEQ ID NO:XX (16478) and the Fc domain has a polypeptide sequence of SEQ ID NO:XX (8924). The heterodimeric protein can be XENP21478.

[0011] In another aspect, the invention provides a 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; b) a second Fc domain; and c) a second protein domain noncovalently attached to the first protein domain; 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 IL15Rα and the second protein domain comprises an IL15 protein.

[0012] In some embodiments, the heterodimer protein comprises: (i) the fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP16481), the second Fc domain having a polypeptide sequence of SEQ ID NO:XX (8793), and a second protein domain having a polypeptide sequence of SEQ ID NO:XX (16484); (ii) the fusion protein having a polypeptide sequence of SEQ ID NO:XX (17034), the second Fc domain having a polypeptide sequence of SEQ ID NO:XX (8793), and a second protein domain having a polypeptide sequence of SEQ ID NO:XX (16484); (iii) the fusion protein having a polypeptide sequence of SEQ ID NO:XX (17038), the second Fc domain having a polypeptide sequence of SEQ ID NO:XX (8793), and a second protein domain having a polypeptide sequence of SEQ ID NO:XX (16484); (iv) the fusion protein having a polypeptide sequence of SEQ ID NO:XX (17036), the second Fc domain having a

polypeptide sequence of SEQ ID NO:XX (8793), and a second protein domain having a polypeptide sequence of SEQ ID NO:XX (16484); (v) the fusion protein having a polypeptide sequence of SEQ ID NO:XX (17038), the second Fc domain having a polypeptide sequence of SEQ ID NO:XX (8793), and a second protein domain having a polypeptide sequence of SEQ ID NO:XX (17074); (vi) the fusion protein having a polypeptide sequence of SEQ ID NO:XX (17039), the second Fc domain having a polypeptide sequence of SEQ ID NO:XX (8793), and a second protein domain having a polypeptide sequence of SEQ ID NO:XX (17074); (vii) the fusion protein having a polypeptide sequence of SEQ ID NO:XX (17040), the second Fc domain having a polypeptide sequence of SEQ ID NO:XX (8793), and a second protein domain having a polypeptide sequence of SEQ ID NO:XX (17074); (viii) the fusion protein having a polypeptide sequence of SEQ ID NO:XX (17044), the second Fc domain having a polypeptide sequence of SEQ ID NO:XX (8793), and a second protein domain having a polypeptide sequence of SEQ ID NO:XX (17071); (ix) the fusion protein having a polypeptide sequence of SEQ ID NO:XX (17044), the second Fc domain having a polypeptide sequence of SEQ ID NO:XX (8793), and a second protein domain having a polypeptide sequence of SEQ ID NO:XX (17072); (x) the fusion protein having a polypeptide sequence of SEQ ID NO:XX (17075), the second Fc domain having a polypeptide sequence of SEQ ID NO:XX (8793), and a second protein domain having a polypeptide sequence of SEQ ID NO:XX (17041); (xi) the fusion protein having a polypeptide sequence of SEQ ID NO:XX (17043), the second Fc domain having a polypeptide sequence of SEQ ID NO:XX (8793), and a second protein domain having a polypeptide sequence of SEQ ID NO:XX (17070); (xii) the fusion protein having a polypeptide sequence of SEQ ID NO:XX (17045), the second Fc domain having a polypeptide sequence of SEQ ID NO:XX (8793), and a second protein domain having a polypeptide sequence of SEQ ID NO:XX (17073); (xiii) the fusion protein having a polypeptide sequence of SEQ ID NO:XX (17042), the second Fc domain having a polypeptide sequence of SEQ ID NO:XX (8793), and a second protein domain having a polypeptide sequence of SEQ ID NO:XX (17083); or (xiv) the fusion protein having a polypeptide sequence of SEQ ID NO:XX (15908), the second Fc domain having a polypeptide sequence of SEQ ID NO:XX (8793), and a second protein domain having a polypeptide sequence of SEQ ID NO:XX (16484). The heterodimer protein can be selected

from the group consisting of XENP21479, XENP22357, XENP22354, XENP22355, XENP22356, XENP22357, XENP22358, XENP22359, XENP22360, XENP22361, XENP22362, XENP22363, XENP22364, XENP22365, XENP22366, and XENP22637.

[0013] In an additional aspect, the 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 said first Fc domain using a domain linker; b) a second fusion protein comprising a second heavy chain comprising a second protein domain and a first second heavy chain comprising a second Fc domain, wherein the second protein domain is covalently attached to the C-terminus of the second Fc domain using a domain linker; c) a third protein domain noncovalently attached to the first protein domain of the first fusion protein; and d) a fourth protein domain noncovalently attached to the second protein domain of the second 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/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 andwherein the first protein domain and the second protein domain comprise an IL15Rα protein, and wherein the third protein domain and the fourth protein domain comprises an IL15 protein.

[0014] In some embodiments, the heterodimer protein comprises (i) the first fusion protein has a polypeptide sequence of SEQ ID NO:XX (17023) the second fusion protein has a polypeptide sequence of SEQ ID NO:XX (17023), the third protein domain has a polypeptide sequence of SEQ ID NO:XX (16484), and the fourth protein domain has a polypeptide sequence of SEQ ID NO:XX (16484) or (ii) the first fusion protein has a polypeptide sequence of SEQ ID NO:XX (17581), the second fusion protein has a polypeptide sequence of SEQ ID NO:XX (17581), the third protein domain has a polypeptide sequence of SEQ ID NO:XX (17074), and the fourth protein domain has a polypeptide sequence of SEQ ID NO:XX (17074). The heterodimer protein can be XENP21978 or XENP22634.

[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/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 IL15Rα 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 invention, 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 invention, 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 IL15Rα protein has a polypeptide sequence selected from the group consisting of SEQ ID NO:3 (full-length human IL15Rα) and SEQ ID NO:4 (sushi domain of human IL15Rα). In some cases, the IL15

protein and the IL15Rα 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] In an additional aspect, the present invention provides 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. In some aspects, the present invention provides 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. 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.

BRIEF DESCRIPTION OF THE DRAWINGS

[0020] Figure 1 depicts the structure of IL-15 in complex with its receptors IL-15Rα (CD215), IL-15Rβ (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-15Rα and human IL-15Rβ. Figure 2A 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.

[0023] 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).

[0024] Figure 5 depict useful ablation variants that ablate FcγR binding (sometimes referred to as "knock outs" or "KO" variants). Generally, ablation variants are found on both monomers, although in some cases they may be on only one monomer.

[0025] Figures 6A-6E show a particularly useful embodiments of "non-cytokine" components of the invention.

[0026] Figure 7 depicts a number of exemplary variable length linkers. In some embodiments, these linkers find use linking the C-terminus of IL-15 and/or IL-15Rα(sushi) to the N-terminus of the Fc region. In some embodiments, these linkers find use fusing IL-15 to the IL-15Rα(sushi).

[0027] Figures 8A-8E show the sequences of several useful IL-15/Rα-Fc format backbones based on human IgG1, without the cytokine sequences (e.g., the Il-15 and/or IL-15Rα(sushi)). Backbone 1 is based on human IgG1 (356E/358M allotype), and includes C220S on both chain, the S364K/E357Q : L368D/K370S skew variants, the Q295E/N384D/Q418E/N421D pI variants on the chain with L368D/K370S skew variants and the E233P/L234V/L235A/G236del/S267K ablation variants on both chains. Backbone 2 is based on human IgG1 (356E/358M allotype), and includes C220S on both chain, the S364K : L368D/K370S skew variants, the Q295E/N384D/Q418E/N421D pI variants on the chain with L368D/K370S skew variants and the E233P/L234V/L235A/G236del/S267K ablation variants on both chains. Backbone 3 is based on human IgG1 (356E/358M allotype), and includes C220S on both chain, the S364K : L368E/K370S skew variants, the Q295E/N384D/Q418E/N421D pI variants on the chain with L368E/K370S skew variants and the E233P/L234V/L235A/G236del/S267K ablation variants on both chains. Backbone 4 is based on human IgG1 (356E/358M allotype), and includes C220S on both chain, the D401K : K360E/Q362E/T411E skew variants, the Q295E/N384D/Q418E/N421D pI variants on the

chain with K360E/Q362E/T411E skew variants and the E233P/L234V/L235A/G236del/S267K ablation variants on both chains. Backbone 5 is based on human IgG1 (356D/358L allotype), and includes C220S on both chain, the S364K/E357Q : L368D/K370S skew variants, the Q295E/N384D/Q418E/N421D pI variants on the chain with L368D/K370S skew variants and the E233P/L234V/L235A/G236del/S267K ablation variants on both chains. Backbone 6 is based on human IgG1 (356E/358M allotype), and includes C220S on both chain, the S364K/E357Q : L368D/K370S skew variants, Q295E/N384D/Q418E/N421D pI variants on the chain with L368D/K370S skew variants and the E233P/L234V/L235A/G236del/S267K ablation variants on both chains, as well as an N297A variant on both chains. Backbone 7 is identical to 6 except the mutation is N297S. Alternative formats for backbones 6 and 7 can exclude the ablation variants E233P/L234V/L235A/G236del/S267K in both chains. Backbone 8 is based on human IgG4, and includes the S364K/E357Q : L368D/K370S skew variants, the Q295E/N384D/Q418E/N421D pI variants on the chain with L368D/K370S skew variants, as well as a S228P (EU numbering, this is S241P in Kabat) variant on both chains that ablates Fab arm exchange as is known in the art. Backbone 9 is based on human IgG2, and includes the S364K/E357Q : L368D/K370S skew variants, the Q295E/N384D/Q418E/N421D pI variants on the chain with L368D/K370S skew variants. Backbone 10 is based on human IgG2, and includes the S364K/E357Q : L368D/K370S skew variants, the Q295E/N384D/Q418E/N421D pI variants on the chain with L368D/K370S skew variants as well as a S267K variant on both chains. Backbone 11 is identical to backbone 1, except it includes M428L/N434S Xtend mutations. Backbone 12 is based on human IgG1 (356E/358M allotype), and includes C220S on both identical chain, the the E233P/L234V/L235A/G236del/S267K ablation variants on both identical chains. Backbone 13 is based on human IgG1 (356E/358M allotype), and includes C220S on both chain, the S364K/E357Q : L368D/K370S skew variants, the P217R/P229R/N276K pI variants on the chain with S364K/E357Q skew variants and the E233P/L234V/L235A/G236del/S267K ablation variants on both chains.

[0028] As will be appreciated by those in the art and outlined below, these sequences can be used with any IL-15 and IL-15Rα(sushi) pairs outlined herein, including but not limited to IL-15/Rα-heteroFc, ncIL-15/Rα, scIL-15/Rα, and dsIL-15/Rα as schematically depicted in Figures 9A-9G, and Figures 39A-39D. Additionally, any IL-15 and/or IL-

15Rα(sushi) variants can be incorporated into these Figures 8A-8E backbones in any combination.

[0029] Included within each of these backbones are sequences that are 90%, 95%, 98% and 99% identical (as defined herein) to the recited sequences, and/or contain from 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 additional amino acid substitutions (as compared to the "parent" of the Figure, which, as will be appreciated by those in the art, already contain a number of amino acid modifications as compared to the parental human IgG1 (or IgG2 or IgG4, depending on the backbone). That is, the recited backbones may contain additional amino acid modifications (generally amino acid substitutions) in addition to the skew, pI and ablation variants contained within the backbones of this figure (Figure 8).

[0030] Figures 9A-9G depict several formats for the IL-15/Rα-Fc fusion proteins of the present invention. IL-15Rα Heterodimeric Fc fusion or "IL-15/Rα-heteroFc" (Figure 9A) comprises IL-15 recombinantly fused to one side of a heterodimeric Fc and IL-15Rα(sushi) recombinantly fused to the other side of a heterodimeric Fc. The IL-15 and IL-15Rα(sushi) may have a variable length Gly-Ser linker between the C-terminus and the N-terminus of the Fc region. Single-chain IL-15/Rα-Fc fusion or "scIL-15/Rα-Fc" (Figure 9B) comprises IL-15Rα(sushi) fused to IL-15 by a variable length linker (termed a "single-chain" IL-15/IL-15Rα(sushi) complex or "scIL-15/Rα") which is then fused to the N-terminus of a heterodimeric Fc-region, with the other side of the molecule being "Fc-only" or "empty Fc". Non-covalent IL-15/Rα-Fc or "ncIL-15/Rα-Fc" (Figure 9C) comprises IL-15Rα(sushi) fused to a heterodimeric Fc region, while IL-15 is transfected separatedly so that a non-covalent IL-15/Rα complex is formed, with the other side of the molecule being "Fc-only" or "empty Fc". Bivalent non-covalent IL-15/Rα-Fc fusion or "bivalent ncIL-15/Rα-Fc" (Figure 9D) comprises IL-15Rα(sushi) fused to the N-terminus of a homodimeric Fc region, while IL-15 is transfected separately so that a non-covalent IL-15/Rα complex is formed. Bivalent single-chain IL-15/Rα-Fc fusion or "bivalent scIL-15/Rα-Fc" (Figure 9E) comprises IL-15 fused to IL-15Rα(sushi) by a variable length linker (termed a "single-chain" IL-15/IL-15Rα(sushi) complex or "scIL-15/Rα") which is then fused to the N-terminus of a homodimeric Fc-region. Fc-non-covalent IL-15/Rα fusion or "Fc-ncIL-15/Rα" (Figure 9F) comprises IL-15Rα(sushi) fused to the C-terminus of a heterodimeric Fc region, while IL-15 is transfected

separately so that a non-covalent IL-15/Rα complex is formed, with the other side of the molecule being "Fc-only" or "empty Fc". Fc-single-chain IL-15/Rα fusion or "Fc-scIL-15/Rα" (Figure 9G) comprises IL-15 fused to IL-15Rα(sushi) by a variable length linker (termed a "single-chain" IL-15/IL-15Rα(sushi) complex or "scIL-15/Rα") which is then fused to the C-terminus of a heterodimeric Fc region, with the other side of the molecule being "Fc-only" or "empty Fc".

[0031] Figure 10 depicts sequences of XENP20818 and XENP21475, illustrative IL-15/Rα-Fc fusion proteins of the "IL-15/Rα-heteroFc" format, with additional sequences being listed as XENPs 20819, 21471, 21472, 21473, 21474, 21476, and 21477 in the sequence listing. IL-15 and IL-15Rα(sushi) are underlined, linkers are double underlined (although as will be appreciated by those in the art, the linkers can be replaced by other linkers, some of which are depicted in Figure 7), and slashes (/) indicate the border(s) between IL-15, IL-15Rα, linkers, and Fc regions.

[0032] Figure 11 depicts sequences of XENP21478, an illustrative IL-15/Rα-Fc fusion protein of the "scIL-15/Rα-Fc" format, with additional sequences being listed as XENPs 21993, 21994, 21995, 23174, 23175, 24477, and 24480 in the sequence listing. IL-15 and IL-15Rα(sushi) are underlined, linkers are double underlined (although as will be appreciated by those in the art, the linkers can be replaced by other linkers, some of which are depicted in Figure 7), and slashes (/) indicate the border(s) between IL-15, IL-15Rα, linkers, and Fc regions.

[0033] Figures 12A-12B depict sequences of XENP21479, XENP22366 and XENP24348, illustrative IL-15/Rα-Fc fusion proteins of the "ncIL-15/Rα-Fc" format. IL-15 and IL-15Rα(sushi) are underlined, linkers are double underlined (although as will be appreciated by those in the art, the linkers can be replaced by other linkers, some of which are depicted in Figure 7), and slashes (/) indicate the border(s) between IL-15, IL-15Rα, linkers, and Fc regions.

[0034] Figure 13 depicts sequences of XENP21978, an illustrative IL-15/Rα-Fc fusion protein of the "bivalent ncIL-15/Rα-Fc" format, with additional sequences being listed as XENP21979 in the sequence listing. IL-15 and IL-15Rα(sushi) are underlined, linkers are

double underlined (although as will be appreciated by those in the art, the linkers can be replaced by other linkers, some of which are depicted in Figure 7), and slashes (/) indicate the border(s) between IL-15, IL-15Rα, linkers, and Fc regions.

[0035] Figure 14 depicts sequences of an illustrative IL-15/Rα-Fc fusion protein of the "bivalent scIL-15/Rα-Fc" format. IL-15 and IL-15Rα(sushi) are underlined, linkers are double underlined (although as will be appreciated by those in the art, the linkers can be replaced by other linkers, some of which are depicted in Figure 7), and slashes (/) indicate the border(s) between IL-15, IL-15Rα, linkers, and Fc regions.

[0036] Figure 15 depicts sequences of XENP22637, an illustrative IL-15/Rα-Fc fusion protein of the "Fc-ncIL-15/Rα" format, with additional sequences being listed as XENP22638 in the sequence listing. IL-15 and IL-15Rα(sushi) are underlined, linkers are double underlined (although as will be appreciated by those in the art, the linkers can be replaced by other linkers, some of which are depicted in Figure 7), and slashes (/) indicate the border(s) between IL-15, IL-15Rα, linkers, and Fc regions.

[0037] Figure 16 depicts sequences of an illustrative IL-15/Rα-Fc fusion protein of the "Fc-scIL-15/Rα" format. IL-15 and IL-15Rα(sushi) are underlined, linkers are double underlined (although as will be appreciated by those in the art, the linkers can be replaced by other linkers, some of which are depicted in Figure 7), and slashes (/) indicate the border(s) between IL-15, IL-15Rα, linkers, and Fc regions.

[0038] Figures 17A-17E depict A) the IL-15/Rα-Fc fusion protein format for XENP20818, the purity and homogeneity of XENP20818 as determined by B) SEC and C) CEF, D) the affinity of XENP20818 for IL-2Rβ as determined by Octet, and E) the stability of XENP20818 as determined by DSF

[0039] Figures 18A-18E depict A) the IL-15/Rα-Fc fusion protein format for XENP21478, the purity and homogeneity of XENP21478 as determined by B) SEC and C) CEF, D) the affinity of XENP21478 for IL-2Rβ as determined by Octet, and E) the stability of XENP21478 as determined by DSF.

[0040] Figures 19A-19E depicts A) the IL-15/Rα-Fc fusion protein format for XENP21479, the purity and homogeneity of XENP21479 as determined by B) SEC and C)

CEF, D) the affinity of XENP21479 for IL-2Rβ as determined by Octet, and E) the stability of XENP21479 as determined by DSF.

[0041] Figures 20A-20C depict the induction of A) NK (CD56+/CD16+) cells, B) CD4+ T cells, and C) CD8+ T cells proliferation by illustrative IL-15/Rα-Fc fusion proteins of the IL-15/Rα-heteroFc format with different linker lengths based on Ki67 expression as measured by FACS.

[0042] Figures 21A-21C depict the induction of A) NK (CD56+/CD16+) cells, B) CD4+ T cells, and C) CD8+ T cells proliferation by illustrative IL-15/Rα-Fc fusion proteins of the scIL-15/Rα-Fc format (XENP21478) and the ncIL-15/Rα-Fc format (XENP21479) based on Ki67 expression as measured by FACS.

[0043] Figure 22 depicts enhancement of IL-2 secretion by illustrative IL-15/Rα-Fc fusion proteins, an isotype control, and a bivalent anti-PD-1 antibody over PBS control in an SEB-stimulated PBMC assay.

[0044] Figure 23 depicts the survival curve for PBMC-engrafted NSG mice following treatment with XENP20818 and recombinant IL-15.

[0045] Figure 24 depicts the concentration of IFNγ in serum of NSG mice on Day 7 after engraftment with human PBMCs and treatment with XENP20818 at the indicated concentrations.

[0046] Figures 25A-25C depict A) CD4+ T cell, B) CD8+ T cell, and C) CD45+ cell counts in whole blood of human PBMC-engrafted NSG mice 7 days after treatment with XENP20818 at the indicated concentrations.

[0047] Figure 26 depicts a structural model of the IL-15/Rα heterodimer showing locations of engineered disulfide bond pairs.

[0048] Figure 27 depicts sequences for illustrative IL-15Rα(sushi) variants engineered with additional residues at the C-terminus to serve as a scaffold for engineering cysteine residues.

[0049] Figure 28 depicts sequences for illustrative IL-15 variants engineered with cysteines in order to form covalent disulfide bonds with IL-15Rα(sushi) variants engineered with cysteines.

[0050] Figure 29 depicts sequences for illustrative IL-15Rα(sushi) variants engineered with cysteines in order to form covalent disulfide bonds with IL-15 variants engineered with cysteines.

[0051] Figures 30A-30C depict IL-15/Rα heterodimers with and without engineered disulfide bonds between IL-15 and IL-15Rα(sushi). Non-covalent IL-15/Rα heterodimer or "ncIL-15/Rα heterodimer" (Figure 30A) comprises IL-15Rα(sushi) and IL-15 transfected separately and non-covalently linked. Disulfide-bonded IL-15/Rα heterodimer or "dsIL-15/Rα heterodimer" (Figure 30B) comprises IL-15Rα(sushi) and IL-15 transfected separately and covalently linked as a result of engineered cysteines. Single-chain IL-15/Rα heterodimer or "scIL-15/Rα Heterodimer" (Figure 30C) comprises IL-15Rα(sushi) fused to IL-15 by a variable length Gly-Ser linker.

[0052] Figure 31 depicts sequences of XENP21996, an illustrative ncIL-15/Rα heterodimer. It is important to note that these sequences were generated using polyhistidine (Hisx6 or HHHHHH) C-terminal tags at the C-terminus of IL-15Rα(sushi).

[0053] Figure 32 depicts sequences of XENP22004, XENP22005, XENP22006, XENP22008, and XENP22494, illustrative dsIL-15/Rα heterodimers, with additional sequences depicted as XENPs 22007, 22009, 22010, 22011, 22012, and 22493 in the sequence listing. It is important to note that these sequences were generated using polyhistidine (Hisx6 or HHHHHH) C-terminal tags at the C-terminus of IL-15Rα(sushi).

[0054] Figure 33 depicts the sequence for XENP22049, an illustrative scIL-15/Rα Heterodimer. It is important to note that these sequences were generated using polyhistidine (Hisx6 or HHHHHH) C-terminal tags at the C-terminus of IL-15. IL-15 and IL-15Rα(sushi) are underlined, linkers are double underlined (although as will be appreciated by those in the art, the linkers can be replaced by other linkers, some of which are depicted in Figure 7), and slashes (/) indicate the border(s) between IL-15, IL-15Rα, and linker

[0055] Figure 34 depicts the purity and homogeneity of illustrative IL-15/Rα heterodimers with and without engineered disulfide bonds as determined by CEF.

[0056] Figure 35 depicts the purity and homogeneity of illustrative IL-15/Rα heterodimers with and without engineered disulfide bonds as determined by CEF.

[0057] Figure 36 depicts the stability and melting temperatures of illustrative IL-15/Rα heterodimers with and without engineered disulfide bonds as indicated by melting curves from DSF.

[0058] Figure 37 depicts the stability and melting temperatures of illustrative IL-15/Rα heterodimers with and without engineered disulfide bonds as indicated by melting curves from DSF.

[0059] Figure 38 depicts the expression yield, molecular weight, predicted change in affinity between IL-15 and IL-15Rα(sushi) as calculated by MOE software, melting temperature, and affinity for IL-2Rß for IL-15/Rα heterodimers with and without engineered disulfide bonds. Mutations are indicated in parentheses after the relevant monomer.

[0060] Figures 39A-39D depict additional formats for the IL-15/Rα-Fc fusion proteins of the present invention with engineered disulfide bonds. Disulfide-bonded IL-15/Rα heterodimeric Fc fusion or "dsIL-15/Rα-heteroFc" (Figure 39A) is the same as "IL-15/Rα-heteroFc", but wherein IL-15Rα(sushi) and IL-15 are further covalently linked as a result of engineered cysteines. Disulfide-bonded IL-15/Rα Fc fusion or "dsIL-15/Rα-Fc" (Figure 39B) is the same as "ncIL-15/Rα-Fc", but wherein IL-15Rα(sushi) and IL-15 are further covalently linked as a result of engineered cysteines. Bivalent disulfide-bonded IL-15/Rα-Fc or "bivalent dsIL-15/Rα-Fc" (Figure 39C) is the same as "bivalent ncIL-15/Rα-Fc", but wherein IL-15Rα(sushi) and IL-15 are further covalently linked as a result of engineered cysteines. Fc-disulfide-bonded IL-15/Rα fusion or "Fc-dsIL-15/Rα" (Figure 39D) is the same as "Fc-ncIL-15/Rα", but wherein IL-15Rα(sushi) and IL-15 are further covalently linked as a result of engineered cysteines.

[0061] Figures 40A-40B depicts sequences of XENP22013, XENP22014, XENP22015, and XENP22017, illustrative IL-15/Rα-Fc fusion protein of the "dsIL-15/Rα-heteroFc" format. IL-15 and IL-15Rα(sushi) are underlined, linkers are double underlined (although as will be

appreciated by those in the art, the linkers can be replaced by other linkers, some of which are depicted in Figure 7), and slashes (/) indicate the border(s) between IL-15, IL-15Rα, linkers, and Fc regions.

[0062] Figures 41A-41B depict sequences of XENP22357, XENP22358, XENP22359, XENP22684, and XENP22361, illustrative IL-15/Rα-Fc fusion proteins of the "dsIL-15/Rα-Fc" format. Additional sequences are depicted as XENPs 22360, 22362, 22363, 22364, 22365, and 22366 in the sequence listing. IL-15 and IL-15Rα(sushi) are underlined, linkers are double underlined (although as will be appreciated by those in the art, the linkers can be replaced by other linkers, some of which are depicted in Figure 7), and slashes (/) indicate the border(s) between IL-15, IL-15Rα, linkers, and Fc regions.

[0063] Figure 42 depicts sequences of XENP22634, XENP22635, and XENP22636, illustrative IL-15/Rα-Fc fusion proteins of the "bivalent dsIL-15/Rα-Fc" format. Additional sequences are depicted as XENP22687 in the sequence listing. IL-15 and IL-15Rα(sushi) are underlined, linkers are double underlined (although as will be appreciated by those in the art, the linkers can be replaced by other linkers, some of which are depicted in Figure 7), and slashes (/) indicate the border(s) between IL-15, IL-15Rα, linkers, and Fc regions.

[0064] Figure 43 depicts sequences of XENP22639 and XENP22640, illustrative IL-15/Rα-Fc fusion proteins of the "Fc-dsIL-15/Rα" format. IL-15 and IL-15Rα(sushi) are underlined, linkers are double underlined (although as will be appreciated by those in the art, the linkers can be replaced by other linkers, some of which are depicted in Figure 7), and slashes (/) indicate the border(s) between IL-15, IL-15Rα, linkers, and Fc regions.

[0065] Figure 44 depicts the purity and homogeneity of illustrative IL-15/Rα-Fc fusion proteins with and without engineered disulfide bonds as determined by CEF.

[0066] Figures 45A-45C depict the induction of A) NK (CD56+/CD16+) cell, B) CD8+ T cell, and C) CD4+ T cell proliferation by illustrative IL-15/Rα-Fc fusion proteins with and without engineered disulfide bonds based on Ki67 expression as measured by FACS.

[0067] Figure 46 depicts the structure of IL-15 complexed with IL-15Rα, IL-2Rß, and common gamma chain. Locations of substitutions designed to reduce potency are shown.

[0068] Figures 47A-47C depict sequences for illustrative IL-15 variants engineered for reduced potency. Included within each of these variant IL-15 sequences are sequences that are 90%, 95%, 98%, and 99% identical (as defined herein) to the recited sequences, and/or contain from 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 additional amino acid substitutions. In a non-limiting example, the recited sequences may contain additional amino acid modifications such as those contributing to formation of covalent disulfide bonds as described in Example 2.

[0069] Figures 48A-48D depict sequences of XENP22821, XENP22822, XENP23554, XENP23557, XENP23561, XENP24018, XENP24019, XENP24045, XENP24051, and XENP24052, illustrative IL-15/Rα-Fc fusion proteins of the "IL-15/Rα-heteroFc" format engineered for lower potency. Additional sequences are depicted as XENPs 22815, 22816, 22817, 22818, 22819, 22820, 22823, 22824, 22825, 22826, 22827, 22828, 22829, 22830, 22831, 22832, 22833, 22834, 23555, 23559, 23560, 24017, 24020, 24043, and 24048 in the sequence listing. IL-15 and IL-15Rα(sushi) are underlined, linkers are double underlined (although as will be appreciated by those in the art, the linkers can be replaced by other linkers, some of which are depicted in Figure 7), and slashes (/) indicate the border(s) between IL-15, IL-15Rα, linkers, and Fc regions..

[0070] Figures 49A-49C depict sequences of XENP24015, XENP24050, XENP24475, XENP24476, XENP24478, XENP24479, and XENP24481, illustrative IL-15/Rα-Fc fusion proteins of the "scIL-15/Rα-Fc" format engineered for lower potency. Additional sequences are depicted as XENPs 24013, 24014, and 24016 in the sequence listing. IL-15 and IL-15Rα(sushi) are underlined, linkers are double underlined (although as will be appreciated by those in the art, the linkers can be replaced by other linkers, some of which are depicted in Figure 7), and slashes (/) indicate the border(s) between IL-15, IL-15Rα, linkers, and Fc regions.

[0071] Figures 50A-50B depict sequences of XENP24349, XENP24890, and XENP25138, illustrative IL-15/Rα-Fc fusion proteins of the "ncIL-15/Rα-Fc" format engineered for lower potency. IL-15 and IL-15Rα(sushi) are underlined, linkers are double underlined (although as will be appreciated by those in the art, the linkers can be replaced by other linkers, some of which are depicted in Figure 7), and slashes (/) indicate the border(s) between IL-15, IL-15Rα, linkers, and Fc regions.

[0072] Figure 51 depicts sequences of XENP22801 and XENP22802, illustrative ncIL-15/Rα heterodimers engineered for lower potency. Additional sequences are depicted as XENPs 22791, 22792, 22793, 22794, 22795, 22796, 22803, 22804, 22805, 22806, 22807, 22808, 22809, 22810, 22811, 22812, 22813, and 22814 in the sequence listing. It is important to note that these sequences were generated using polyhistidine (Hisx6 or HHHHHH) C-terminal tags at the C-terminus of IL-15Rα(sushi)..

[0073] Figure 52 depicts sequences of XENP24342, an illustrative IL-15/Rα-Fc fusion protein of the "bivalent ncIL-15/Rα-Fc" format engineered for lower potency. IL-15 and IL-15Rα(sushi) are underlined, linkers are double underlined (although as will be appreciated by those in the art, the linkers can be replaced by other linkers, some of which are depicted in Figure 7), and slashes (/) indicate the border(s) between IL-15, IL-15Rα, linkers, and Fc regions.

[0074] Figure 53 depicts sequences of XENP23472 and XENP23473, illustrative IL-15/Rα-Fc fusion proteins of the "dsIL-15/Rα-Fc" format engineered for lower potency. IL-15 and IL-15Rα(sushi) are underlined, linkers are double underlined (although as will be appreciated by those in the art, the linkers can be replaced by other linkers, some of which are depicted in Figure 7), and slashes (/) indicate the border(s) between IL-15, IL-15Rα, linkers, and Fc regions.

[0075] Figures 54A-54C depict the induction of (A) NK cell, (B) CD8+ (CD45RA-) T cell, and (C) CD4+ (CD45RA-) T cell proliferation by variant IL-15/Rα-Fc fusion proteins based on Ki67 expression as measured by FACS.

[0076] Figure 55 depicts EC50 for induction of NK and CD8+ T cells proliferation by variant IL-15/Rα-Fc fusion proteins, and fold reduction in EC50 relative to XENP20818.

[0077] Figures 56A-56C depict the gating of lymphocytes and subpopulations for the experiments depicted in Figures 59A-59D. Figure 56A shows the gated lymphocyte population. Figure 56B shows the CD3-negative and CD3-positive subpopulations. Figure 56C shows the CD16=negative and CD16-positive subpopulations of the CD3-negative cells.

[0078] Figures 57A-57C depict the gating of CD3+ lymphocyte subpopulations for the experiments depicted in Figures 59A-59D. Figure 57A shows the CD4+, CD8+ and γδ T cell

subpopulations of the CD3+ T cells. Figure 57B shows the CD45RA(-) and CD45RA(+) subpopulations of the CD4+ T cells. Figure 57C shows the CD45RA(-) and CD45RA(+) subpopulation s of the CD8+ T cells.

[0079] Figures 58A-58B depict CD69 and CD25 expression before (Figure 58A) and after (Figure 58B) incubation of human PBMCs with XENP22821.

[0080] Figures 59A-59D depict cell proliferation in human PBMCs incubated for four days with the indicated variant IL-15/Rα-Fc fusion proteins. Figures 59A-C show the percentage of proliferating NK cells (CD3-CD16+) (Figure 59A), CD8+ T cells (CD3+CD8+CD45RA-) (Figure 59B) and CD4+ T cells (CD3+CD4+CD45RA-) (Figure 59C). Figure 59D shows the fold change in EC50 of various IL15/IL15Rα Fc heterodimers relative to control (XENP20818).

[0081] Figures 60A-60D depict cell proliferation in human PBMCs incubated for three days with the indicated variant IL-15/Rα-Fc fusion proteins. Figures 60A-C show the percentage of proliferating CD8+ (CD45RA-) T cells (Figure A), CD4+ (CD45RA-) T cells (Figure 60B), γδ T cells (Figure 60C), and NK cells (Figure 60D).

[0082] Figures 61A-61C depict the percentage of Ki67 expression on (A) CD8+ T cells, (B) CD4+ T cells, and (C) NK cells following treatment with additional IL-15/Rα variants.

[0083] Figures 62A-62E depict the percentage of Ki67 expression on (A) CD8+ (CD45RA-) T cells, (B) CD4+ (CD45RA-) T cells, (C) γδ T cells, (D) NK (CD16+CD8α-) cells, and (E) NK (CD56+CD8α-) cells following treatment with IL-15/Rα variants.

[0084] Figures 63A-63E depict the percentage of Ki67 expression on (A) CD8+ (CD45RA-) T cells, (B) CD4+ (CD45RA-) T cells, (C) γδ T cells, (D) NK (CD16+CD8α-) cells, and (E) NK (CD56+CD8α-) cells following treatment with IL-15/Rα variants.

[0085] Figures 64A-64D depict the percentage of Ki67 expression on (A) CD8+ T cells, (B) CD4+ T cells, (C) γδ T cells and (D) NK (CD16+) cells following treatment with additional IL-15/Rα variants engineered for decreased potency with different linker lengths.

[0086] Figures 65A-65D depict the percentage of Ki67 expression on (A) CD8+ T cells, (B) CD4+ T cells, (C) γδ T cells and (D) NK (CD16+) cells following treatment with additional IL-15/Rα variants.

[0087] Figures 66A-66D depict gating of lymphocytes and subpopulations thereof for the experiments depicted in Figure 67. Figure 66A shows gating of the lymphocyte population. Figure 66B shows CD4+ and CD8+ T cells. Figure 66C shows the CD45RA and CD27 expressing subpopulations of CD4+ T cells. Figure 66D shows the CD45RA and CD27 expressing subpopulations of CD8+ T cells.

[0088] Figures 67A-67C depict STAT5 phosphorylation on A) CD8+ T cells (CD45RA-CD27-) and B) CD4+ T cells (CD45RA-CD27-) following incubation of PBMCs for 4 days with the indicated variant IL15/IL15Rα-Fc fusion proteins at the indicated concentrations. Figure 67C shows the fold change in EC50 of various IL15/IL15Rα Fc heterodimers relative to control (XENP20818).

[0089] Figure 68 depicts IV-TV Dose PK of various IL-15/Rα-Fc fusion proteins or controls in C57BL/6 mice at 0.1 mg/kg single dose.

[0090] Figure 69 depicts the correlation of half-life vs NK cell potency.

[0091] Figure 70 shows that CD45+ cell levels are predictive of disease.

[0092] Figures 71A-71B depict the enhancement of engraftment by variant IL-15/Rα-Fc fusion proteins as indicated by CD45+ cell counts on Days A) 4 and B) 8.

[0093] Figures 72A-72C depict IFNγ levels on Days (A) 4, (B) 7 and (C) 11 after treatment of NSG mice engrafted with human PBMCs with the indicated variant IL15/Rα-Fc fusion proteins or control.

[0094] Figures 73A-73C depict CD45+ lymphocyte cell counts on Days (A) 4, (B) 7, and (C) 11 after treatment of NSG mice engrafted with human PBMCs with the indicated variant IL15/Rα-Fc fusion proteins or control.

[0095] Figures 74A-74C depict NK cell (CD16+CD56+CD45RA+) counts on Days A) 4, B) 7 and C) 11 after treatment of NSG mice engrafted with human PBMCs with the indicated IL15/Rα-Fc fusion proteins or control.

[0096] Figures 75A-75B depict CD8+ T cell (CD8+CD45RA+) counts on Days (A) 7 and (B) 11 after treatment of NSG mice engrafted with human PBMCs with the indicated IL15/Rα-Fc fusion proteins or control.

[0097] Figures 76A-76B depict CD4+ T cell (CD4+CD45RA+) counts on Days A) 7 and B) 11 after treatment of NSG mice engrafted with human PBMCs with the indicated IL15/Rα-Fc fusion proteins or control.

[0098] Figure 77 depicts IFNγ level on Days 4, 7, and 11 in serum of huPBMC engrafted mice following treatment with additional variant IL-15/Rα-Fc fusion proteins.

[0099] Figures 78A-78C depict CD8+ T cell count on Days (A) 4, (B) 7, and (C) 11 in whole blood of huPBMC engrafted mice following treatment with additional variant IL-15/Rα-Fc fusion proteins.

[00100] Figures 79A-79C depict CD4+ T cell count on Days (A) 4, (B) 7, and (C) 11 in whole blood of huPBMC engrafted mice following treatment with additional variant IL-15/Rα-Fc fusion proteins.

[00101] Figures 80A-80C depict CD45+ cell count on Days (A) 4, (B) 7, and (C) 11 in whole blood of huPBMC engrafted mice following treatment with additional variant IL-15/Rα-Fc fusion proteins.

[00102] Figures 81A-81C depict the body weight as a percentage of initial body weight of huPBMC engrafted mice on Days (A) 4, (B) 7, and (C) 11 following treatment with additional IL-15/Rα variants. Each point represents a single NSG mouse. Mice whose body weights dropped below 70% initial body weight were euthanized. Dead mice are represented as 70%.

[00103] Figures 82A-82E depict lymphocyte counts after dosing cynomolgus monkeys with XENP20818. Figures 82A-E respectively show the fold change in absolute count of CD56+ NK cells (Figure 82A), CD16+ NK cells (Figure 82B), γδ T cells (CD45RA+CD3+CD4-CD8-) (Figure 82C), CD8+ T cells (Figure 82D), and CD4+ T cells (Figure 82E).

[00104] Figures 83A-83E depict proliferation of CD56+ NK cells (Figure 83A), CD16+ NK cells (Figure 83B), CD8+ T cells (CD45RA+) (Figure 83C), CD8+ T cells (CD45RA-) (Figure

83D), and CD4+ T cells (CD45RA-) (Figure 83E) after dosing cynomolgus monkeys with XENP20818.

[00105] Figures 84A-84E depict lymphocyte counts after dosing cynomolgus monkeys with XENP22819. Figures 84A-E respectively show the fold change in absolute count of CD56+ NK cells (Figure 84A), CD16+ NK cells (Figure 84B), γδ T cells (CD45RA+CD3+CD4-CD8-) (Figure 84C), CD8+ T cells (Figure 84D), and CD4+ T cells (Figure 84E).

[00106] Figures 85A-85E depict proliferation of CD56+ NK cells (Figure 85A), CD16+ NK cells (Figure 85B), CD8+ T cells (CD45RA+) (Figure 85C), CD8+ T cells (CD45RA-) (Figure 85D), and CD4+ T cells (CD45RA-) (Figure 85E) after dosing cynomolgus monkeys with XENP22819.

[00107] Figures 86A-86Edepict lymphocyte counts after dosing cynomolgus monkeys with XENP22821. Figures 86A-E respectively show the fold change in absolute count of CD56+ NK cells (Figure 86A), CD16+ NK cells (Figure 86B), γδ T cells (CD45RA+CD3+CD4-CD8-) (Figure 86C), CD8+ T cells (Figure 86D), and CD4+ T cells (Figure 86E).

[00108] Figures 87A-87D depict proliferation of CD56+ NK cells (Figure 87A), CD16+ NK cells (Figure 87B), CD8+ T cells (CD45RA+) (Figure 87C), CD8+ T cells (CD45RA-) (Figure 87D), and CD4+ T cells (CD45RA-) (Figure 87E) after dosing cynomolgus monkeys with XENP22821.

[00109] Figures 88A-88E depict lymphocyte counts after dosing cynomolgus monkeys with XENP22822. Figures 88A-E respectively show the fold change in absolute count of CD56+ NK cells (Figure 88A), CD16+ NK cells (Figure 88B), γδ T cells (CD45RA+CD3+CD4-CD8-) (Figure 88C), CD8+ T cells (Figure 88D), and CD4+ T cells (Figure 88E).

[00110] Figures 89A-89E depict proliferation of CD56+ NK cells (Figure 89A), CD16+ NK cells (Figure 89B), CD8+ T cells (CD45RA+) (Figure 89C), CD8+ T cells (CD45RA-) (Figure 89D), and CD4+ T cells (CD45RA-) (Figure 89E) after dosing cynomolgus monkeys with XENP22822.

[00111] Figures 90A-90E depict lymphocyte counts after dosing cynomolgus monkeys with XENP22834. Figures 90A-E respectively show the fold change in absolute count of

CD56+ NK cells (Figure 90A), CD16+ NK cells (Figure 90B), γδ T cells (CD45RA+CD3+CD4-CD8-) (Figure 90C), CD8+ T cells (Figure 90D), and CD4+ T cells (Figure 90E).

[00112] Figures 91A-91E depict proliferation of CD56+ NK cells (Figure 91A), CD16+ NK cells (Figure 91B), CD8+ T cells (CD45RA+) (Figure 91C), CD8+ T cells (CD45RA-) (Figure 91D), and CD4+ T cells (CD45RA-) (Figure 91E) after dosing cynomolgus monkeys with XENP22834.

[00113] Figures 92A-92E depict lymphocyte counts after dosing cynomolgus monkeys with XENP23343. Figures 92A-E respectively show the fold change in absolute count of CD56+ NK cells (Figure 92A), CD16+ NK cells (Figure 92B), γδ T cells (CD45RA+CD3+CD4-CD8-) (Figure 92C), CD8+ T cells (Figure 92D), and CD4+ T cells (Figure 92E).

[00114] Figures 93A-93E depict proliferation of CD56+ NK cells (Figure 93A), CD16+ NK cells (Figure 93B), CD8+ T cells (CD45RA+) (Figure 93C), CD8+ T cells (CD45RA-) (Figure 93D), and CD4+ T cells (CD45RA-) (Figure 93E) after dosing cynomolgus monkeys with XENP23343.

[00115] Figures 94A-94D depict sequences of XENP23343, XENP23504, XENP24113, XENP24301, XENP24306, and XENP24341, illustrative IL-15/Rα-Fc fusion proteins of the "IL-15/Rα-heteroFc" format with M428L/N434S substitutions. IL-15 and IL-15Rα(sushi) are underlined, linkers are double underlined (although as will be appreciated by those in the art, the linkers can be replaced by other linkers, some of which are depicted in Figure 7), and slashes (/) indicate the border(s) between IL-15, IL-15Rα, linkers, and Fc regions. Figure 94D depicts sequences of XENP25938, an illustrative IL-15/Rα-Fc fusion protein of the "scIL-15/Rα-Fc" format with M428L/N434S substitutions.

[00116] Figure 95 depicts sequences of XENP24383, an illustrative IL-15/Rα-Fc fusion protein of the "ncIL-15/Rα-Fc" format with M428L/N434S substitutions. IL-15 and IL-15Rα(sushi) are underlined, linkers are double underlined (although as will be appreciated by those in the art, the linkers can be replaced by other linkers, some of which are depicted in Figure 7), and slashes (/) indicate the border(s) between IL-15, IL-15Rα, linkers, and Fc regions.

[00117] Figure 96 depicts sequences of XENP24346 and XENP24351, illustrative IL-15/Rα-Fc fusion proteins of the "bivalent ncIL-15/Rα-Fc" format with M428L/N434S substitutions. IL-15 and IL-15Rα(sushi) are underlined, linkers are double underlined (although as will be appreciated by those in the art, the linkers can be replaced by other linkers, some of which are depicted in Figure 7), and slashes (/) indicate the border(s) between IL-15, IL-15Rα, linkers, and Fc regions.

[00118] Figures 97A-97C depict the percentage of Ki67 expression on (A) human CD8+ T cells, (B) human CD4+ T cells and (C) human NK cells following treatment with IL-15/Rα variants with M428L/N434S Fc mutations.

[00119] Figures 98A-98C depict the percentage of Ki67 expression on (A) cyno CD8+ T cells, (B) cyno CD4+ T cells and (C) cyno NK cells following treatment with IL-15/Rα variants with M428L/N434S Fc mutations.

[00120] Figures 99A-99C depict CD4+ T cell count on (A) Day 4 and (B) Day 7 in whole blood and (C) Day 8 in spleen of huPBMC engrafted mice following treatment with additional variant IL-15/Rα-Fc fusion proteins.

[00121] Figures 100A-100C depict CD8+ T cell count on (A) Day 4 and (B) Day 7 in whole blood and (C) Day 8 in spleen of huPBMC engrafted mice following treatment with additional variant IL-15/Rα-Fc fusion proteins.

[00122] Figures 101A-101C depicts CD8+ T cell count on (A) Day 4 and (B) Day 7 in whole blood and (C) Day 8 in spleen of huPBMC engrafted mice following treatment with additional variant IL-15/Rα-Fc fusion proteins.

[00123] Figures 102A-102E depict the body weight as a percentage of initial body weight of huPBMC engrafted mice on Days (A) -2, (B) 1, (C) 5, (D) 8, and (E) 11 following treatment with additional IL-15/Rα variants. Each point represents a single NSG mouse. Figure 102F depicts a time-course of body weight in huPBMC engrafted mice following treatment with the IL-15/Rα variants.

[00124] Figures 103A-103C depict (A) CD8+ T cell, (B) CD4+ T cell, and (C) NK cell counts in cynomolgus monkeys after treatment with IL-15/Rα variants on Day 1.

[00125] Figures 104A-104Z, 104AA-104AZ, and 104BA-104BL depicts sequences of the invention. The CDRs are in bold, IL-15 and IL15-Rα(sushi) are underlined, linkers are double underlined, and slashes (/) are between IL-15, IL15-Rα(sushi), linkers, and Fc domains.

[00126] Figure 105 depicts some preferred embodiments of the invention. "Xtend" versions contain the 428L/434S variants in the Fc domains of each monomer.

[00127] Figure 106 depicts a list of engineered heterodimer-skewing (e.g. "steric heterodimerization") Fc variants with heterodimer yields (determined by HPLC-CIEX) and thermal stabilities (determined by DSC). Not determined thermal stability is denoted by "n.d.".

DETAILED DESCRIPTION OF THE INVENTION

I. Definitions

[00128] In order that the application may be more completely understood, several definitions are set forth below. Such definitions are meant to encompass grammatical equivalents.

[00129] By "ablation" herein is meant a decrease or removal of activity. Thus for example, "ablating FcγR binding" means the Fc region amino acid variant has less than 50% starting binding as compared to an Fc region not containing the specific variant, with less than 70-80-90-95-98% loss of activity being preferred, and in general, with the activity being below the level of detectable binding in a Biacore assay. Of particular use in the ablation of FcγR binding are those shown in Figure 86. However, unless otherwise noted, the Fc monomers of the invention retain binding to the FcRn receptor.

[00130] By "ADCC" or "antibody dependent cell-mediated cytotoxicity" as used herein is meant the cell-mediated reaction wherein nonspecific cytotoxic cells that express FcγRs recognize bound antibody on a target cell and subsequently cause lysis of the target cell. ADCC is correlated with binding to FcγRIIIa; increased binding to FcγRIIIa leads to an increase in ADCC activity. As is discussed herein, many embodiments of the invention ablate ADCC activity entirely.

[00131] By "ADCP" or antibody dependent cell-mediated phagocytosis as used herein is meant the cell-mediated reaction wherein nonspecific cytotoxic cells that express FcγRs recognize bound antibody on a target cell and subsequently cause phagocytosis of the target cell.

[00132] By "modification" herein is meant an amino acid substitution, insertion, and/or deletion in a polypeptide sequence or an alteration to a moiety chemically linked to a protein. For example, a modification may be an altered carbohydrate or PEG structure attached to a protein. By "amino acid modification" herein is meant an amino acid substitution, insertion, and/or deletion in a polypeptide sequence. For clarity, unless otherwise noted, the amino acid modification is always to an amino acid coded for by DNA, e.g., the 20 amino acids that have codons in DNA and RNA.

[00133] By "amino acid substitution" or "substitution" herein is meant the replacement of an amino acid at a particular position in a parent polypeptide sequence with a different amino acid. In particular, in some embodiments, the substitution is to an amino acid that is not naturally occurring at the particular position, either not naturally occurring within the organism or in any organism. For example, the substitution E272Y refers to a variant polypeptide, in this case an Fc variant, in which the glutamic acid at position 272 is replaced with tyrosine. For clarity, a protein which has been engineered to change the nucleic acid coding sequence but not change the starting amino acid (for example exchanging CGG (encoding arginine) to CGA (still encoding arginine) to increase host organism expression levels) is not an "amino acid substitution"; that is, despite the creation of a new gene encoding the same protein, if the protein has the same amino acid at the particular position that it started with, it is not an amino acid substitution.

[00134] By "amino acid insertion" or "insertion" as used herein is meant the addition of an amino acid sequence at a particular position in a parent polypeptide sequence. For example, -233E or 233E designates an insertion of glutamic acid after position 233 and before position 234. Additionally, -233ADE or A233ADE designates an insertion of AlaAspGlu after position 233 and before position 234.

[00135] By "amino acid deletion" or "deletion" as used herein is meant the removal of an amino acid sequence at a particular position in a parent polypeptide sequence. For example, E233- or E233#, E233() or E233del designates a deletion of glutamic acid at position 233. Additionally, EDA233- or EDA233# designates a deletion of the sequence GluAspAla that begins at position 233.

[00136] By "variant protein" or "protein variant", or "variant" as used herein is meant a protein that differs from that of a parent protein by virtue of at least one amino acid modification. Protein variant may refer to the protein itself, a composition comprising the protein, or the amino sequence that encodes it. Preferably, the protein variant has at least one amino acid modification compared to the parent protein, e.g. from about one to about seventy amino acid modifications, and preferably from about one to about five amino acid modifications compared to the parent. As described below, in some embodiments the parent polypeptide, for example an Fc parent polypeptide, is a human wild type sequence, such as the Fc region from IgG1, IgG2, IgG3 or IgG4, although human sequences with variants can also serve as "parent polypeptides", for example the IgG1/2 hybrid can be included. The protein variant sequence herein will preferably possess at least about 80% identity with a parent protein sequence, and most preferably at least about 90% identity, more preferably at least about 95-98-99% identity . Variant protein can refer to the variant protein itself, compositions comprising the protein variant, or the DNA sequence that encodes it.

[00137] Accordingly, by "antibody variant" or "variant antibody" as used herein is meant an antibody that differs from a parent antibody by virtue of at least one amino acid modification, "IgG variant" or "variant IgG" as used herein is meant an antibody that differs from a parent IgG (again, in many cases, from a human IgG sequence) by virtue of at least one amino acid modification, and "immunoglobulin variant" or "variant immunoglobulin" as used herein is meant an immunoglobulin sequence that differs from that of a parent immunoglobulin sequence by virtue of at least one amino acid modification. "Fc variant" or "variant Fc" as used herein is meant a protein comprising an amino acid modification in an Fc domain. The Fc variants of the present invention are defined according to the amino acid modifications that compose them. Thus, for example, N434S or 434S is an Fc variant with the substitution serine at position 434 relative to the parent Fc polypeptide, wherein the

numbering is according to the EU index. Likewise, M428L/N434S defines an Fc variant with the substitutions M428L and N434S relative to the parent Fc polypeptide. The identity of the WT amino acid may be unspecified, in which case the aforementioned variant is referred to as 428L/434S. It is noted that the order in which substitutions are provided is arbitrary, that is to say that, for example, 428L/434S is the same Fc variant as M428L/N434S, and so on. For all positions discussed in the present invention that relate to antibodies, unless otherwise noted, amino acid position numbering is according to the EU index. The EU index or EU index as in Kabat or EU numbering scheme refers to the numbering of the EU antibody (Edelman et al., 1969, Proc Natl Acad Sci USA 63:78-85, hereby entirely incorporated by reference). The modification can be an addition, deletion, or substitution. Substitutions can include naturally occurring amino acids and, in some cases, synthetic amino acids. Examples include U.S. Pat. No. 6,586,207; WO 98/48032; WO 03/073238; US2004-0214988A1; WO 05/35727A2; WO 05/74524A2; J. W. Chin et al., (2002), Journal of the American Chemical Society 124:9026-9027; J. W. Chin, & P. G. Schultz, (2002), ChemBioChem 11:1135-1137; J. W. Chin, et al., (2002), PICAS United States of America 99:11020-11024; and, L. Wang, & P. G. Schultz, (2002), Chem. 1-10, all entirely incorporated by reference.

[00138] As used herein, "protein" herein is meant at least two covalently attached amino acids, which includes proteins, polypeptides, oligopeptides and peptides. The peptidyl group may comprise naturally occurring amino acids and peptide bonds, or synthetic peptidomimetic structures, i.e. "analogs", such as peptoids (see Simon et al., PNAS USA 89(20):9367 (1992), entirely incorporated by reference). The amino acids may either be naturally occurring or synthetic (e.g. not an amino acid that is coded for by DNA); as will be appreciated by those in the art. For example, homo-phenylalanine, citrulline, ornithine and noreleucine are considered synthetic amino acids for the purposes of the invention, and both D- and L-(R or S) configured amino acids may be utilized. The variants of the present invention may comprise modifications that include the use of synthetic amino acids incorporated using, for example, the technologies developed by Schultz and colleagues, including but not limited to methods described by Cropp & Shultz, 2004, Trends Genet. 20(12):625-30, Anderson et al., 2004, Proc Natl Acad Sci USA 101 (2):7566-71, Zhang et al., 2003, 303(5656):371-3, and Chin et al., 2003, Science 301(5635):964-7, all entirely incorporated

by reference. In addition, polypeptides may include synthetic derivatization of one or more side chains or termini, glycosylation, PEGylation, circular permutation, cyclization, linkers to other molecules, fusion to proteins or protein domains, and addition of peptide tags or labels.

[00139] By "residue" as used herein is meant a position in a protein and its associated amino acid identity. For example, Asparagine 297 (also referred to as Asn297 or N297) is a residue at position 297 in the human antibody IgG1.

[00140] By "IgG subclass modification" or “isotype modification” as used herein is meant an amino acid modification that converts one amino acid of one IgG isotype to the corresponding amino acid in a different, aligned IgG isotype. For example, because IgG1 comprises a tyrosine and IgG2 a phenylalanine at EU position 296, a F296Y substitution in IgG2 is considered an IgG subclass modification.

[00141] By "non-naturally occurring modification" as used herein is meant an amino acid modification that is not isotypic. For example, because none of the IgGs comprise a serine at position 434, the substitution 434S in IgG1, IgG2, IgG3, or IgG4 (or hybrids thereof) is considered a non-naturally occurring modification.

[00142] By "amino acid" and "amino acid identity" as used herein is meant one of the 20 naturally occurring amino acids that are coded for by DNA and RNA.

[00143] By "effector function" as used herein is meant a biochemical event that results from the interaction of an antibody Fc region with an Fc receptor or ligand. Effector functions include but are not limited to ADCC, ADCP, and CDC.

[00144] By "IgG Fc ligand" as used herein is meant a molecule, preferably a polypeptide, from any organism that binds to the Fc region of an IgG antibody to form an Fc/Fc ligand complex. Fc ligands include but are not limited to FcγRIs, FcγRIIs, FcγRIIIs, FcRn, C1q, C3, mannan binding lectin, mannose receptor, staphylococcal protein A, streptococcal protein G, and viral FcγR. Fc ligands also include Fc receptor homologs (FcRH), which are a family of Fc receptors that are homologous to the FcγRs (Davis et al., 2002, Immunological Reviews 190:123-136, entirely incorporated by reference). Fc ligands may include undiscovered molecules that bind Fc. Particular IgG Fc ligands are FcRn and Fc gamma receptors. By "Fc

ligand" as used herein is meant a molecule, preferably a polypeptide, from any organism that binds to the Fc region of an antibody to form an Fc/Fc ligand complex.

[00145] By "Fc gamma receptor", "FcγR" or "FcgammaR" as used herein is meant any member of the family of proteins that bind the IgG antibody Fc region and is encoded by an FcγR gene. In humans this family includes but is not limited to FcγRI (CD64), including isoforms FcγRIa, FcγRIb, and FcγRIc; FcγRII (CD32), including isoforms FcγRIIa (including allotypes H131 and R131), FcγRIIb (including FcγRIIb-1 and FcγRIIb-2), and FcγRIIc; and FcγRIII (CD16), including isoforms FcγRIIIa (including allotypes V158 and F158) and FcγRIIIb (including allotypes FcγRIIb-NA1 and FcγRIIb-NA2) (Jefferis et al., 2002, Immunol Lett 82:57-65, entirely incorporated by reference), as well as any undiscovered human FcγRs or FcγR isoforms or allotypes. An FcγR may be from any organism, including but not limited to humans, mice, rats, rabbits, and monkeys. Mouse FcγRs include but are not limited to FcγRI (CD64), FcγRII (CD32), FcγRIII (CD16), and FcγRIII-2 (CD16-2), as well as any undiscovered mouse FcγRs or FcγR isoforms or allotypes.

[00146] By "FcRn" or "neonatal Fc Receptor" as used herein is meant a protein that binds the IgG antibody Fc region and is encoded at least in part by an FcRn gene. The FcRn may be from any organism, including but not limited to humans, mice, rats, rabbits, and monkeys. As is known in the art, the functional FcRn protein comprises two polypeptides, often referred to as the heavy chain and light chain. The light chain is beta-2-microglobulin and the heavy chain is encoded by the FcRn gene. Unless otherwise noted herein, FcRn or an FcRn protein refers to the complex of FcRn heavy chain with beta-2-microglobulin. A variety of FcRn variants can be used to increase binding to the FcRn receptor, and in some cases, to increase serum half-life. In general, unless otherwise noted, the Fc monomers of the invention retain binding to the FcRn receptor (and, as noted below, can include amino acid variants to increase binding to the FcRn receptor).

[00147] By "parent polypeptide" as used herein is meant a starting polypeptide that is subsequently modified to generate a variant. The parent polypeptide may be a naturally occurring polypeptide, or a variant or engineered version of a naturally occurring polypeptide. Parent polypeptide may refer to the polypeptide itself, compositions that comprise the parent polypeptide, or the amino acid sequence that encodes it. Accordingly,

by "parent immunoglobulin" as used herein is meant an unmodified immunoglobulin polypeptide that is modified to generate a variant, and by "parent antibody" as used herein is meant an unmodified antibody that is modified to generate a variant antibody. It should be noted that "parent antibody" includes known commercial, recombinantly produced antibodies as outlined below.

[00148] By "Fc" or "Fc region" or "Fc domain" as used herein is meant the polypeptide comprising the constant region of an antibody excluding, in some instances, the first constant region immunoglobulin domain (e.g., CH1) or a portion thereof, and in some cases, part of the hinge. Thus, an Fc can refer to the last two constant region immunoglobulin domains (e.g., CH2 and CH3) of IgA, IgD, and IgG, the last three constant region immunoglobulin domains of IgE and IgM, and the flexible hinge N-terminal to these domains. For IgA and IgM, Fc may include the J chain. For IgG, the Fc domain comprises immunoglobulin domains Cγ2 and Cγ3 (Cγ2 and Cγ3) and the lower hinge region between Cγ1 (Cγ1) and Cγ2 (Cγ2). In some embodiments, an Fc refers to a truncated CH1 domain, and CH2 and CH3 of an immunoglobulin. Although the boundaries of the Fc region may vary, the human IgG heavy chain Fc region is usually defined to include residues E216 or C226 or P230 to its carboxyl-terminus, wherein the numbering is according to the EU index as in Kabat. In some embodiments, as is more fully described below, amino acid modifications are made to the Fc region, for example to alter binding to one or more FcγR receptors or to the FcRn receptor.

[00149] By "Fc fusion protein" or "immunoadhesin" herein is meant a protein comprising an Fc region, generally linked (optionally through a linker moiety, as described herein) to a different protein, such as to IL-15 and/or IL-15R, as described herein. In some instances, two Fc fusion proteins can form a homodimeric Fc fusion protein or a heterodimeric Fc fusion protein with the latter being preferred. In some cases, one monomer of the heterodimeric Fc fusion protein comprises an Fc domain alone (e.g., an empty Fc domain) and the other monomer is an Fc fusion, comprising a variant Fc domain and a protein domain, such as a receptor, ligand or other binding partner.

[00150] By "position" as used herein is meant a location in the sequence of a protein. Positions may be numbered sequentially, or according to an established format, for example the EU index for antibody numbering.

[00151] By "strandedness" in the context of the monomers of the heterodimeric antibodies of the invention herein is meant that, similar to the two strands of DNA that "match", heterodimerization variants are incorporated into each monomer so as to preserve the ability to "match" to form heterodimers. For example, if some pI variants are engineered into monomer A (e.g. making the pI higher) then steric variants that are "charge pairs" that can be utilized as well do not interfere with the pI variants, e.g. the charge variants that make a pI higher are put on the same "strand" or "monomer" to preserve both functionalities. Similarly, for "skew" variants that come in pairs of a set as more fully outlined below, the skilled artisan will consider pI in deciding into which strand or monomer that incorporates one set of the pair will go, such that pI separation is maximized using the pI of the skews as well.

[00152] By "wild type or WT" herein is meant an amino acid sequence or a nucleotide sequence that is found in nature, including allelic variations. A WT protein has an amino acid sequence or a nucleotide sequence that has not been intentionally modified.

[00153] The heterodimeric proteins of the present invention are generally isolated or recombinant. "Isolated," when used to describe the various polypeptides disclosed herein, means a polypeptide that has been identified and separated and/or recovered from a cell or cell culture from which it was expressed. Ordinarily, an isolated polypeptide will be prepared by at least one purification step. An "isolated protein," refers to aa protein which is substantially free of other antibodies having different antigenic specificities. "Recombinant" means the proteins are generated using recombinant nucleic acid techniques in exogeneous host cells.

[00154] "Percent (%) amino acid sequence identity" with respect to a protein sequence is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the specific (parental) sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and

not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. One particular program is the ALIGN-2 program outlined at paragraphs [0279] to [0280] of US Pub. No. 20160244525, hereby incorporated by reference.

[00155] The degree of identity between an amino acid sequence of the present invention ("invention sequence") and the parental amino acid sequence is calculated as the number of exact matches in an alignment of the two sequences, divided by the length of the "invention sequence," or the length of the parental sequence, whichever is the shortest. The result is expressed in percent identity.

[00156] In some embodiments, two or more amino acid sequences are at least 50%, 60%, 70%, 80%, or 90% identical. In some embodiments, two or more amino acid sequences are at least 95%, 97%, 98%, 99%, or even 100% identical.

[00157] "Specific binding" or "specifically binds to" or is "specific for" a particular antigen or an epitope means binding that is measurably different from a non-specific interaction. Specific binding can be measured, for example, by determining binding of a molecule compared to binding of a control molecule, which generally is a molecule of similar structure that does not have binding activity. For example, specific binding can be determined by competition with a control molecule that is similar to the target.

[00158] Before the invention is further described, it is to be understood that this invention is not limited to particular embodiments described, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

II. Heterodimeric Fc Fusion Proteins

[00159] The present invention relates to heterodimeric Fc fusion proteins that include IL-15 and IL-15 receptor alpha (IL-15Rα) protein domains in different orientations. The Fc domains can be derived from IgG Fc domains, e.g., IgG1, IgG2, IgG3 or IgG4 Fc domains, with IgG1 Fc domains finding particular use in the invention.

[00160] The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function. Kabat et al. collected numerous primary sequences of the variable regions of heavy chains and light chains. Based on the degree of conservation of the sequences, they classified individual primary sequences into the CDR and the framework and made a list thereof (see SEQUENCES OF IMMUNOLOGICAL INTEREST, 5th edition, NIH publication, No. 91-3242, E.A. Kabat et al., entirely incorporated by reference). Throughout the present specification, the Kabat numbering system is generally used when referring to a residue in the variable domain (approximately, residues 1-107 of the light chain variable region and residues 1-113 of the heavy chain variable region) and the EU numbering system for Fc regions (e.g., Kabat et al., supra (1991)).

[00161] In the IgG subclass of immunoglobulins, there are several immunoglobulin domains in the heavy chain. By "immunoglobulin (Ig) domain" herein is meant a region of an immunoglobulin having a distinct tertiary structure. Of interest in the present invention are the heavy chain domains, including, the constant heavy (CH) domains and the hinge domains. In the context of IgG antibodies, the IgG isotypes each have three CH regions. Accordingly, "CH" domains in the context of IgG are as follows: "CH1" refers to positions 118-220 according to the EU index as in Kabat. "CH2" refers to positions 237-340 according to the EU index as in Kabat, and "CH3" refers to positions 341-447 according to the EU index as in Kabat. As shown herein and described below, the pI variants can be in one or more of the CH regions, as well as the hinge region, discussed below.

[00162] Another type of Ig domain of the heavy chain is the hinge region. By "hinge" or "hinge region" or "antibody hinge region" or "immunoglobulin hinge region" herein is meant the flexible polypeptide comprising the amino acids between the first and second constant domains of an antibody. Structurally, the IgG CH1 domain ends at EU position 220, and the IgG CH2 domain begins at residue EU position 237. Thus for IgG the antibody hinge is herein defined to include positions 221 (D221 in IgG1) to 236 (G236 in IgG1),

wherein the numbering is according to the EU index as in Kabat. In some embodiments, for example in the context of an Fc region, the lower hinge is included, with the "lower hinge" generally referring to positions 226 or 230. As noted herein, pI variants can be made in the hinge region as well.

[00163] Thus, the present invention provides different antibody domains. As described herein and known in the art, the heterodimeric proteins of the invention comprise different domains, which can be overlapping as well. These domains include, but are not limited to, the Fc domain, the CH1 domain, the CH2 domain, the CH3 domain, the hinge domain, and the heavy constant domain (CH1-hinge-Fc domain or CH1-hinge-CH2-CH3).

[00164] Thus, the "Fc domain" includes the -CH2-CH3 domain, and optionally a hinge domain. In some embodiments, the Fc domain also includes a truncated CH1 domain. In the embodiments herein, when a protein fragment, e.g., IL-15 or IL-15Rα is attached to an Fc domain, it is the C-terminus of the IL-15 or IL-15Rα construct that is attached to all or part of the hinge of the Fc domain; for example, it is generally attached to the sequence EPKSS which is the beginning of the hinge. In other embodiments, when a protein fragment, e.g., IL-15 or IL-15Rα, is attached to an Fc domain, it is the C-terminus of the IL-15 or IL15Rα construct that is attached to the CH1 domain of the Fc domain.

[00165] In some of the constructs and sequences outlined herein of an Fc domain protein, the C-terminus of the IL-15 or IL-15Rα protein fragment is attached to the N-terminus of a domain linker, the C-terminus of which is attached to the N-terminus of a constant Fc domain (N-IL-15 or IL-15Rα protein fragment-linker-Fc domain-C) although that can be switched (N- Fc domain-linker- IL-15 or IL-15Rα protein fragment -C). In other constructs and sequence outlined herein, C-terminus of a first protein fragment is attached to the N-terminus of a second protein fragment, optionally via a domain linker, the C-terminus of the second protein fragment is attached to the N-terminus of a constant Fc domain, optionally via a domain linker. In yet other constructs and sequences outlined herein, a constant Fc domain that is not attached to a first protein fragment or a second protein fragment is provided. A heterodimer Fc fusion protein can contain two or more of the exemplary monomeric Fc domain proteins described herein.

[00166] In some embodiments, the linker is a "domain linker", used to link any two domains as outlined herein together, some of which are depicted in Figure 87. While any suitable linker can be used, many embodiments utilize a glycine-serine polymer, including for example (GS)n, (GSGGS)n, (GGGGS)n, and (GGGS)n, where n is an integer of at least one (and generally from 0 to 1 to 2 to 3 to 4 to 5) as well as any peptide sequence that allows for recombinant attachment of the two domains with sufficient length and flexibility to allow each domain to retain its biological function. In some cases, and with attention being paid to "strandedness", as outlined below, charged domain linkers.

[00167] In one embodiment, heterodimeric Fc fusion proteins contain at least two constant domains which can be engineered to produce heterodimers, such as pI engineering. Other Fc domains that can be used include fragments that contain one or more of the CH1, CH2, CH3, and hinge domains of the invention that have been pI engineered. In particular, the formats depicted in Figures 9A-9G, and 39A-39D are heterodimeric Fc fusion proteins, meaning that the protein has two associated Fc sequences self-assembled into a heterodimeric Fc domain and at least one protein fragment (e.g., 1, 2 or more protein fragments). In some cases, a first protein fragment is linked to a first Fc sequence and a second protein fragment is linked to a second Fc sequence. In other cases, a first protein fragment is linked to a first Fc sequence, and the first protein fragment is non-covalently attached to a second protein fragment that is not linked to an Fc sequence. In some cases, the heterodimeric Fc fusion protein contains a first protein fragment linked to a second protein fragment which is linked a first Fc sequence, and a second Fc sequence that is not linked to either the first or second protein fragments.

[00168] Accordingly, in some embodiments the present invention provides heterodimeric Fc fusion proteins that rely on the use of two different heavy chain variant Fc sequences, that will self-assemble to form a heterodimeric Fc domain fusion polypeptide.

[00169] The present invention is directed to novel constructs to provide heterodimeric Fc fusion proteins that allow binding to one or more binding partners, ligands or receptors. The heterodimeric Fc fusion constructs are based on the self-assembling nature of the two Fc domains of the heavy chains of antibodies, e.g., two "monomers" that assemble into a "dimer". Heterodimeric Fc fusions are made by altering the amino acid sequence of each

monomer as more fully discussed below. Thus, the present invention is generally directed to the creation of heterodimeric Fc fusion proteins which can co-engage binding partner(s) or ligand(s) or receptor(s) in several ways, relying on amino acid variants in the constant regions that are different on each chain to promote heterodimeric formation and/or allow for ease of purification of heterodimers over the homodimers.

[00170] There are a number of mechanisms that can be used to generate the heterodimers of the present invention. In addition, as will be appreciated by those in the art, these mechanisms can be combined to ensure high heterodimerization. Thus, amino acid variants that lead to the production of heterodimers are referred to as "heterodimerization variants". As discussed below, heterodimerization variants can include steric variants (e.g. the "knobs and holes" or "skew" variants described below and the "charge pairs" variants described below) as well as "pI variants", which allows purification of homodimers away from heterodimers. As is generally described in WO2014/145806, hereby incorporated by reference in its entirety and specifically as below for the discussion of "heterodimerization variants", useful mechanisms for heterodimerization include "knobs and holes" ("KIH"; sometimes herein as "skew" variants (see discussion in WO2014/145806), "electrostatic steering" or "charge pairs" as described in WO2014/145806, pI variants as described in WO2014/145806, and general additional Fc variants as outlined in WO2014/145806 and below.

[00171] In the present invention, there are several basic mechanisms that can lead to ease of purifying heterodimeric antibodies; one relies on the use of pI variants, such that each monomer has a different pI, thus allowing the isoelectric purification of A-A, A-B and B-B dimeric proteins. Alternatively, some formats also allow separation on the basis of size. As is further outlined below, it is also possible to "skew" the formation of heterodimers over homodimers. Thus, a combination of steric heterodimerization variants and pI or charge pair variants find particular use in the invention.

[00172] In general, embodiments of particular use in the present invention rely on sets of variants that include skew variants, that encourage heterodimerization formation over homodimerization formation, coupled with pI variants, which increase the pI difference between the two monomers.

[00173] Additionally, as more fully outlined below, depending on the format of the heterodimer Fc fusion protein, pI variants can be either contained within the constant and/or Fc domains of a monomer, or domain linkers can be used. That is, the invention provides pI variants that are on one or both of the monomers, and/or charged domain linkers as well. In addition, additional amino acid engineering for alternative functionalities may also confer pI changes, such as Fc, FcRn and KO variants.

[00174] In the present invention that utilizes pI as a separation mechanism to allow the purification of heterodimeric proteins, amino acid variants can be introduced into one or both of the monomer polypeptides; that is, the pI of one of the monomers (referred to herein for simplicity as "monomer A") can be engineered away from monomer B, or both monomer A and B change be changed, with the pI of monomer A increasing and the pI of monomer B decreasing. As discussed, the pI changes of either or both monomers can be done by removing or adding a charged residue (e.g., a neutral amino acid is replaced by a positively or negatively charged amino acid residue, e.g., glycine to glutamic acid), changing a charged residue from positive or negative to the opposite charge (e.g. aspartic acid to lysine) or changing a charged residue to a neutral residue (e.g., loss of a charge; lysine to serine.). A number of these variants are shown in the Figures.

[00175] Accordingly, this embodiment of the present invention provides for creating a sufficient change in pI in at least one of the monomers such that heterodimers can be separated from homodimers. As will be appreciated by those in the art, and as discussed further below, this can be done by using a "wild type" heavy chain constant region and a variant region that has been engineered to either increase or decrease its pI (wt A-+B or wt A - -B), or by increasing one region and decreasing the other region (A+ -B- or A- B+).

[00176] Thus, in general, a component of some embodiments of the present invention are amino acid variants in the constant regions that are directed to altering the isoelectric point (pI) of at least one, if not both, of the monomers of a dimeric protein by incorporating amino acid substitutions ("pI variants" or "pI substitutions") into one or both of the monomers. As shown herein, the separation of the heterodimers from the two homodimers can be accomplished if the pIs of the two monomers differ by as little as 0.1 pH unit, with 0.2, 0.3, 0.4 and 0.5 or greater all finding use in the present invention.

[00177] As will be appreciated by those in the art, the number of pI variants to be included on each or both monomer(s) to get good separation will depend in part on the starting pI of the components. As is known in the art, different Fcs will have different starting pIs which are exploited in the present invention. In general, as outlined herein, the pIs are engineered to result in a total pI difference of each monomer of at least about 0.1 logs, with 0.2 to 0.5 being preferred as outlined herein.

[00178] As will be appreciated by those in the art, the number of pI variants to be included on each or both monomer(s) to get good separation will depend in part on the starting pI of the components. That is, to determine which monomer to engineer or in which "direction" (e.g., more positive or more negative), the sequences of the Fc domains, and in some cases, the protein domain(s) linked to the Fc domain are calculated and a decision is made from there. As is known in the art, different Fc domains and/or protein domains will have different starting pIs which are exploited in the present invention. In general, as outlined herein, the pIs are engineered to result in a total pI difference of each monomer of at least about 0.1 logs, with 0.2 to 0.5 being preferred as outlined herein.

[00179] Furthermore, as will be appreciated by those in the art and outlined herein, in some embodiments, heterodimers can be separated from homodimers on the basis of size. As shown in the Figures, for example, several of the formats allow separation of heterodimers and homodimers on the basis of size.

[00180] In the case where pI variants are used to achieve heterodimerization, by using the constant region(s) of Fc domains(s), a more modular approach to designing and purifying heterodimeric Fc fusion proteins is provided. Thus, in some embodiments, heterodimerization variants (including skew and purification heterodimerization variants) must be engineered. In addition, in some embodiments, the possibility of immunogenicity resulting from the pI variants is significantly reduced by importing pI variants from different IgG isotypes such that pI is changed without introducing significant immunogenicity. Thus, an additional problem to be solved is the elucidation of low pI constant domains with high human sequence content, e.g. the minimization or avoidance of non-human residues at any particular position.

[00181] A side benefit that can occur with this pI engineering is also the extension of serum half-life and increased FcRn binding. That is, as described in USSN 13/194,904 (incorporated by reference in its entirety), lowering the pI of antibody constant domains (including those found in antibodies and Fc fusions) can lead to longer serum retention in vivo. These pI variants for increased serum half life also facilitate pI changes for purification.

[00182] In addition, it should be noted that the pI variants of the heterodimerization variants give an additional benefit for the analytics and quality control process of Fc fusion proteins, as the ability to either eliminate, minimize and distinguish when homodimers are present is significant. Similarly, the ability to reliably test the reproducibility of the heterodimeric Fc fusion protein production is important.

A. Heterodimerization Variants

[00183] The present invention provides heterodimeric proteins, including heterodimeric Fc fusion proteins in a variety of formats, which utilize heterodimeric variants to allow for heterodimeric formation and/or purification away from homodimers. The heterodimeric fusion constructs are based on the self-assembling nature of the two Fc domains, e.g., two "monomers" that assemble into a "dimer".

[00184] There are a number of suitable pairs of sets of heterodimerization skew variants. These variants come in "pairs" of "sets". That is, one set of the pair is incorporated into the first monomer and the other set of the pair is incorporated into the second monomer. It should be noted that these sets do not necessarily behave as "knobs in holes" variants, with a one-to-one correspondence between a residue on one monomer and a residue on the other; that is, these pairs of sets form an interface between the two monomers that encourages heterodimer formation and discourages homodimer formation, allowing the percentage of heterodimers that spontaneously form under biological conditions to be over 90%, rather than the expected 50% (25 % homodimer A/A:50% heterodimer A/B:25% homodimer B/B).

B. Steric Variants

[00185] In some embodiments, the formation of heterodimers can be facilitated by the addition of steric variants. That is, by changing amino acids in each heavy chain, different heavy chains are more likely to associate to form the heterodimeric structure than to form homodimers with the same Fc amino acid sequences. Suitable steric variants are included in in the Figure 29 of USSN 15/141,350, all of which is hereby incorporated by reference in its entirety, as well as in Figure 84.

[00186] One mechanism is generally referred to in the art as "knobs and holes", referring to amino acid engineering that creates steric influences to favor heterodimeric formation and disfavor homodimeric formation can also optionally be used; this is sometimes referred to as "knobs and holes", as described in USSN 61/596,846, Ridgway et al., Protein Engineering 9(7):617 (1996); Atwell et al., J. Mol. Biol. 1997 270:26; US Patent No. 8,216,805, all of which are hereby incorporated by reference in their entirety. The Figures identify a number of "monomer A – monomer B" pairs that rely on "knobs and holes". In addition, as described in Merchant et al., Nature Biotech. 16:677 (1998), these "knobs and hole" mutations can be combined with disulfide bonds to skew formation to heterodimerization.

[00187] An additional mechanism that finds use in the generation of heterodimers is sometimes referred to as "electrostatic steering" as described in Gunasekaran et al., J. Biol. Chem. 285(25):19637 (2010), hereby incorporated by reference in its entirety. This is sometimes referred to herein as "charge pairs". In this embodiment, electrostatics are used to skew the formation towards heterodimerization. As those in the art will appreciate, these may also have an effect on pI, and thus on purification, and thus could in some cases also be considered pI variants. However, as these were generated to force heterodimerization and were not used as purification tools, they are classified as "steric variants". These include, but are not limited to, D221E/P228E/L368E paired with D221R/P228R/K409R (e.g., these are "monomer corresponding sets) and C220E/P228E/368E paired with C220R/E224R/P228R/K409R.

[00188] Additional monomer A and monomer B variants that can be combined with other variants, optionally and independently in any amount, such as pI variants outlined herein or other steric variants that are shown in Figure 37 of US 2012/0149876, all of which are incorporated expressly by reference herein.

[00189] In some embodiments, the steric variants outlined herein can be optionally and independently incorporated with any pI variant (or other variants such as Fc variants, FcRn variants, etc.) into one or both monomers, and can be independently and optionally included or excluded from the proteins of the invention.

[00190] A list of suitable skew variants is found in Figure 84. Of particular use in many embodiments are the pairs of sets including, but not limited to, S364K/E357Q : L368D/K370S; L368D/K370S : S364K; L368E/K370S : S364K; T411T/E360E/Q362E : D401K; L368D/K370S : S364K/E357L, K370S : S364K/E357Q and T366S/L368A/Y407V : T366W (optionally including a bridging disulfide, T366S/L368A/Y407V/Y349C : T366W/S354C). In terms of nomenclature, the pair "S364K/E357Q : L368D/K370S" means that one of the monomers has the double variant set S364K/E357Q and the other has the double variant set L368D/K370S; as above, the "strandedness" of these pairs depends on the starting pI.

C. pI (Isoelectric point) Variants for Heterodimers

[00191] In general, as will be appreciated by those in the art, there are two general categories of pI variants: those that increase the pI of the protein (basic changes) and those that decrease the pI of the protein (acidic changes). As described herein, all combinations of these variants can be done: one monomer may be wild type, or a variant that does not display a significantly different pI from wild-type, and the other can be either more basic or more acidic. Alternatively, each monomer is changed, one to more basic and one to more acidic.

[00192] Preferred combinations of pI variants are shown in Figure 30 of USSN 15/141,350, all of which are herein incorporated by reference in its entirety. As outlined herein and shown in the figures, these changes are shown relative to IgG1, but all isotypes can be altered this way, as well as isotype hybrids. In the case where the heavy chain constant domain is from IgG2-4, R133E and R133Q can also be used.

[00193] In one embodiment, a preferred combination of pI variants has one monomer comprising 208D/295E/384D/418E/421D variants (N208D/Q295E/N384D/Q418E/N421D when relative to human IgG1) if one of the Fc monomers includes a CH1 domain. In some instances, the second monomer comprising a positively charged domain linker, including

$(GKPGS)_4$. In some cases, the first monomer includes a CH1 domain, including position 208. Accordingly, in constructs that do not include a CH1 domain (for example for heterodimeric Fc fusion proteins that do not utilize a CH1 domain on one of the domains), a preferred negative pI variant Fc set includes 295E/384D/418E/421D variants (Q295E/N384D/Q418E/N421D when relative to human IgG1).

[00194] In some embodiments, mutations are made in the hinge domain of the Fc domain, including positions 221, 222, 223, 224, 225, 233, 234, 235 and 236. It should be noted that changes in 233-236 can be made to increase effector function (along with 327A) in the IgG2 backbone. Thus, pI mutations and particularly substitutions can be made in one or more of positions 221-225, with 1, 2, 3, 4 or 5 mutations finding use in the present invention. Again, all possible combinations are contemplated, alone or with other pI variants in other domains.

[00195] Specific substitutions that find use in lowering the pI of hinge domains include, but are not limited to, a deletion at position 221, a non-native valine or threonine at position 222, a deletion at position 223, a non-native glutamic acid at position 224, a deletion at position 225, a deletion at position 235 and a deletion or a non-native alanine at position 236. In some cases, only pI substitutions are done in the hinge domain, and in others, these substitution(s) are added to other pI variants in other domains in any combination.

[00196] In some embodiments, mutations can be made in the CH2 region, including positions 274, 296, 300, 309, 320, 322, 326, 327, 334 and 339. Again, all possible combinations of these 10 positions can be made; e.g., a pI antibody may have 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 CH2 pI substitutions.

[00197] Specific substitutions that find use in lowering the pI of CH2 domains include, but are not limited to, a non-native glutamine or glutamic acid at position 274, a non-native phenylalanine at position 296, a non native phenylalanine at position 300, a non-native valine at position 309, a non-native glutamic acid at position 320, a non-native glutamic acid at position 322, a non-native glutamic acid at position 326, a non-native glycine at position 327, a non-native glutamic acid at position 334, a non native threonine at position 339, and all possible combinations within CH2 and with other domains.

[00198] In this embodiment, the mutations can be independently and optionally selected from position 355, 359, 362, 384, 389,392, 397, 418, 419, 444 and 447. Specific substitutions that find use in lowering the pI of CH3 domains include, but are not limited to, a non native glutamine or glutamic acid at position 355, a non-native serine at position 384, a non-native asparagine or glutamic acid at position 392, a non-native methionine at position 397, a non native glutamic acid at position 419, a non native glutamic acid at position 359, a non native glutamic acid at position 362, a non native glutamic acid at position 389, a non native glutamic acid at position 418, a non native glutamic acid at position 444, and a deletion or non-native aspartic acid at position 447.

D. Isotypic Variants

[00199] In addition, many embodiments of the invention rely on the "importation" of pI amino acids at particular positions from one IgG isotype into another, thus reducing or eliminating the possibility of unwanted immunogenicity being introduced into the variants. A number of these are shown in Figure 21 of US Publ. App. No. 2014/0370013, hereby incorporated by reference. That is, IgG1 is a common isotype for therapeutic antibodies for a variety of reasons, including high effector function. However, the heavy constant region of IgG1 has a higher pI than that of IgG2 (8.10 versus 7.31). By introducing IgG2 residues at particular positions into the IgG1 backbone, the pI of the resulting monomer is lowered (or increased) and additionally exhibits longer serum half-life. For example, IgG1 has a glycine (pI 5.97) at position 137, and IgG2 has a glutamic acid (pI 3.22); importing the glutamic acid will affect the pI of the resulting protein. As is described below, a number of amino acid substitutions are generally required to significant affect the pI of the variant Fc fusion protein. However, it should be noted as discussed below that even changes in IgG2 molecules allow for increased serum half-life.

[00200] In other embodiments, non-isotypic amino acid changes are made, either to reduce the overall charge state of the resulting protein (e.g., by changing a higher pI amino acid to a lower pI amino acid), or to allow accommodations in structure for stability, etc. as is more further described below.

[00201] In addition, by pI engineering both the heavy and light constant domains, significant changes in each monomer of the heterodimer can be seen. As discussed herein, having the pIs of the two monomers differ by at least 0.5 can allow separation by ion exchange chromatography or isoelectric focusing, or other methods sensitive to isoelectric point.

E. Calculating pI

[00202] The pI of each monomer can depend on the pI of the variant heavy chain constant domain and the pI of the total monomer, including the variant heavy chain constant domain and the fusion partner. Thus, in some embodiments, the change in pI is calculated on the basis of the variant heavy chain constant domain, using the chart in the Figure 19 of US Publ. App. No. 2014/0370013. As discussed herein, which monomer to engineer is generally decided by the inherent pI of each monomer.

F. pI Variants that also confer better FcRn in vivo binding

[00203] In the case where the pI variant decreases the pI of the monomer, they can have the added benefit of improving serum retention in vivo.

[00204] Although still under examination, Fc regions are believed to have longer half-lives in vivo, because binding to FcRn at pH 6 in an endosome sequesters the Fc (Ghetie and Ward, 1997 Immunol Today. 18(12): 592-598, entirely incorporated by reference). The endosomal compartment then recycles the Fc to the cell surface. Once the compartment opens to the extracellular space, the higher pH, ~7.4, induces the release of Fc back into the blood. In mice, Dall' Acqua et al. showed that Fc mutants with increased FcRn binding at pH 6 and pH 7.4 actually had reduced serum concentrations and the same half-life as wild-type Fc (Dall' Acqua et al. 2002, J. Immunol. 169:5171-5180, entirely incorporated by reference). The increased affinity of Fc for FcRn at pH 7.4 is thought to forbid the release of the Fc back into the blood. Therefore, the Fc mutations that will increase Fc's half-life in vivo will ideally increase FcRn binding at the lower pH while still allowing release of Fc at higher pH. The amino acid histidine changes its charge state in the pH range of 6.0 to 7.4. Therefore, it is not surprising to find His residues at important positions in the Fc/FcRn complex.

G. Additional Fc Variants for Additional Functionality

[00205] In addition to pI amino acid variants, there are a number of useful Fc amino acid modification that can be made for a variety of reasons, including, but not limited to, altering binding to one or more FcγR receptors, altered binding to FcRn receptors, etc.

[00206] Accordingly, the proteins of the invention can include amino acid modifications, including the heterodimerization variants outlined herein, which includes the pI variants and steric variants. Each set of variants can be independently and optionally included or excluded from any particular heterodimeric protein.

H. FcγR Variants

[00207] Accordingly, there are a number of useful Fc substitutions that can be made to alter binding to one or more of the FcγR receptors. Substitutions that result in increased binding as well as decreased binding can be useful. For example, it is known that increased binding to FcγRIIIa results in increased ADCC (antibody dependent cell-mediated cytotoxicity; the cell-mediated reaction wherein nonspecific cytotoxic cells that express FcγRs recognize bound antibody on a target cell and subsequently cause lysis of the target cell). Similarly, decreased binding to FcγRIIb (an inhibitory receptor) can be beneficial as well in some circumstances. Amino acid substitutions that find use in the present invention include those listed in USSNs 11/124,620 (particularly Figure 41), 11/174,287, 11/396,495, 11/538,406, all of which are expressly incorporated herein by reference in their entirety and specifically for the variants disclosed therein. Particular variants that find use include, but are not limited to, 236A, 239D, 239E, 332E, 332D, 239D/332E, 267D, 267E, 328F, 267E/328F, 236A/332E, 239D/332E/330Y, 239D, 332E/330L, 243A, 243L, 264A, 264V and 299T.

[00208] In addition, amino acid substitutions that increase affinity for FcγRIIc can also be included in the Fc domain variants outlined herein. The substitutions described in, for example, USSNs 11/124,620 and 14/578,305 are useful.

[00209] In addition, there are additional Fc substitutions that find use in increased binding to the FcRn receptor and increased serum half-life, as specifically disclosed in USSN 12/341,769, hereby incorporated by reference in its entirety, including, but not limited to,

434S, 434A, 428L, 308F, 259I, 428L/434S, 259I/308F, 436I/428L, 436I or V/434S, 436V/428L and 259I/308F/428L.

I. Ablation Variants

[00210] Similarly, another category of functional variants are "FcγR ablation variants" or "Fc knock out (FcKO or KO)" variants. In these embodiments, for some therapeutic applications, it is desirable to reduce or remove the normal binding of the Fc domain to one or more or all of the Fcγ receptors (e.g., FcγR1, FcγRIIa, FcγRIIb, FcγRIIIa, etc.) to avoid additional mechanisms of action. That is, for example, in many embodiments, particularly in the use of bispecific immunomodulatory antibodies desirable to ablate FcγRIIIa binding to eliminate or significantly reduce ADCC activity such that one of the Fc domains comprises one or more Fcγ receptor ablation variants. These ablation variants are depicted in Figure 31 of USSN 15/141,350, all of which are herein incorporated by reference in its entirety, and each can be independently and optionally included or excluded, with preferred aspects utilizing ablation variants selected from the group 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 the EU index. It should be noted that the ablation variants referenced herein ablate FcγR binding but generally not FcRn binding.

J. Combination of Heterodimeric and Fc Variants

[00211] As will be appreciated by those in the art, all of the recited heterodimerization variants (including skew and/or pI variants) can be optionally and independently combined in any way, as long as they retain their "strandedness" or "monomer partition". In addition, all of these variants can be combined into any of the heterodimerization formats.

[00212] In the case of pI variants, while embodiments finding particular use are shown in the Figures, other combinations can be generated, following the basic rule of altering the pI difference between two monomers to facilitate purification.

[00213] In addition, any of the heterodimerization variants, skew and pI, are also independently and optionally combined with Fc ablation variants, Fc variants, FcRn variants, as generally outlined herein.

[00214] In addition, a monomeric Fc domain can comprise a set of amino acid substitutions that includes C220S/S267K/L368D/K370S or C220S/S267K/S364K/E357Q.

[00215] In addition, the heterodimeric Fc fusion proteins can comprise skew variants (e.g., a set of amino acid substitutions as shown in Figures 1A-1C of USSN 15/141,350, all of which are herein incorporated by reference in its entirety), with particularly useful skew variants being selected from the group consisting of S364K/E357Q : L368D/K370S; L368D/K370S : S364K; L368E/K370S : S364K; T411T/E360E/Q362E : D401K; L368D/K370S : S364K/E357L, K370S : S364K/E357Q, T366S/L368A/Y407V : T366W and T366S/L368A/Y407V/Y349C : T366W/S354C, optionally ablation variants, optionally charged domain linkers and the heavy chain comprises pI variants.

[00216] In some embodiments, the Fc domain comprising an amino acid substitution selected from the group consisting of: 236R, 239D, 239E, 243L, M252Y, V259I, 267D, 267E, 298A, V308F, 328F, 328R, 330L, 332D, 332E, M428L, N434A, N434S, 236R/328R, 239D/332E, M428L, 236R/328F, V259I/V308F, 267E/328F, M428L/N434S, Y436I/M428L, Y436V/M428L, Y436I/N434S, Y436V/N434S, 239D/332E/330L, M252Y/S254T/T256E, V259I/V308F/M428L, E233P/L234V/L235A/G236del/S267K, G236R/L328R and PVA/S267K. In some cases, the Fc domain comprises the amino acid substitution 239D/332E. In other cases, the Fc domain comprises the amino acid substitution G236R/L328R or PVA/S267K.

[00217] In one embodiment, a particular combination of skew and pI variants that finds use in the present invention is T366S/L368A/Y407V : T366W (optionally including a bridging disulfide, T366S/L368A/Y407V/Y349C : T366W/S354C) with one monomer comprises Q295E/N384D/Q418E/N481D and the other a positively charged domain linker. As will be appreciated in the art, the "knobs in holes" variants do not change pI, and thus can be used on either monomer.

III. IL-15 and IL15Rα Protein Domains

[00218] The present invention provides heterodimeric Fc fusion proteins containing IL-15 and IL-15Rα proteins. As shown in the figures, the IL-15 complex can take several forms. As stated above, the IL-15 protein on its own is less stable than when complexed with the IL-15Rα protein. As is known in the art, the IL-15Rα protein contains a "sushi domain", which is the shortest region of the receptor that retains IL-15 binding activity. Thus, while heterodimeric fusion proteins comprising the entire IL-15Rα protein can be made, preferred embodiments herein include complexes that just use the sushi domain, the sequence of which is shown in the figures.

[00219] Accordingly, the IL-15 complexes generally comprises the IL-15 protein and the sushi domain of IL IL-15Rα (unless otherwise noted that the full length sequence is used, "IL-15Rα", "IL-15Rα(sushi)" and "sushi" are used interchangeably throughout). This complex can be used in three different formats. As shown in Figures 9A, the IL-15 protein and the IL-15Rα(sushi) are not covalently attached, but rather are self-assembled through regular ligand-ligand interactions. As is more fully described herein, it can be either the IL-15 domain or the sushi domain that is covalently linked to the Fc domain (generally using an optional domain linker). Alternatively, they can be covalently attached using a domain linker as generally shown in Figures 9B, 9E, 9G Figure 9B depicts the sushi domain as the N-terminal domain, although this can be reversed. Finally, each of the IL-15 and sushi domains can be engineered to contain a cysteine amino acid, that forms a disulfide bond to form the complex as is generally shown in Figures39A-39D, again, with either the IL-15 domain or the sushi domain being covalently attached (using an optional domain linker) to the Fc domain.

[00220] In some embodiments, the human IL-15 protein has the amino acid sequence set forth in NCBI Ref. Seq. No. NP_000576.1 or SEQ ID NO:1. In some cases, the coding sequence of human IL-15 is set forth in NCBI Ref. Seq. No. NM_000585. An exemplary IL-15 protein of the Fc fusion heterodimeric protein outlined herein can have the amino acid sequence of SEQ ID NO:2 or amino acids 49-162 of SEQ ID NO:1. In some embodiments, the IL-15 protein has at least 90%, e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to SEQ ID NO:2. In some embodiments, the IL-15 protein has the amino acid sequence set forth in SEQ ID NO:2 and the amino acid substitution N72D. In

other embodiments, the IL-15 protein has the amino acid sequence of SEQ ID NO:2 and one or more amino acid substitutions selected from the group consisting of C42S, L45C, Q48C, V49C, L52C, E53C, E87C, and E89C. Optionally, the IL-15 protein also has an N72D substitution. The IL-15 protein of the Fc fusion protein can have 1, 2, 3, 4, 5, 6, 7, 8 or 9 amino acid substitutions.

[00221] The amino acid substitution(s) may be isosteric substitutions at the IL-15:IL-2β and IL-15:common gamma chain interface. In some embodiments, the human IL-15 protein has one or more amino acid substitutions selected from the group consisting of N1D, N4D, D8N, D30N, D61N, E64Q, N65D, Q108E, and any combination thereof. In some embodiments, the IL-15 protein has the amino acid substitution Q108E. In some cases, the IL-15 protein has 1, 2, 3, 4, 5, 6, 7, 8, or more amino acid substitutions. The IL-15 protein can have a N1D, N4D, D8N, D30N, D61N, E64Q, N65D, or Q108E substitution. In some embodiments, the amino acid substitution can include N1D/D61N, N1D/E64Q, N4D/D61N, N4D/E64Q, D8N/D61N, D8N/E64Q, D61N/E64Q, E64Q/Q108E, N1D/N4D/D8N, D61N/E64Q/N65D, N1D/D61N/E64Q, N1D/D61N/E64Q/Q108E, or N4D/D61N/E64Q/Q108E. In some embodiments, the IL-15 protein has the amino acid substitutions D30N/E64Q/N65D.

[00222] In some embodiments, the human IL-15 receptor alpha (IL-15Rα) protein has the amino acid sequence set forth in NCBI Ref. Seq. No. NP_002180.1 or SEQ ID NO:3. In some cases, the coding sequence of human IL-15Rα is set forth in NCBI Ref. Seq. No. NM_002189.3. An exemplary the IL-15Rα protein of the Fc fusion heterodimeric protein outlined herein can comprise or consist of the sushi domain of SEQ ID NO:3 (e.g., amino acids 31-95 of SEQ ID NO:3), or in other words, the amino acid sequence of SEQ ID NO:4. In some embodiments, the IL-15Rα protein has the amino acid sequence of SEQ ID NO:4 and an amino acid insertion selected from the group consisting of D96, P97, A98, D96/P97, D96/C97, D96/P97/A98, D96/P97/C98, and D96/C97/A98, wherein the amino acid position is relative to full-length human IL-15Rα protein or SEQ ID NO:3. For instance, amino acid(s) such as D (e.g., Asp), P (e.g., Pro), A (e.g., Ala), DP (e.g., Asp-Pro), DC (e.g., Asp-Cys), DPA (e.g., Asp-Pro-Ala), DPC (e.g., Asp-Pro-Cys), or DCA (e.g., Asp-Cys-Ala) can be added to the C-terminus of the IL-15Rα protein of SEQ ID NO:4. In some embodiments, the IL-15Rα protein has the amino acid sequence of SEQ ID NO:4 and one or more amino acid

substitutions selected from the group consisting of K34C, A37C, G38C, S40C, and L42C, wherein the amino acid position is relative to SEQ ID NO:4. The IL-15Rα protein can have 1, 2, 3, 4, 5, 6, 7, 8 or more amino acid mutations (e.g., substitutions, insertions and/or deletions).

IV. Domain Linkers

[00223] In some embodiments, the IL-15 protein and IL-15Rα protein are attached together via a linker. Optionally, the proteins are not attached via a linker. In other embodiments, the IL-15 protein and IL-15Rα protein are noncovalently attached. In some embodiments, the IL-15 protein is attached to an Fc domain via a linker. In certain embodiments, the IL-15 protein is attached to an Fc domain directly, such as without a linker. In other embodiments, the IL-15Rα protein is attached to an Fc domain via a linker. In other embodiments, the IL-15Rα protein is attached to an Fc domain directly. In some cases, a linker is not used to attach the IL-15 protein or IL-15Rα protein to an Fc domain.

[00224] In some embodiments, the linker is a "domain linker", used to link any two domains as outlined herein together. While any suitable linker can be used, many embodiments utilize a glycine-serine polymer, including for example (GS)n, (GSGGS)n, (GGGGS)n, and (GGGS)n, where n is an integer of at least 0 (and generally from 0 to 1 to 2 to 3 to 4 to 5) as well as any peptide sequence that allows for recombinant attachment of the two domains with sufficient length and flexibility to allow each domain to retain its biological function. In certain cases, useful linkers include $(GGGGS)_0$ or $(GGGGS)_1$ or $(GGGGS)_2$. In some cases, and with attention being paid to "strandedness", as outlined below, charged domain linkers can be used as discussed herein and shown in Figure 7.

V. Useful formats of the Invention

[00225] As shown in Figures 9A-9G and 39A-39D there are a number of useful formats of the bispecific heterodimeric fusion proteins of the invention. In general, the heterodimeric fusion proteins of the invention have two functional components: an IL-15/IL-15Rα(sushi) component and an Fc component, both of which can take different forms as

outlined herein and both of which can be combined with the other component in any configuration.

[00226] The first and the second Fc domains can have a set of amino acid substitutions selected from the group consisting of a) S267K/L368D/K370S : S267K/LS364K/E357Q; b) S364K/E357Q : L368D/K370S; c) L368D/K370S : S364K; d) L368E/K370S : S364K; e) T411T/E360E/Q362E : D401K; f) L368D/K370S : S364K/E357L and g) K370S : S364K/E357Q, according to EU numbering.

[00227] In some embodiments, 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.

[00228] 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.

[00229] Optionally, the first and/or second Fc domains have 428L/434S variants for half life extension.

A. IL-15/Rα-heteroFc format

[00230] In this embodiment, as shown in Figure 9A, the heterodimeric fusion protein comprises two monomers. The first monomer comprises (from N-to C-terminus) IL-15-optional domain linker-CH2-CH3, where the domain linker sometimes comprises all or part of the hinge. The second monomer comprises the IL-15/Rα(sushi) domain-optional domain linker-CH2-CH3, where the domain linker sometimes comprises all or part of the hinge.

[00231] In the IL-15/Rα-heteroFc format, a preferred embodiment utilizes the skew variant pair S364K/E357Q : L368D/K370S.

[00232] In the IL-15/Rα-heteroFc format, a preferred embodiment utilizes the IL-15 variant Q108E.

[00233] In the IL-15/Rα-heteroFc format, a preferred embodiment utilizes the IL-15 variant Q108E and the skew variant pair S364K/E357Q : L368D/K370S.

[00234] In the IL-15/Rα-heteroFc format, a preferred embodiment utilizes the IL-15 variant Q108E and the skew variant pair S364K/E357Q : L368D/K370S and the 428L/434S variants on both monomers.

[00235] In the IL-15/Rα-heteroFc format, a preferred embodiment utilizes the IL-15 D30N/E64Q/N65D variants.

[00236] In the IL-15/Rα-heteroFc format, a preferred embodiment utilizes the IL-15 D30N/E64Q/N65D variants and the skew variant pair S364K/E357Q : L368D/K370S.

[00237] In the IL-15/Rα-heteroFc format, a preferred embodiment utilizes the IL-15 D30N/E64Q/N65D variants, the skew variant pair S364K/E357Q : L368D/K370S and the 428L/434S variants on each Fc monomer.

[00238] In the IL-15/Rα-heteroFc format, a preferred embodiment utilizes the IL-15 N65D variant.

[00239] In the IL-15/Rα-heteroFc format, a preferred embodiment utilizes the IL-15 N65D variant, and the skew variant pair S364K/E357Q : L368D/K370S

[00240] In the IL-15/Rα-heteroFc format, a preferred embodiment utilizes the IL-15 N65D variant, the skew variant pair S364K/E357Q : L368D/K370S and the 428L/434S variants on each Fc monomer.

[00241] In the IL-15/Rα-heteroFc format, a preferred embodiment utilizes the IL-15 N4D/N65D variant.

[00242] In the IL-15/Rα-heteroFc format, a preferred embodiment utilizes the IL-15 N4D/N65D variant and the skew variant pair S364K/E357Q : L368D/K370S.

[00243] In the IL-15/Rα-heteroFc format, a preferred embodiment utilizes the IL-15 N4D/N65D variant, the skew variant pair S364K/E357Q : L368D/K370S and the 428L/434S variants on each Fc monomer.

[00244] In the IL-15/Rα-heteroFc format, a preferred embodiment utilizes the IL-15 N1D/N65D variant.

[00245] In the IL-15/Rα-heteroFc format, a preferred embodiment utilizes the IL-15 N1D/N65D variant and the skew variant pair S364K/E357Q : L368D/K370S.

[00246] In the IL-15/Rα-heteroFc format, a preferred embodiment utilizes the IL-15 N1D/N65D variant, the skew variant pair S364K/E357Q : L368D/K370S and the 428L/434S variants on each Fc monomer.

[00247] In the IL-15/Rα-heteroFc format, preferred embodiments are shown in Figure 48A (XENP22822 including chain 1 (17693) and chain 2 (15908)), Figure 94A (XENP23504 including chain 1 and chain 2), Figure 104AO (XENP24045 including chain 1 and chain 2), Figure 104AQ (XENP24306 including chain 1 and chain 2), Figure 48A (XENP22821 including chain 1 and chain 2), Figure 94A (XENP23343 including chain 1 and chain 2), Figure 104AJ (XENP23557 including chain 1 and chain 2), Figure 104AP (XENP24113 including chain 1 and chain 2), Figure 104AP (XENP24051 including chain 1 and chain 2), Figure 104AR (XENP24341 including chain 1 and chain 2), Figure 104AP (XENP24052 including chain 1 and chain 2), and Figure 104AP (XENP24301 including chain 1 and chain 2).

B. scIL-15-Rα-Fc

[00248] In this embodiment, as shown in Figure 9B, the heterodimeric fusion protein comprises two monomers. The first monomer comprises (from N-to C-terminus) IL-15/Rα(sushi)-domain linker-IL-15-optional domain linker-CH2-CH3, where the domain linker sometimes comprises all or part of the hinge. The second monomer comprises and "empty" Fc, comprising all or part of the hinge-CH2-CH3. This is referred to as "scIL-15/Rα-Fc" with the "sc" standing for "single chain" (e.g. of the IL-15/sushi complex).

[00249] In the scIL-15/Rα-Fc format, a preferred embodiment utilizes the skew variant pair S364K/E357Q : L368D/K370S.

[00250] In the scIL-15/Rα-Fc format, a preferred embodiment utilizes the IL-15 variant Q108E.

[00251] In the scIL-15/Rα-Fc format, a preferred embodiment utilizes the IL-15 variant Q108E and the skew variant pair S364K/E357Q : L368D/K370S.

[00252] In the scIL-15/Rα-Fc format, a preferred embodiment utilizes the IL-15 variant Q108E and the skew variant pair S364K/E357Q : L368D/K370S and the 428L/434S variants on both monomers.

[00253] In the scIL-15/Rα-Fc format, a preferred embodiment utilizes the IL-15 D30N/E64Q/N65D variants.

[00254] In the scIL-15/Rα-Fc format, a preferred embodiment utilizes the IL-15 D30N/E64Q/N65D variants and the skew variant pair S364K/E357Q : L368D/K370S.

[00255] In the scIL-15/Rα-Fc format, a preferred embodiment utilizes the IL-15 D30N/E64Q/N65D variants, the skew variant pair S364K/E357Q : L368D/K370S and the 428L/434S variants on each Fc monomer.

[00256] In the scIL-15/Rα-Fc format, a preferred embodiment utilizes the IL-15 N65D variant.

[00257] In the scIL-15/Rα-Fc format, a preferred embodiment utilizes the IL-15 N65D variant, and the skew variant pair S364K/E357Q : L368D/K370S

[00258] In the scIL-15/Rα-Fc format, a preferred embodiment utilizes the IL-15 N65D variant, the skew variant pair S364K/E357Q : L368D/K370S and the 428L/434S variants on each Fc monomer.

[00259] In the scIL-15/Rα-Fc format, a preferred embodiment utilizes the IL-15 N4D/N65D variant.

[00260] In the scIL-15/Rα-Fc format, a preferred embodiment utilizes the IL-15 N4D/N65D variant and the skew variant pair S364K/E357Q : L368D/K370S.

[00261] In the scIL-15/Rα-Fc format, a preferred embodiment utilizes the IL-15 N4D/N65D variant, the skew variant pair S364K/E357Q : L368D/K370S and the 428L/434S variants on each Fc monomer.

[00262] In the scIL-15/Rα-Fc format, a preferred embodiment utilizes the IL-15 N1D/N65D variant.

[00263] In the scIL-15/Rα-Fc format, a preferred embodiment utilizes the IL-15 N1D/N65D variant and the skew variant pair S364K/E357Q : L368D/K370S.

[00264] In the IL-15/Rα-heteroFc format, a preferred embodiment utilizes the IL-15 N1D/N65D variant, the skew variant pair S364K/E357Q : L368D/K370S and the 428L/434S variants on each Fc monomer.

C. ncIL-15/Rα-Fc

[00265] In this embodiment, as shown in Figure 9C, the heterodimeric fusion protein comprises three monomers. The first monomer comprises (from N-to C-terminus) IL-15/Rα(sushi)-domain linker-CH2-CH3, where the domain linker sometimes comprises all or part of the hinge. The second monomer comprises and "empty" Fc, comprising all or part of the hinge-CH2-CH3. The third monomer is IL-15. This is referred to as "ncIL-15/Rα-Fc" with the "nc" standing for "non-covalent").

[00266] In the ncIL-15/Rα-Fc format, a preferred embodiment utilizes the skew variant pair S364K/E357Q : L368D/K370S.

[00267] In the ncIL-15/Rα-Fc format, a preferred embodiment utilizes the IL-15 variant Q108E.

[00268] In the ncIL-15/Rα-Fc format, a preferred embodiment utilizes the IL-15 variant Q108E and the skew variant pair S364K/E357Q : L368D/K370S.

[00269] In the ncIL-15/Rα-Fc format, a preferred embodiment utilizes the IL-15 variant Q108E and the skew variant pair S364K/E357Q : L368D/K370S and the 428L/434S variants on both monomers.

[00270] In the ncIL-15/Rα-Fc format, a preferred embodiment utilizes the IL-15 D30N/E64Q/N65D variants.

[00271] In the ncIL-15/Rα-Fc format, a preferred embodiment utilizes the IL-15 D30N/E64Q/N65D variants and the skew variant pair S364K/E357Q : L368D/K370S.

[00272] In the ncIL-15/Rα-Fc format, a preferred embodiment utilizes the IL-15 D30N/E64Q/N65D variants, the skew variant pair S364K/E357Q : L368D/K370S and the 428L/434S variants on each Fc monomer.

[00273] In the ncIL-15/Rα-Fc format, a preferred embodiment utilizes the IL-15 N65D variant.

[00274] In the ncIL-15/Rα-Fc format, a preferred embodiment utilizes the IL-15 N65D variant, and the skew variant pair S364K/E357Q : L368D/K370S

[00275] In the ncIL-15/Rα-Fc format, a preferred embodiment utilizes the IL-15 N65D variant, the skew variant pair S364K/E357Q : L368D/K370S and the 428L/434S variants on each Fc monomer.

[00276] In the ncIL-15/Rα-Fc format, a preferred embodiment utilizes the IL-15 N4D/N65D variant.

[00277] In the ncIL-15/Rα-Fc format, a preferred embodiment utilizes the IL-15 N4D/N65D variant and the skew variant pair S364K/E357Q : L368D/K370S.

[00278] In the ncIL-15/Rα-Fc format, a preferred embodiment utilizes the IL-15 N4D/N65D variant, the skew variant pair S364K/E357Q : L368D/K370S and the 428L/434S variants on each Fc monomer.

[00279] In the ncIL-15/Rα-Fc format, a preferred embodiment utilizes the IL-15 N1D/N65D variant.

[00280] In the ncIL-15/Rα-Fc format, a preferred embodiment utilizes the IL-15 N1D/N65D variant and the skew variant pair S364K/E357Q : L368D/K370S.

[00281] In the ncIL-15/Rα-heteroFc format, a preferred embodiment utilizes the IL-15 N1D/N65D variant, the skew variant pair S364K/E357Q : L368D/K370S and the 428L/434S variants on each Fc monomer.

[00282] In the ncIL-15/Rα-heteroFc format, preferred embodiments are shown in Figure 104AS (XENP24349including chain 1 and chain 2) and Figure 104AT (XENP24383 including chain 1 and chain 2).

D. Bivalent ncIL-15/Rα-Fc

[00283] In this embodiment, as shown in Figure 9D, the heterodimeric fusion protein comprises four monomers. The first and second monomers comprise (from N-to C-terminus) IL-15/Rα(sushi)-domain linker-CH2-CH3, where the domain linker sometimes comprises all or part of the hinge. The third and fourth monomers comprise IL-15. This is referred to as "bivalent ncIL-15/Rα-Fc" with the "nc" standing for "non-covalent").

[00284] In the bivalent ncIL-15/Rα-Fc format, a preferred embodiment utilizes the skew variant pair S364K/E357Q : L368D/K370S.

[00285] In the bivalent ncIL-15/Rα-Fc format, a preferred embodiment utilizes the IL-15 variant Q108E.

[00286] In the bivalent ncIL-15/Rα-Fc format, a preferred embodiment utilizes the IL-15 variant Q108E and the skew variant pair S364K/E357Q : L368D/K370S.

[00287] In the bivalent ncIL-15/Rα-Fc format, a preferred embodiment utilizes the IL-15 variant Q108E and the skew variant pair S364K/E357Q : L368D/K370S and the 428L/434S variants on both monomers.

[00288] In the bivalent ncIL-15/Rα-Fc format, a preferred embodiment utilizes the IL-15 D30N/E64Q/N65D variants.

[00289] In the bivalent ncIL-15/Rα-Fc format, a preferred embodiment utilizes the IL-15 D30N/E64Q/N65D variants and the skew variant pair S364K/E357Q : L368D/K370S.

[00290] In the bivalent ncIL-15/Rα-Fc format, a preferred embodiment utilizes the IL-15 D30N/E64Q/N65D variants, the skew variant pair S364K/E357Q : L368D/K370S and the 428L/434S variants on each Fc monomer.

[00291] In the bivalent ncIL-15/Rα-Fc format, a preferred embodiment utilizes the IL-15 N65D variant.

[00292] In the bivalent ncIL-15/Rα-Fc format, a preferred embodiment utilizes the IL-15 N65D variant, and the skew variant pair S364K/E357Q : L368D/K370S

[00293] In the bivalent ncIL-15/Rα-Fc format, a preferred embodiment utilizes the IL-15 N65D variant, the skew variant pair S364K/E357Q : L368D/K370S and the 428L/434S variants on each Fc monomer.

[00294] In the bivalent ncIL-15/Rα-Fc format, a preferred embodiment utilizes the IL-15 N4D/N65D variant.

[00295] In the bivalent ncIL-15/Rα-Fc format, a preferred embodiment utilizes the IL-15 N4D/N65D variant and the skew variant pair S364K/E357Q : L368D/K370S.

[00296] In the bivalent ncIL-15/Rα-Fc format, a preferred embodiment utilizes the IL-15 N4D/N65D variant, the skew variant pair S364K/E357Q : L368D/K370S and the 428L/434S variants on each Fc monomer.

[00297] In the bivalent ncIL-15/Rα-Fc format, a preferred embodiment utilizes the IL-15 N1D/N65D variant.

[00298] In the bivalent ncIL-15/Rα-Fc format, a preferred embodiment utilizes the IL-15 N1D/N65D variant and the skew variant pair S364K/E357Q : L368D/K370S.

[00299] In the bivalent ncIL-15/Rα-Fc format, a preferred embodiment utilizes the IL-15 N1D/N65D variant, the skew variant pair S364K/E357Q : L368D/K370S and the 428L/434S variants on each Fc monomer.

[00300] In the bivalent ncIL-15/Rα-Fc format, preferred embodiments are shown in Figure 104AR (XENP24342 including chain 1 and chain 2) and (XENP24346 including chain 1 and chain 2).

VI. Useful Embodiments of the Invention

[00301] As will be appreciated by those in the art and discussed more fully below, the heterodimeric fusion proteins of the present invention can take on a wide variety of configurations, as are generally depicted in Figures 9A-9G and Figures 39A-39D. The amino acid sequences of exemplary fusion proteins are provided in 8A-8E, 10, 11, 12A, 12B, 13-15, 40A, 40B, 41A, 41B, 42, 43, 48A-48D, 49A-49C, 50A, 50B, 51, 52, 53, and 94A-94D.

[00302] Many of the embodiments outlined herein rely in general on the format comprising a first monomer (first fusion protein) comprising an IL-15 protein domain covalently attached using a first domain linker to the N-terminus of a first Fc domain, and a second monomer (second fusion protein) comprising an IL-15Rα protein domain covalently attached using a second domain linker to the N-terminus of a second Fc domain. Exemplary

embodiments of this format ("IL-15/Rα hetero Fc" and "dsIL-15/Rα hetero Fc") include, but are not limited to, 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, XENP22815, XENP22816, XENP22817, XENP22818, XENP22819, XENP22820, XENP22821, XENP23343, XENP23554, XENP23555, XENP23557, XENP23559,XENP23561,XENP24018, XENP24019, XENP24020, XENP24051, XENP24052, XENP23504, XENP24306, XENP24306, XENP23343, XENO24113, XENP24341, and XENP24301.

[00303] A useful format of a heterodimer Fc fusion protein comprises a fusion protein comprising a first protein domain covalently attached to the N-terminus of a second protein domain via a first domain linker that is covalently attached to the N-terminus of a first Fc domain via a second domain linker, and a second Fc domain (e.g., an empty Fc domain). In some cases, the first protein domain is an IL-15Rα protein domain and the second protein domain is an IL-15 protein domain. An exemplary embodiment of this format ("scIL-15/ Rα-Fc") includes, but is not limited to, XENP21478.

[00304] Yet another useful of a heterodimer Fc fusion protein outlined herein comprises a fusion protein comprising a first protein domain covalently attached to the N-terminus a first Fc domain via a domain linker, a second Fc domain (e.g., an empty Fc domain), and a second protein domain that is noncovalently attached to the first protein domain. In some cases, the first protein domain is an IL-15 protein domain and the second protein domain is an IL-15Rα protein domain. An exemplary embodiment of this format ("ncIL-15/ Rα-Fc" or "dsIL-15/ Rα-Fc") includes, but is not limited to, XENP21479, XENP22357, XENP22354, XENP22355, XENP22356, XENP22357, XENP22358, XENP22359, XENP22360, XENP22361, XENP22362, XENP22363, XENP22364, XENP22365, XENP22366, XENP22637, XENP24348, XENP24349, and XENP24383.

[00305] Another useful format of a heterodimer Fc fusion protein outlined herein comprises a first fusion protein comprising a first protein domain covalently attached to the N-terminus of said first Fc domain via a first domain linker, a second fusion protein

comprising a second protein domain covalently attached to the N-terminus of said second Fc domain via a second domain linker, a third protein domain noncovalently attached to said first protein domain of said first fusion protein, and a fourth protein domain noncovalently attached to said second protein domain of said second fusion protein. In some cases, the first and second protein domains are IL-15 Rα protein domains, and the third and fourth protein domains are IL-15 protein domains. An exemplary embodiment of this format ("bivalent ncIL-15/ Rα-Fc"or "bivalent dsIL-15/ Rα-Fc") includes, but is not limited to, XENP21978, XENP22634, XENP24342, and XENP24346.

[00306] Another useful format ("bivalent scIL-15/ Rα-Fc") is outlined herein in Figure 14.

[00307] Yet another useful format of a heterodimer Fc fusion protein outlined herein comprises a first fusion protein comprising a first Fc domain covalently attached to the N-terminus of a first protein domain using a domain linker, a second Fc domain (e.g., an empty Fc domain), and a second protein domain noncovalently attached to said first protein domain. An exemplary embodiment of this format ("Fc-ncIL-15/Rα" or "Fc-dsIL-15/Rα") includes, but is not limited to, XENP22637 and XENP22639, and those depicted in Figure 16. In some embodiments, the first protein and the second protein are attached via a linker (Figure 9G).

[00308] For any of the heterodimer Fc fusion proteins outlined herein, the first domain linker and the second domain linker can be the same or different. In addition, the first Fc domain and the second Fc domain of the heterodimeric protein can have different amino acid sequences.

[00309] The Fc domains of the present invention comprise IgG Fc domains, e.g., IgG1 Fc domains. In some embodiments, the first and second Fc domains comprising a set of amino acid substitutions selected from the group consisting of: L368D/K370S and S364K; L368D/K370S and S364K/E357L; L368D/K370S and S364K/E357Q; T411E/K360E/Q362E and D401K; L368E/K370S and S364K; K370S and S364K/E357Q; K370S and S364K/E357Q; S267K/L368D/K370S and S267K/S364K/E357Q, according to EU numbering. In some instances, the first and/or the second Fc domains of any of the heterodimeric Fc fusion formats outlined herein can have an additional set of amino acid substitutions comprising

Q295E/N384D/Q418E/N421D, according to EU numbering. In some embodiments, 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.

[00310] Additional heterodimerization variants can be independently and optionally included and selected from variants outlined in the figures. These compositions can further comprise ablation variants, pI variants, charged variants, isotypic variants, etc.

VII. Nucleic Acids of the Invention

[00311] The invention further provides nucleic acid compositions encoding the heterodimeric Fc fusion protein of the invention (or, in the case of a monomer Fc domain protein, nucleic acids encoding those as well).

[00312] As will be appreciated by those in the art, the nucleic acid compositions will depend on the format of the heterodimeric protein. Thus, for example, when the format requires three amino acid sequences, three nucleic acid sequences can be incorporated into one or more expression vectors for expression. Similarly, some formats only two nucleic acids are needed; again, they can be put into one or two expression vectors.

[00313] As is known in the art, the nucleic acids encoding the components of the invention can be incorporated into expression vectors as is known in the art, and depending on the host cells used to produce the heterodimeric Fc fusion proteins of the invention. Generally the nucleic acids are operably linked to any number of regulatory elements (promoters, origin of replication, selectable markers, ribosomal binding sites, inducers, etc.). The expression vectors can be extra-chromosomal or integrating vectors.

[00314] The nucleic acids and/or expression vectors of the invention are then transformed into any number of different types of host cells as is well known in the art, including mammalian, bacterial, yeast, insect and/or fungal cells, with mammalian cells (e.g. CHO cells), finding use in many embodiments.

[00315] In some embodiments, nucleic acids encoding each monomer, as applicable depending on the format, are each contained within a single expression vector, generally under different or the same promoter controls. In embodiments of particular use in the present invention, each of these two or three nucleic acids are contained on a different expression vector.

[00316] The heterodimeric Fc fusion protein of the invention are made by culturing host cells comprising the expression vector(s) as is well known in the art. Once produced, traditional fusion protein or antibody purification steps are done, including an ion exchange chromotography step. As discussed herein, having the pIs of the two monomers differ by at least 0.5 can allow separation by ion exchange chromatography or isoelectric focusing, or other methods sensitive to isoelectric point. That is, the inclusion of pI substitutions that alter the isoelectric point (pI) of each monomer so that such that each monomer has a different pI and the heterodimer also has a distinct pI, thus facilitating isoelectric purification of the heterodimer (e.g., anionic exchange columns, cationic exchange columns). These substitutions also aid in the determination and monitoring of any contaminating homodimers post-purification (e.g., IEF gels, clEF, and analytical IEX columns).

VIII. Biological and Biochemical Functionality of IL-15/IL15Rα Heterodimeric Immunomodulatory Fc Fusion Proteins

[00317] Generally the heterodimeric Fc fusion proteins of the invention are administered to patients with cancer, and efficacy is assessed, in a number of ways as described herein. Thus, while standard assays of efficacy can be run, such as cancer load, size of tumor, evaluation of presence or extent of metastasis, etc., immuno-oncology treatments can be assessed on the basis of immune status evaluations as well. This can be done in a number of ways, including both in vitro and in vivo assays. For example, evaluation of changes in immune status (e.g., presence of ICOS+ CD4+ T cells following ipi treatment) along with "old fashioned" measurements such as tumor burden, size, invasiveness, LN involvement, metastasis, etc. can be done. Thus, any or all of the following can be evaluated: the inhibitory effects of PVRIG on $CD4^+$ T cell activation or proliferation, $CD8^+$ T (CTL) cell activation or proliferation, $CD8^+$ T cell-mediated cytotoxic activity and/or CTL mediated cell depletion, NK cell activity and NK mediated cell depletion, the potentiating effects of

PVRIG on Treg cell differentiation and proliferation and Treg- or myeloid derived suppressor cell (MDSC)- mediated immunosuppression or immune tolerance, and/or the effects of PVRIG on proinflammatory cytokine production by immune cells, e.g., IL-2, IFN-ɣ or TNF-α production by T or other immune cells.

[00318] In some embodiments, assessment of treatment is done by evaluating immune cell proliferation, using for example, CFSE dilution method, Ki67 intracellular staining of immune effector cells, and ^{3}H-thymidine incorporation method,

[00319] In some embodiments, assessment of treatment is done by evaluating the increase in gene expression or increased protein levels of activation-associated markers, including one or more of: CD25, CD69, CD137, ICOS, PD1, GITR, OX40, and cell degranulation measured by surface expression of CD107A.

[00320] In general, gene expression assays are done as is known in the art.

[00321] In general, protein expression measurements are also similarly done as is known in the art.

[00322] In some embodiments, assessment of treatment is done by assessing cytotoxic activity measured by target cell viability detection via estimating numerous cell parameters such as enzyme activity (including protease activity), cell membrane permeability, cell adherence, ATP production, co-enzyme production, and nucleotide uptake activity. Specific examples of these assays include, but are not limited to, Trypan Blue or PI staining, ^{51}Cr or ^{35}S release method, LDH activity, MTT and/or WST assays, Calcein-AM assay, Luminescent based assay, and others.

[00323] In some embodiments, assessment of treatment is done by assessing T cell activity measured by cytokine production, measure either intracellularly in culture supernatant using cytokines including, but not limited to, IFNγ, TNFα, GM-CSF, IL2, IL6, IL4, IL5, IL10, IL13 using well known techniques.

[00324] Accordingly, assessment of treatment can be done using assays that evaluate one or more of the following: (i) increases in immune response, (ii) increases in activation of αβ and/or γδ T cells, (iii) increases in cytotoxic T cell activity, (iv) increases in NK and/or NKT cell activity, (v) alleviation of αβ and/or γδ T-cell suppression, (vi) increases in pro-

inflammatory cytokine secretion, (vii) increases in IL-2 secretion; (viii) increases in interferon-ɣ production, (ix) increases in Th1 response, (x) decreases in Th2 response, (xi) decreases or eliminates cell number and/or activity of at least one of regulatory T cells (Tregs).

A. Assays to Measure Efficacy

[00325] In some embodiments, T cell activation is assessed using a Mixed Lymphocyte Reaction (MLR) assay as is known in the art. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[00326] In one embodiment, the signaling pathway assay measures increases or decreases in immune response as measured for an example by phosphorylation or de-phosphorylation of different factors, or by measuring other post translational modifications. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[00327] In one embodiment, the signaling pathway assay measures increases or decreases in activation of $\alpha\beta$ and/or $\gamma\delta$ T cells as measured for an example by cytokine secretion or by proliferation or by changes in expression of activation markers like for an example CD137, CD107a, PD1, etc. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[00328] In one embodiment, the signaling pathway assay measures increases or decreases in cytotoxic T cell activity as measured for an example by direct killing of target cells like for an example cancer cells or by cytokine secretion or by proliferation or by changes in expression of activation markers like for an example CD137, CD107a, PD1, etc. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[00329] In one embodiment, the signaling pathway assay measures increases or decreases in NK and/or NKT cell activity as measured for an example by direct killing of target cells like for an example cancer cells or by cytokine secretion or by changes in expression of activation markers like for an example CD107a, etc. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[00330] In one embodiment, the signaling pathway assay measures increases or decreases in αβ and/or γδ T-cell suppression, as measured for an example by cytokine secretion or by proliferation or by changes in expression of activation markers like for an example CD137, CD107a, PD1, etc. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[00331] In one embodiment, the signaling pathway assay measures increases or decreases in pro-inflammatory cytokine secretion as measured for example by ELISA or by Luminex or by Multiplex bead based methods or by intracellular staining and FACS analysis or by Alispot etc. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[00332] In one embodiment, the signaling pathway assay measures increases or decreases in IL-2 secretion as measured for example by ELISA or by Luminex or by Multiplex bead based methods or by intracellular staining and FACS analysis or by Alispot etc. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[00333] In one embodiment, the signaling pathway assay measures increases or decreases in interferon-γ production as measured for example by ELISA or by Luminex or by Multiplex bead based methods or by intracellular staining and FACS analysis or by Alispot etc. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[00334] In one embodiment, the signaling pathway assay measures increases or decreases in Th1 response as measured for an example by cytokine secretion or by changes in expression of activation markers. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[00335] In one embodiment, the signaling pathway assay measures increases or decreases in Th2 response as measured for an example by cytokine secretion or by changes in expression of activation markers. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[00336] In one embodiment, the signaling pathway assay measures increases or decreases cell number and/or activity of at least one of regulatory T cells (Tregs), as measured for example by flow cytometry or by IHC. A decrease in response indicates immunostimulatory activity. Appropriate decreases are the same as for increases, outlined below.

[00337] In one embodiment, the signaling pathway assay measures increases or decreases in M2 macrophages cell numbers, as measured for example by flow cytometry or by IHC. A decrease in response indicates immunostimulatory activity. Appropriate decreases are the same as for increases, outlined below.

[00338] In one embodiment, the signaling pathway assay measures increases or decreases in M2 macrophage pro-tumorigenic activity, as measured for an example by cytokine secretion or by changes in expression of activation markers. A decrease in response indicates immunostimulatory activity. Appropriate decreases are the same as for increases, outlined below.

[00339] In one embodiment, the signaling pathway assay measures increases or decreases in N2 neutrophils increase, as measured for example by flow cytometry or by IHC. A decrease in response indicates immunostimulatory activity. Appropriate decreases are the same as for increases, outlined below.

[00340] In one embodiment, the signaling pathway assay measures increases or decreases in N2 neutrophils pro-tumorigenic activity, as measured for an example by cytokine secretion or by changes in expression of activation markers. A decrease in response indicates immunostimulatory activity. Appropriate decreases are the same as for increases, outlined below.

[00341] In one embodiment, the signaling pathway assay measures increases or decreases in inhibition of T cell activation, as measured for an example by cytokine secretion or by proliferation or by changes in expression of activation markers like for an example CD137, CD107a, PD1, etc. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[00342] In one embodiment, the signaling pathway assay measures increases or decreases in inhibition of CTL activation as measured for an example by direct killing of target cells like for an example cancer cells or by cytokine secretion or by proliferation or by changes in expression of activation markers like for an example CD137, CD107a, PD1, etc. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[00343] In one embodiment, the signaling pathway assay measures increases or decreases in $\alpha\beta$ and/or $\gamma\delta$ T cell exhaustion as measured for an example by changes in expression of activation markers. A decrease in response indicates immunostimulatory activity. Appropriate decreases are the same as for increases, outlined below.

[00344] In one embodiment, the signaling pathway assay measures increases or decreases $\alpha\beta$ and/or $\gamma\delta$ T cell response as measured for an example by cytokine secretion or by proliferation or by changes in expression of activation markers like for an example CD137, CD107a, PD1, etc. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[00345] In one embodiment, the signaling pathway assay measures increases or decreases in stimulation of antigen-specific memory responses as measured for an example by cytokine secretion or by proliferation or by changes in expression of activation markers like for an example CD45RA, CCR7 etc. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below. .

[00346] In one embodiment, the signaling pathway assay measures increases or decreases in apoptosis or lysis of cancer cells as measured for an example by cytotoxicity assays such as for an example MTT, Cr release, Calcine AM, or by flow cytometry based assays like for an example CFSE dilution or propidium iodide staining etc. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[00347] In one embodiment, the signaling pathway assay measures increases or decreases in stimulation of cytotoxic or cytostatic effect on cancer cells, as measured for an example by cytotoxicity assays such as for an example MTT, Cr release, Calcine AM, or by

flow cytometry based assays like for an example CFSE dilution or propidium iodide staining etc. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[00348] In one embodiment, the signaling pathway assay measures increases or decreases direct killing of cancer cells as measured for an example by cytotoxicity assays such as for an example MTT, Cr release, Calcine AM, or by flow cytometry based assays like for an example CFSE dilution or propidium iodide staining etc. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[00349] In one embodiment, the signaling pathway assay measures increases or decreases Th17 activity as measured for an example by cytokine secretion or by proliferation or by changes in expression of activation markers. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[00350] In one embodiment, the signaling pathway assay measures increases or decreases in induction of complement dependent cytotoxicity and/or antibody dependent cell-mediated cytotoxicity, as measured for an example by cytotoxicity assays such as for an example MTT, Cr release, Calcine AM, or by flow cytometry based assays like for an example CFSE dilution or propidium iodide staining etc. An increase in activity indicates immunostimulatory activity. Appropriate increases in activity are outlined below.

[00351] In one embodiment, T cell activation is measured for an example by direct killing of target cells like for an example cancer cells or by cytokine secretion or by proliferation or by changes in expression of activation markers like for an example CD137, CD107a, PD1, etc. For T-cells, increases in proliferation, cell surface markers of activation (e.g., CD25, CD69, CD137, PD1), cytotoxicity (ability to kill target cells), and cytokine production (e.g., IL-2, IL-4, IL-6, IFNγ, TNF-a, IL-10, IL-17A) would be indicative of immune modulation that would be consistent with enhanced killing of cancer cells.

[00352] In one embodiment, NK cell activation is measured for example by direct killing of target cells like for an example cancer cells or by cytokine secretion or by changes in expression of activation markers like for an example CD107a, etc. For NK cells, increases in proliferation, cytotoxicity (ability to kill target cells and increases CD107a, granzyme, and

perforin expression), cytokine production (e.g., IFNγ and TNF), and cell surface receptor expression (e.g. CD25) would be indicative of immune modulation that would be consistent with enhanced killing of cancer cells.

[00353] In one embodiment, γδ T cell activation is measured for example by cytokine secretion or by proliferation or by changes in expression of activation markers.

[00354] In one embodiment, Th1 cell activation is measured for example by cytokine secretion or by changes in expression of activation markers.

[00355] Appropriate increases in activity or response (or decreases, as appropriate as outlined above), are increases of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or 98 to 99% percent over the signal in either a reference sample or in control samples, for example test samples that do not contain an anti-PVRIG antibody of the invention. Similarly, increases of at least one-, two-, three-, four- or five-fold as compared to reference or control samples show efficacy.

IX. Treatments

[00356] Once made, the compositions of the invention find use in a number of oncology applications, by treating cancer, generally by promoting T cell activation (e.g., T cells are no longer suppressed) with the binding of the heterodimeric Fc fusion proteins of the invention.

[00357] Accordingly, the heterodimeric compositions of the invention find use in the treatment of these cancers.

A. Heterodimeric Protein Compositions for *In Vivo* Administration

[00358] Formulations of the antibodies used in accordance with the present invention are prepared for storage by mixing an antibody having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers (as generally outlined in Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. [1980]), in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, buffers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol,

butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG).

B. Administrative Modalities

[00359] The heterodimeric proteins and chemotherapeutic agents of the invention are administered to a subject, in accord with known methods, such as intravenous administration as a bolus or by continuous infusion over a period of time.

C. Treatment Modalities

[00360] In the methods of the invention, therapy is used to provide a positive therapeutic response with respect to a disease or condition. By "positive therapeutic response" is intended an improvement in the disease or condition, and/or an improvement in the symptoms associated with the disease or condition. For example, a positive therapeutic response would refer to one or more of the following improvements in the disease: (1) a reduction in the number of neoplastic cells; (2) an increase in neoplastic cell death; (3) inhibition of neoplastic cell survival; (5) inhibition (i.e., slowing to some extent, preferably halting) of tumor growth; (6) an increased patient survival rate; and (7) some relief from one or more symptoms associated with the disease or condition.

[00361] Positive therapeutic responses in any given disease or condition can be determined by standardized response criteria specific to that disease or condition. Tumor response can be assessed for changes in tumor morphology (i.e., overall tumor burden, tumor size, and the like) using screening techniques such as magnetic resonance imaging (MRI) scan, x-radiographic imaging, computed tomographic (CT) scan, bone scan imaging, endoscopy, and tumor biopsy sampling including bone marrow aspiration (BMA) and counting of tumor cells in the circulation.

[00362] In addition to these positive therapeutic responses, the subject undergoing therapy may experience the beneficial effect of an improvement in the symptoms associated with the disease.

[00363] Treatment according to the present invention includes a "therapeutically effective amount" of the medicaments used. A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve a desired therapeutic result.

[00364] A therapeutically effective amount may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the medicaments to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the antibody or antibody portion are outweighed by the therapeutically beneficial effects.

[00365] A "therapeutically effective amount" for tumor therapy may also be measured by its ability to stabilize the progression of disease. The ability of a compound to inhibit cancer may be evaluated in an animal model system predictive of efficacy in human tumors.

[00366] Alternatively, this property of a composition may be evaluated by examining the ability of the compound to inhibit cell growth or to induce apoptosis by in vitro assays known to the skilled practitioner. A therapeutically effective amount of a therapeutic compound may decrease tumor size, or otherwise ameliorate symptoms in a subject. One of ordinary skill in the art would be able to determine such amounts based on such factors as the subject's size, the severity of the subject's symptoms, and the particular composition or route of administration selected.

[00367] Dosage regimens are adjusted to provide the optimum desired response (e.g., a therapeutic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. Parenteral compositions may be formulated in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit contains a predetermined quantity of active

compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier.

[00368] The specification for the dosage unit forms of the present invention are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

[00369] The efficient dosages and the dosage regimens for the bispecific antibodies used in the present invention depend on the disease or condition to be treated and may be determined by the persons skilled in the art.

[00370] An exemplary, non-limiting range for a therapeutically effective amount of an bispecific antibody used in the present invention is about 0.1-100 mg/kg.

[00371] All cited references are herein expressly incorporated by reference in their entirety.

[00372] Whereas particular embodiments of the invention have been described above for purposes of illustration, it will be appreciated by those skilled in the art that numerous variations of the details may be made without departing from the invention as described in the appended claims.

EXAMPLES

[00373] Examples are provided below to illustrate the present invention. These examples are not meant to constrain the present invention to any particular application or theory of operation. For all constant region positions discussed in the present invention, numbering is according to the EU index as in Kabat (Kabat et al., 1991, Sequences of Proteins of Immunological Interest, 5th Ed., United States Public Health Service, National Institutes of Health, Bethesda, entirely incorporated by reference). Those skilled in the art of antibodies will appreciate that this convention consists of nonsequential numbering in specific regions of an immunoglobulin sequence, enabling a normalized reference to conserved positions in immunoglobulin families. Accordingly, the positions of any given immunoglobulin as defined by the EU index will not necessarily correspond to its sequential sequence.

[00374] General and specific scientific techniques are outlined in US Publications 2015/0307629, 2014/0288275 and WO2014/145806, all of which are expressly incorporated by reference in their entirety and particularly for the techniques outlined therein.

X. Example 1: IL-15/IL-15Rα(sushi) Fc Fusion Proteins

[00375] In order to address the short half-life of IL-15/IL-15Rα heterodimers, we generated the IL-15/IL-15Rα(sushi) complex as a Fc fusion (hereon referred to as IL-15/Rα-Fc fusion proteins) with the goal of facilitating production and promoting FcRn-mediated recycling of the complex and prolonging half-life.

A. Example 1A: Engineering IL-15/Rα-Fc fusion proteins

[00376] Plasmids coding for IL-15 or IL-15Rα sushi domain were constructed by standard gene synthesis, followed by subcloning into a pTT5 expression vector containing Fc fusion partners (e.g., constant regions as depicted in Figures 8). Cartoon schematics of illustrative IL-15/Rα-Fc fusion protein formats are depicted in Figures 9A-G.

[00377] The IL-15Rα heterodimeric Fc fusion or "IL-15/Rα-heteroFc" format comprises IL-15 recombinantly fused to one side of a heterodimeric Fc and IL-15Rα sushi domain recombinantly fused to the other side of the heterodimeric Fc (Figure 9A). The IL-15 and IL-15Rα may have a variable length linker (see Figure 7) between their respective C-terminus and the N-terminus of the Fc region. Illustrative proteins of this format include XENP20818 and XENP21475, sequences for which are depicted in Figure 10 (see also Table 1). Sequences for additional proteins of this format are listed as XENPs 20819, 21471, 21472, 21473, 21474, 21476, and 21477 in the figures and in the sequence listing.

Table 1

XENP	IL-15-Fc Linker	IL-15Rα(sushi)-Fc Linker
20818	$(GGGGS)_1$	$(GGGGS)_1$
20819	$(GGGGS)_1$	$(GGGGS)_4$
21471	NONE	$(GGGGS)_1$
21472	$(GGGGS)_1$	NONE
21473	$(GGGGS)_1$	$(GGGGS)_3$
21474	NONE	$(GGGGS)_4$

21475	NONE	NONE
21476	$(GGGGS)_2$	$(GGGGS)_2$
21477	$(GGGGS)_2$	$(GGGGS)_4$

[00378] The single-chain IL-15/Rα-Fc fusion or "scIL-15/Rα-Fc" format comprises IL-15Rα sushi domain fused to IL-15 by a variable length linker (termed a "single-chain" IL-15/IL-15Rα complex or "scIL-15/Rα") which is then fused to the N-terminus of a heterodimeric Fc-region, with the other side of the molecule being a "Fc-only" or "empty-Fc" heterodimeric Fc (Figure 9B). Sequences for illustrative linkers are depicted in Figure 7. An illustrative protein of this format is XENP21478, sequences for which are depicted in Figure 11 (also see Table 2). Sequences for additional proteins of this format are listed as XENPs 21993, 21994, 21995, 23174, 23175, 24477, and 24480 in the figures and the sequence listing.

Table 2

XENP	**Linker between IL-15 and IL-15Rα**
21478	$(GGGGS)_6$
21993	$(GGGGS)_5$
21994	$(GGGGS)_4$
21995	$(GGGGS)_3$
23174	$(GKPGS)_6$
23175	$(GKPGS)_5$
24477	$(GGGGS)_7$
24480	30AA-linker

[00379] The non-covalent IL-15/Rα-Fc fusion or "ncIL-15/Rα-Fc" format comprises IL-15Rα sushi domain fused to a heterodimeric Fc region, while IL-15 is transfected separately so that a non-covalent IL-15/IL-15Rα complex is formed, with the other side of the molecule being a "Fc-only" or "empty-Fc" heterodimeric Fc (Figure 9C). Illustrative proteins of this format include XENP21479, XENP22366 and XENP24348, sequences for which are depicted in Figure 12.

[00380] The bivalent non-covalent IL-15/Rα-Fc fusion or "bivalent ncIL-15/Rα-Fc" format (Figure 9D) comprises IL-15Rα(sushi) fused to the N-terminus of a homodimeric Fc region, while IL-15 is transfected separately so that a non-covalent IL-15/Rα complex is formed. An illustrative protein of this format is XENP21978, sequences for which are depicted in Figure 13. Sequences for additional proteins of this format are listed as XENP21979 in the figures and in the sequence listing.

[00381] The bivalent single-chain IL-15/Rα-Fc fusion or "bivalent scIL-15/Rα-Fc" format (Figure 9E) comprises IL-15 fused to IL-15Rα(sushi) by a variable length linker (termed a "single-chain" IL-15/IL-15Rα(sushi) complex or "scIL-15/Rα") which is then fused to the N-terminus of a homodimeric Fc-region. Sequences for illustrative linkers are depicted in Figure 7. Sequences for an illustrative protein of this format are depicted in Figure 14.

[00382] The Fc-non-covalent IL-15/Rα fusion or "Fc-ncIL-15/Rα" format (Figure 9E) comprises IL-15Rα(sushi) fused to the C-terminus of a heterodimeric Fc region, while IL-15 is transfected separately so that a non-covalent IL-15/Rα complex is formed, with the other side of the molecule being "Fc-only" or "empty Fc". An illustrative protein of this format is XENP22637, sequences for which are depicted in Figure 15. Sequences for additional proteins of this format are listed XENP22638 in the figures and the sequence listing.

[00383] The Fc-single-chain IL-15/Rα fusion or "Fc-scIL-15/Rα" format (Figure 9G) comprises IL-15 fused to IL-15Rα(sushi) by a variable length linker ("scIL-15/Rα") which is then fused to the C-terminus of a heterodimeric Fc region, with the other side of the molecule being "Fc-only" or "empty Fc". Sequences for illustrative linkers are depicted in Figure 7. Sequences for an illustrative protein of this format are depicted in Figure 16.

[00384] Proteins were produced by transient transfection in HEK293E cells and were purified by a two-step purification process comprising protein A chromatography (GE Healthcare) and anion exchange chromatography (HiTrapQ 5 mL column with a 5-40% gradient of 50 mM Tris pH 8.5 and 50 mM Tris pH 8.5 with 1 M NaCl).

B. Example 1B: Engineering IL-15/Rα-Fc fusion proteins

[00385] IL-15/Rα-Fc fusion proteins produced in several of the formats as described above were characterized by size-exclusion chromatography (SEC) and capillary isoelectric focusing (CEF) for purity and homogeneity as generally described below.

[00386] The proteins were analyzed using SEC to measure their size (i.e. hydrodynamic volume) and determine the native-like behavior of the purified samples. The analysis was performed on an Agilent 1200 high-performance liquid chromatography (HPLC) system. Samples were injected onto a Superdex™ 200 10/300 GL column (GE Healthcare Life Sciences) at 1.0 mL/min using 1 x PBS, pH 7.4 as the mobile phase at 4oC for 25 minutes with UV detection wavelength at 280 nM. Analysis was performed using Agilent OpenLab Chromatography Data System (CDS) ChemStation Edition AIC version C.01.07. Chromatograms for selected IL-15/Rα-Fc fusion proteins are shown in Figures 17B, 18B, and 19B.

[00387] The proteins were analyzed electrophoretically via CEF using LabChip GXII Touch HT (PerkinElmer, Waltham, Mass.) using Protein Express Assay LabChip and Protein Express Assay Reagent Kit carried out using the manufacturer's instructions. Samples were run in duplicate, one under reducing (with dithiothreitol) and the other under non-reducing conditions. Gel images for selected IL-15/Rα-Fc fusion proteins are shown in Figures 17C, 18C, and 19C.

[00388] The symmetry of the peaks and the relatively low populations of other species for each of the fusion proteins indicate that the various formats were robust.

C. Example 1C: Characterization of IL-15/Rα-Fc fusion proteins for affinity and stability

[00389] Affinity screens of IL-15/Rα-Fc fusion proteins were performed using Octet, a BioLayer Interferometry (BLI)-based method. Experimental steps for Octet generally included the following: Immobilization (capture of ligand or test article onto a biosensor); Association (dipping of ligand- or test article-coated biosensors into wells containing serial dilutions of the corresponding test article or ligand); and Dissociation (returning of biosensors to well containing buffer) in order to determine the affinity of the test articles. A reference well containing buffer alone was also included in the method for background

correction during data processing. In particular, anti-human Fc (AHC) biosensors were used to capture the test articles and then dipped into multiple concentration of IL-2Rβ (R&D Systems, Minneapolis, Minn.) for KD determination. The affinity results and corresponding sensorgrams are depicted in Figures 17D, 18D, and 19D. Each of the three constructs showed high affinity binding (3-8 nM) for IL-1Rβ.

[00390] Stability of IL-15/Rα-Fc fusion proteins were evaluated using Differential Scanning Fluorimetry (DSF). DSF experiments were performed using a Bio-Rad CFX Connect Real-Time PCR Detection System. Proteins were mixed with SYPRO Orange fluorescent dye and diluted to 0.2 mg/mL in PBS. The final concentration of SYPRO Orange was 10X. After an initial 10 minute incubation period at 25°C, proteins were heated from 25 to 95oC using a heating rate of 1oC/min. A fluorescence measurement was taken every 30 sec. Melting temperatures (Tm) were calculated using the instrument software. The stability results and corresponding melting curves are depicted in Figures 17E, 18E, and 19E. Each of the constructs showed favorable overall stability with Tm ~68°C.

D. Example 1D: Activity of IL-15/Rα-Fc fusion proteins in cell proliferation assays

[00391] IL-15/Rα-Fc fusion proteins in the various formats as described above were tested in a cell proliferation assay. Human PBMCs were treated with the test articles at the indicated concentrations. 4 days after treatment, the PBMCs were stained with anti-CD8-FITC (RPA-T8), anti-CD4-PerCP/Cy5.5 (OKT4), anti-CD27-PE (M-T271), anti-CD56-BV421 (5.1H11), anti-CD16-BV421 (3G8), and anti-CD45RA-BV605 (Hi100) to gate for the following cell types: CD4+ T cells, CD8+ T cells, and NK cells (CD56+/CD16+). Ki67 is a protein strictly associated with cell proliferation, and staining for intracellular Ki67 was performed using anti-Ki67-APC (Ki-67) and Foxp3/Transcription Factor Staining Buffer Set (Thermo Fisher Scientific, Waltham, Mass.). The percentage of Ki67 on the above cell types was measured using FACS (depicted in Figures 20A-20C and 21A-21C).

[00392] The various IL-15/Rα-Fc fusion proteins induced strong proliferation of CD8+ T cells and NK cells. Notably, differences in proliferative activity were dependent on the linker

length on the IL-15-Fc side. In particular, constructs having no linker (hinge only), including XENP21471, XENP21474, and XENP21475, demonstrated weaker proliferative activity.

E. Example 1E: Activity of IL-15/Rα-Fc fusion proteins in an SEB-stimulated PBMC assay

[00393] As described above, IL-15/Rα heterodimers can potently activate T cells. IL-15/Rα-Fc fusion proteins in the various formats as described above were tested in an SEB-stimulated PBMC assay. Staphylococcal Enterotoxin B (SEB) is a superantigen that causes T cell activation and proliferation in a manner similar to that achieved by activation via the T cell receptor (TCR). Stimulating human PBMC with SEB is a common method for assaying T cell activation and proliferation.

[00394] Human PBMCs from multiple donors were stimulated with 10 ng/mL of SEB for 72 hours in combination with 20 μg/mL of various IL-15/Rα-Fc fusion proteins or controls (PBS, an isotype control, and a bivalent anti-PD-1 antibody). After treatment, supernatant was collected and assayed for IL-2, data for which is depicted in Figure 22. The data clearly show that the IL-15/Rα-Fc fusion proteins enhanced IL-2 secretion more than PBS and isotype control. Notably, a number of the IL-15/Rα-Fc fusion proteins have activity equivalent to or better than that of the anti-PD-1 antibody.

F. Example 1F: IL-15/Rα-Fc fusion proteins enhance engraftment and disease activity in human PBMC-engrafted NSG mice

[00395] IL-15/Rα-Fc fusion protein XENP20818 was evaluated in a Graft-versus-Host Disease (GVHD) model conducted in female NSG (NOD-SCID-gamma) immunodeficient mice. When the NSG mice were injected with human PBMCs, the human PBMCs developed an autoimmune response against mouse cells. Treatment of NSG mice injected with human PBMCs followed with IL-15/Rα-Fc fusion proteins enhances proliferation of the engrafted T cells.

[00396] 10 million human PBMCs were engrafted into NSG mice via IV-OSP on Day 0 followed by dosing of XENP20818 (1 mg/kg on Day 1 and then weekly thereafter) and recombinant IL-15 (Biolegend; 0.17 mg/kg on Day 1 and then weekly thereafter). The

survival curve is shown in Figure 23. The data show that mice receiving the IL-15/Rα-Fc fusion protein demonstrated rapid morbidity and mortality (all dead by Day 10) compared with mice receiving recombinant IL-15 (all alive by Day 14). This is likely due to the expected longer half-life of the IL-15/Rα-Fc fusion protein.

[00397] In another experiment, 10 million human PBMCs were engrafted in NSG mice via IV-OSP on Day 0 followed by dosing of XENP20818 (1 mg/kg, 0.3 mg/kg, 0.1 mg/kg, or 0.03 mg/kg on Day 1 and then weekly thereafter) or PBS. Control groups in which mice were not engrafted with PBMCs were included to investigate any effect of XENP20818 on wild-type NSG mice. Blood was collected on Day 7 to measure IFNγ, data for which is depicted in Figure 24, and to measure CD4+ T cell, CD8+ T cell, and CD45+ cell counts, data for which are depicted in Figure 25. The data shows a clear dose response for XENP20818.

XI. Example 2: IL-15/Rα-Fc Heterodimeric Fusion Proteins with Engineered Disulfide Bonds

[00398] To further improve stability and prolong the half-life of IL-15/Rα-Fc fusion proteins, we engineered disulfide bonds into the IL-15/Rα interface.

A. Example 2A: Engineering and characterization of IL-15/Rα heterodimers with engineered disulfide bonds

[00399] By examining the crystal structure of the IL-15/Rα complex, as well as by modeling using Molecular Operating Environment (MOE; Chemical Computing Group, Montreal, Quebec, Canada) software, we predicted residues at the IL-15/Rα interface that may be substituted with cysteine in order to form covalent disulfide bonds, as depicted in Figure 26.

[00400] Plasmids coding for IL-15 or IL-15Rα(sushi) were constructed by standard gene synthesis, followed by subcloning into a pTT5 expression vector. The IL-15Rα(sushi) chain included a C-terminal polyhistidine tag. Residues identified as described above were substituted with cysteines by standard mutagenesis techniques. Additionally, up to three amino acids following the sushi domain in IL-15Rα were added to the C-terminus of IL-15Rα(sushi) as a scaffold for engineering cysteines (illustrative sequences for which are

depicted in Figure 27). Sequences for illustrative IL-15 and IL-15Rα(sushi) variants engineered with cysteines are respectively depicted in Figure 28 and 29.

[00401] Cartoon schematics of IL-15/Rα heterodimers with and without engineered disulfides are depicted in Figures 30A-C. Sequences for an illustrative ncIL-15/Rα heterodimer XENP21996 is depicted in Figure 31. Sequences for illustrative dsIL-15/Rα heterodimers XENP22004, XENP22005, XENP22006, XENP22008, and XENP22494 are depicted in Figure 32. Sequences for an illustrative scIL-15/Rα heterodimer are depicted in Figure 33. "Wild-type" IL-15/Rα heterodimers, with additional residues at the C-terminus but without engineered cysteines, were generated as controls. Sequences for these control IL-15/Rα heterodimers are listed as XENPs 22001, 22002, and 22003 in the figures and the sequence listing. Proteins were produced by transient transfection in HEK293E cells and purified by Ni-NTA chromatography.

[00402] After the proteins were purified, they were characterized by capillary isoelectric focusing (CEF) for purity and homogeneity as generally described in Example 1B, gel images for which are depicted in Figures 34-35. The proteins were then screened for stability using DSF as generally described in Example 1C, data for which are depicted in Figures 36-38. Finally, the proteins were screened for binding to IL-2Rβ by Octet as generally described in Example 1C, data for which is depicted in Figure 38.

[00403] Many of the disulfide bonds were correctly formed as indicated by denaturing non-reducing CEF, where the larger molecular weight of the covalent complex can be seen when compared to the controls without engineered disulfide bonds (Figures 34-35). The disulfide bonded IL-15/Rα heterodimers had increased thermostability of up to +13°C (Figure 38). Binding to IL-2Rβ was not affected by the inclusion of engineered disulfide bonds (Figure 38). Favorite disulfide bonded pairs were XENP22005, XENP22006, XENP22008, and XENP22494 and were constructed as Fc fusion proteins as described below.

B. Example 2B: Characterization of IL-15/Rα-Fc fusion proteins with engineered disulfide bonds

[00404] Plasmids coding for IL-15 or IL-15Rα sushi domain with the above-described mutations were subcloned into a pTT5 expression vector containing Fc fusion partners (e.g.,

constant regions as depicted in Figures 8). Cartoon schematics of IL-15/Rα-Fc fusion proteins with engineered disulfide bonds are depicted in Figures 39A-D.

[00405] Disulfide-bonded IL-15/Rα heterodimeric Fc fusion or "dsIL-15/Rα-heteroFc" (Figure 39A) is the same as "IL-15/Rα-heteroFc", but wherein IL-15Rα(sushi) and IL-15 are further covalently linked as a result of engineered cysteines. Illustrative proteins of this format include XENP22013, XENP22014, XENP22015, and XENP22017, sequences for which are depicted in Figure 40.

[00406] Disulfide-bonded IL-15/Rα Fc fusion or "dsIL-15/Rα-Fc" (Figure 39B) is the same as "ncIL-15/Rα-Fc", but wherein IL-15Rα(sushi) and IL-15 are further covalently linked as a result of engineered cysteines. Illustrative proteins of this format include XENP22357, XENP22358, XENP22359, XENP22684, and XENP22361, sequences for which are depicted in Figure 41. Sequences for additional proteins of this format are listed as XENPs 22360, 22362, 22363, 22364, 22365, and 22366 in the figures and the sequence listing.

[00407] Bivalent disulfide-bonded IL-15/Rα-Fc or "bivalent dsIL-15/Rα-Fc" (Figure 39C) is the same as "bivalent ncIL-15/Rα-Fc", but wherein IL-15Rα(sushi) and IL-15 are further covalently linked as a result of engineered cysteines. Illustrative proteins of this format include XENP22634, XENP22635, and XENP22636, sequences for which are depicted in Figure 42. Sequences for additional proteins of this format are listed as XENP22687 in the figures and the sequence listing.

[00408] Fc-disulfide-bonded IL-15/Rα fusion or "Fc-dsIL-15/Rα" (Figure 39D) is the same as "Fc-ncIL-15/Rα", but wherein IL-15Rα(sushi) and IL-15 are further covalently linked as a result of engineered cysteines. Illustrative proteins of this format include XENP22639 and XENP22640, sequences for which are depicted in Figure 43.

[00409] "Wild-type" IL-15/Rα-Fc fusion proteins, with additional residues at the C-terminus but without engineered cysteines, were generated as controls. Sequences for these control IL-15/Rα-Fc fusion proteins are listed as XENPs 21988, 21989, 21990, 21991, 21992, 22354, 22355, and 22356 in the figures and the sequence listing.

[00410] Proteins were produced by transient transfection in HEK293E cells and were purified by a two-step purification process comprising protein A chromatography (GE

Healthcare) and anion exchange chromatography (HiTrapQ 5 mL column with a 5-40% gradient of 50 mM Tris pH 8.5 and 50 mM Tris pH 8.5 with 1 M NaCl).

[00411] After the proteins were purified, they were characterized by capillary isoelectric focusing (CEF) for purity and homogeneity as generally described in Example 1B. As above, many of the disulfide bonds were correctly formed as indicated by denaturing non-reducing CEF, where the larger molecular weight of the covalent complex can be seen when compared to the controls without engineered disulfide bonds (Figure 44).

[00412] The proteins were then tested in a cell proliferation assay. IL-15/Rα-Fc fusion proteins (with or without engineered disulfide bonds) or controls were incubated with PBMCs for 4 days. Following incubation, PBMCs were stained with anti-CD4-PerCP/Cy5.5 (RPA-T4), anti-CD8-FITC (RPA-T8), anti-CD45RA-BV510 (HI100), anti-CD16-BV421 (3G8), anti-CD56-BV421 (HCD56), anti-CD27-PE (O323), and anti-Ki67-APC (Ki-67) to mark various cell populations and analyzed by FACS as generally described in Example 1D. Proliferation of NK cells, CD4+ T cells, and CD8+ T cells as indicated by Ki67 expression are depicted in Figures 45A-C. Each of the IL-15/Rα-Fc fusion proteins and the IL-15 control induced strong proliferation of NK cells, CD8+ T cells, and CD4+ T cells.

XII. Example 3: IL-15/Rα-Fc fusion proteins engineered for lower potency and increased PK and half-life

[00413] In order to further improve PK and prolong half-life, we reasoned that decreasing the potency of IL-15 would decrease the antigen sink, and thus, increase the half-life.

A. Example 3A: Engineering and production of variant IL-15/Rα-Fc fusion proteins

[00414] By examining the crystal structure of the IL-15:IL-2Rß and IL-15:common gamma chain interfaces, as well as by modeling using MOE software, we predicted residues at these interfaces that may be substituted in order to reduce potency. Figure 46 depicts a structural model of the IL-15:receptor complexes showing locations of the predicted residues where we engineered isosteric substitutions (in order to reduce the risk of immunogenicity).

Sequences for illustrative IL-15 variants engineered for reduced potency are depicted in Figure 47.

[00415] Plasmids coding for IL-15 or IL-15Rα(sushi) were constructed by standard gene synthesis, followed by subcloning into a pTT5 expression vector containing Fc fusion partners (e.g., constant regions as depicted in Figures 8). Substitutions identified as described above were incorporated by standard mutagenesis techniques. Sequences for illustrative IL-15/Rα-Fc fusion proteins of the "IL-15/Rα-heteroFc" format engineered for reduced potency are depicted in Figure 48, with additional sequences listed as XENPs 22815, 22816, 22817, 22818, 22819, 22820, 22823, 22824, 22825, 22826, 22827, 22828, 22829, 22830, 22831, 22832, 22833, 22834, 23555, 23559, 23560, 24017, 24020, 24043, and 24048 in the figures and the sequence listing.

[00416] Sequences for illustrative IL-15/Rα-Fc fusion proteins of the "scIL-15/Rα-Fc" format engineered for lower potency are depicted in Figure 49, with additional sequences listed as XENPs 24013, 24014, and 24016 in the figures and the sequence listing. Sequences for illustrative IL-15/Rα-Fc fusion proteins of the "ncIL-15/Rα-Fc" format engineered for lower potency are depicted in Figure 50. Sequences for illustrative ncIL-15/Rα heterodimers engineered for lower potency are depicted in Figure 51, with additional sequences listed as XENPs 22791, 22792, 22793, 22794, 22795, 22796, 22803, 22804, 22805, 22806, 22807, 22808, 22809, 22810, 22811, 22812, 22813, and 22814 in the figures and the sequence listing. Sequences for an illustrative IL-15/Rα-Fc fusion protein of the "bivalent ncIL-15/Rα-Fc" format engineered for lower potency are depicted in Figure 52. Sequences for illustrative IL-15/Rα-Fc fusion proteins of the "dsIL-15/Rα-Fc" format engineered for lower potency are depicted in Figure 53.

[00417] Proteins were produced by transient transfection in HEK293E cells and were purified by a two-step purification process comprising protein A chromatography (GE Healthcare) and anion exchange chromatography (HiTrapQ 5 mL column with a 5-40% gradient of 50 mM Tris pH 8.5 and 50 mM Tris pH 8.5 with 1 M NaCl).

B. Example 3B: In vitro activity of variant IL-15/Rα-heteroFc and scIL-15/Rα-Fc fusion proteins engineered for decreased potency

[00418] The variant IL-15/Rα-Fc fusion proteins were tested in a number of cell proliferation assays.

[00419] In a first cell proliferation assay, IL-15/Rα-Fc fusion proteins (with or without engineered substitutions) or control were incubated with PBMCs for 4 days. Following incubation, PBMCs were stained with anti-CD4-Evolve605 (SK-3), anti-CD8-PerCP/Cy5.5 (RPA-T8), anti-CD45RA-APC/Cy7 (HI100), anti-CD16-eFluor450 (CB16), anti-CD56-eFluor450 (TULY56), anti-CD3-FITC (OKT3), and anti-Ki67-APC (Ki-67) to mark various cell populations and analyzed by FACS as generally described in Example 1D. Proliferation of NK cells, CD8+ T cells, and CD4+ T cells as indicated by Ki67 expression are depicted in Figures 54-55. Most of the IL-15/Rα-Fc fusion proteins induced proliferation of each cell population; however, activity varied depending on the particular engineered substitutions.

[00420] In a second cell proliferation assay, IL-15/Rα-Fc fusion proteins (with or without engineered substitutions) were incubated with PBMCs for 3 days. Following incubation, PBMCs were stained with anti-CD3-FITC (OKT3), anti-CD4-Evolve604 (SK-3), anti-CD8-PerCP/Cy5.5 (RPA-T8), anti-CD16-eFluor450 (CB16), anti-CD56-eFluor450 (TULY56), anti-CD27-PE (O323), anti-CD45RA-APC/Cy7 (HI100) and anti-Ki67-APC (20Raj1) antibodies to mark various cell populations. Figures 56-57 depict selection of various cell populations following incubation with XENP22821 by FACS. Lymphocytes were first gated on the basis of side scatter (SSC) and forward scatter (FSC) (Figure 56A). Lymphocytes were then gated based on CD3 expression (Figure 56B). Cells negative for CD3 expression were further gated based on CD16 expression to identify NK cells (CD16+) (Figure 56C). CD3+ T cells were further gated based on CD4 and CD8 expression to identify CD4+ T cells, CD8+ T cells, and γδ T cells (CD3+CD4-CD8-) (Figure 57A). The CD4+ and CD8+ T cells were gated for CD45RA expression as shown respectively in Figures 57B-C. Finally, the proliferation of the various cell populations were determined based on percentage Ki67 expression, and the data are shown in Figures 59A-D. NK and CD8+ T cells are more sensitive than CD4+ T cells to IL-15/Rα-Fc fusion proteins, and as above, proliferative activity varied depending on the particular engineered substitutions. Figure 59D shows the fold change in EC50 of various IL-15/Rα-Fc fusion proteins relative to control XENP20818. Figure 58A and B further depict the activation of lymphocytes following treatment with IL-15/Rα-Fc fusion proteins by gating

for the expression of CD69 and CD25 (T cell activation markers) before and after incubation of PBMCs with XENP22821.

[00421] In a third experiment, additional variant IL-15/Rα-Fc fusion proteins were incubated with human PBMCs for 3 days at 37°C. Following incubation, PBMCs were stained with anti-CD3-FITC (OKT3), anti-CD4-SB600 (SK-3), anti-CD8-PerCP/Cy5.5 (RPA-T8), anti-CD45RA-APC/Cy7 (HI100), anti-CD16-eFluor450 (CB16), anti-CD25-PE (M-A251), and anti-Ki67-APC (Ki-67) to mark various cell populations and analyzed by FACS as generally described in Example 1D. Proliferation of CD8+ (CD45RA-) T cells, CD4+ (CD45RA-) T cells, γδ T cells, and NK cells as indicated by Ki67 expression are depicted in Figures 60A-D.

[00422] In a fourth experiment, human PBMCs were incubated with the additional IL-15/Rα-Fc variants at the indicated concentrations for 3 days. Following incubation, PBMCs were stained with anti-CD3-FITC (OKT3), anti-CD4 (SB600), anti-CD8-PerCP/Cy5.5 (RPA-T8), anti-CD16-eFluor450 (CB16), anti-CD25-PE (M-A251), anti-CD45RA-APC/Cy7 (HI100), and anti-Ki67-APC (Ki67) and analyzed by FACS as generally described in Example 1D. Percentage of Ki67 on CD8+ T cells, CD4+ T cells and NK cells following treatment are depicted in Figure 61.

[00423] In a fifth experiment, variant IL-15/Rα-Fc fusion proteins were incubated with human PBMCs for 3 days at 37°C. Following incubation, cells were stained with anti-CD3-PE (OKT3), anti-CD4-FITC (RPA-T4), anti-CD8α-BV510 (SK1), anti-CD8ß-APC (2ST8.5H7), anti-CD16-BV421 (3G8), anti-CD25-PerCP/Cy5.5 (M-A251), anti-CD45RA-APC/Cy7 (HI100), anti-CD56-BV605 (NCAM16.2), and anti-Ki67-PE/Cy7 (Ki-67) and analyzed by FACS as generally described in Example 1D. Percentage of Ki67 on CD8+ T cells, CD4+ T cells, γδ T cells, and NK cells are depicted in Figures 62A-E.

[00424] In a sixth experiment, variant IL-15/Rα-Fc fusion proteins were incubated with human PBMCs for 3 days at 37°C. Following incubation, cells were stained with anti-CD3-PE (OKT3), anti-CD4-FITC (RPA-T4), anti-CD8α-BV510 (SK1), anti-CD8ß-APC (SIDI8BEE), anti-CD16-BV421 (3G8), anti-CD25-PerCP/Cy5.5 (M-A251), anti-CD45RA-APC/Cy7 (HI100), anti-CD56-BV605 (NCAM16.2), and anti-Ki67-PE/Cy7 (Ki-67) and analyzed by FACS as

generally described in Example 1D. Percentage of Ki67 on CD8+ T cells, CD4+ T cells, γδ T cells, and NK cells are depicted in Figures 63A-E.

C. Example 3C: In vitro activity of variant scIL-15/Rα-Fc fusion proteins engineered for decreased potency with different linker lengths between IL-15 and IL-15Rα

[00425] IL-15/Rα-Fc fusion proteins with some of the substitutions described above, further with different lengths linkers between IL-15 and IL-15Rα (as depicted in Table 3) were incubated with human PBMCs at the indicated concentrations for 3 days at 37°C. Following incubation, PBMCs were stained with anti-CD3-PE (OKT3), anti-CD4-FITC (RPA-T4), anti-CD8-APC (RPA-T8), anti-CD16-BV605 (3G8), anti-CD25-PerCP/Cy5.5 (M-A251), anti-CD45RA-APC/Fire750 (HI100) and anti-Ki67-PE/Cy7 (Ki-67) and analyzed by FACS as generally described in Example 1D. Percentage Ki67 on CD8+ T cells, CD4+ T cells, γδ T cells and NK (CD16+) cells are depicted in Figures 64A-D. The data show that the ncIL-15/Rα-Fc fusion protein XENP21479 is the most potent inducer of CD8+ T cell, CD4+ T cell, NK (CD16+) cell, and γδ T cell proliferation. Each of the scIL-15/Rα-Fc fusion proteins were less potent than XENP21479 in inducing proliferation, but differences were dependent on both the linker length, as well as the particular engineered substitutions.

Table 3

XENP	**Format**	**Linker between IL-15 and IL-15Rα**	**Mutation**
24013	scIL-15/Rα-Fc	$(GGGGS)_5$	D61N
21014	scIL-15/Rα-Fc	$(GGGGS)_5$	N65D
24015	scIL-15/Rα-Fc	$(GGGGS)_5$	Q108E
24475	scIL-15/Rα-Fc	$(GGGGS)_6$	Q108E
24476	scIL-15/Rα-Fc	$(GGGGS)_6$	N4D/N65D
24478	scIL-15/Rα-Fc	$(GGGGS)_7$	Q108E
24479	scIL-15/Rα-Fc	$(GGGGS)_7$	N4D/N65D
24481	scIL-15/Rα-Fc	30AA-linker	Q108E

D. Example 3D: In vitro activity of variant IL-15/Rα-Fc fusion proteins engineered for decreased potency in additional formats

[00426] Variant IL-15/Rα-Fc fusion proteins in different formats (as depicted in Table 4) were incubated with human PBMCs at the indicated concentrations for 3 days at 37°C. Following incubation, PBMCs were stained with anti-CD3-PE (OKT3), anti-CD4-FITC (RPA-T4), anti-CD8-APC (RPA-T8), anti-CD16-BV605 (3G8), anti-CD25-PerCP/Cy5.5 (M-A251), anti-CD45RA-APC/Fire750 (HI100) and anti-Ki67-PE/Cy7 (Ki-67) and analyzed by FACS as generally described in Example 1D. Percentage Ki67 on CD8+ T cells, CD4+ T cells, γδ T cells and NK (CD16+) cells are respectively depicted in Figures 65A-D. As above, the data show that the ncIL-15/Rα-Fc fusion protein XENP21479 is the most potent inducer of CD8+ T cell, CD4+ T cell, NK (CD16+) cell, and γδ T cell proliferation. Notably, introduction of Q108E substitution into the ncIL-15/Rα-Fc format (XENP24349) drastically reduces its proliferative activity in comparison to wildtype (XENP21479).

Table 4

XENP	Format	Mutation
24351	Bivalent IL-15/Rα-Fc	N4D/N65D
21479	ncIL-15/Rα-Fc	WT
23472	dsIL-15/Rα-Fc	N65D
23557	IL-15/Rα-heteroFc	N4D/N65D
24349	ncIL-15/Rα-Fc	Q108E

E. Example 3E: STAT5 phosphorylation by variant IL-15/Rα-Fc fusion proteins

[00427] Transpresentation of IL-15 and IL-15Rα drives phosphorylation of STAT5 and subsequent proliferation of NK and T cells (CD4+ and CD8+). Accordingly, CD8+ and CD4+ T cells were analyzed for STAT5 phosphorylation following 15 minutes incubation with the indicated IL-15/Rα-Fc test articles. PBMCs were stained with anti-CD4-BV421 (RPA-T4) and anti-CD8-A700 (SK1) for 30-45 minutes at room temperature. Cells were washed and incubated with pre-chilled (-20°C) 90% methanol for 20-60 minutes. After incubation with methanol, cells were washed again and stained with anti-CD45RA-BV510 (HI100), anti-CD27-BV605 (L128), anti-CD25-PE (M-A251), anti-pSTAT5-Alexa647 (pY687), and anti-FoxP3-Alexa488 (259D) to mark various cell populations and STAT5 phosphorylation. Figures 66A-D depict selection of various cell populations following incubation with XENP22821. Lymphocytes were first gated on the basis of SSC and FSC (Figure 66A). The

lymphocytes were then gated based on CD4 and CD8 expression to identify CD4+ and CD8+ T cells (Figure 66B). The CD4+ and CD8+ T cells were then further gated based on CD45RA and CD27 expression to identify further subpopulations depicted respectively in Figures 66C-D. Finally, the phosphorylation of STAT5 in the various cell populations was determined, and the data are shown in Figures 67A-C. STAT5 phosphorylation on T cells was induced in a dose dependent manner and also varied depending on the particular engineered substitutions. Figure 67C shows the fold change in EC50 for STAT5 phosphorylation of the variant IL-15/Rα-Fc fusion proteins relative to control.

F. Example 3F: PK of variant IL-15/Rα-Fc fusion proteins engineered for lower potency

[00428] In order to investigate if IL-15/Rα-Fc fusion proteins engineered for reduced potency had improved half-life and PK, we examined these variants in a PK study in C57BL/6 mice. Two cohorts of mice (5 mice per test article per cohort) were dosed with 0.1 mg/kg of the indicated test articles via IV-TV on Day 0. Serum was collected 60 minutes after dosing and then on Days 2, 4, and 7 for Cohort 1 and Days 1, 3, and 8 for Cohort 2. Serum levels of IL-15/Rα-Fc fusion proteins were determined using anti-IL-15 and anti-IL-15Rα antibodies in a sandwich ELISA. The results are depicted in Figure 68. Figure 69 depicts the correlation between potency and half-life of the test articles.

[00429] As predicted, variants with reduced potency demonstrated substantially longer half-life. Notably, half-life was improved up to almost 9 days (see XENP22821 and XENP22822), as compared to 0.5 days for the wild-type control XENP20818.

G. Example 3G: Variant IL-15/Rα-Fc fusion proteins enhance engraftment and disease activity in human PBMC-engrafted NSG mice

[00430] The variant IL-15/Rα-Fc fusion proteins were evaluated in a GVHD models conducted in female NSG immunodeficient mice as generally described in Example 1F.

[00431] In a first study, 10 million human PBMCs were engrafted into NSG mice via IV-OSP on Day 0 followed by dosing of IL-15/Rα-Fc fusion proteins at the indicated concentrations on Day 1. CD45+ proliferation correlates with decreased body weight (as

shown in Figure 70), and so CD45+ cells were measured on Days 4 and 8 as an indicator of disease activity in this study (Figure 71A-B). The data show that each of the IL-15/Rα-Fc fusion proteins enhance proliferation of CD45+ cells in human PBMC-engrafted NSG mice as compared to control (PBS).

[00432] In another study, 10 million human PBMCs were engrafted into NSG mice via IV-OSP on Day 0 followed by dosing with IL-15/Rα-Fc fusion proteins at the indicated concentrations on Day 1. IFNγ levels and human NK cell, CD45+ lymphocytes, CD8+ T cell and CD4+ T cell counts were measured at days 4, 7, and 11 (Figures 72-76). The data show that the variant IL-15/Rα-Fc fusion proteins enhance IFNγ secretion and proliferation of human NK cell and T cells in a dose dependent manner. Notably, the observed activity is correlated to the in vitro potency of each variant.

[00433] In yet another study, 10 million human PBMCs were engrafted into NSG mice via IV-OSP on Day -8 followed by dosing with the indicated test articles at the indicated concentrations on Day 0. IFNγ levels and human NK cell, CD45+ lymphocytes, CD8+ T cell and CD4+ T cell counts were measured at Days 4, 7, and 11. Figure 77 depicts IFNγ levels in mice serum on Days 4, 7, and 11. Figures 78A-C respectively depict CD8+ T cell counts on Days 4, 7, and 11. Figures 79A-C respectively depict CD4+ T cell counts on Days 4, 7, and 11. Figures 80A-C respectively depict CD45+ cell counts on Days 4, 7, and 11. Body weight of the mice were also measured on Days 4, 7, and 11 and depicted as percentage of initial body weight in Figure 81.

H. Example 3H: IL-15/Rα-Fc fusion proteins are active in cynomolgus monkeys

[00434] Cynomolgus monkeys were administered a single intravenous (i.v.) dose of XENP20818 (n=3), XENP22819 (n=1), XENP22821 (n=3), XENP22822 (n=3), XENP22834 (n=3), and XENP23343 (n=3). Lymphocyte counts (Figures 82, 84, 86, 88, 90, and 92) and proliferation (Figures 83, 85, 87, 89, 91, and 93) were assessed over time. The data show significant changes in CD56+ NK cells (Figure 86A), CD16+ NK cells (Figure 86B), γδ T cells (Figure 86C), CD8+ T cells (CD45RA+) (Figure 86D), CD8+ T cells (CD45RA-) (Figure 86E), and CD4+ T cells (Figure 86F) following treatment with XENP22821 peaking at Day 6 with

subsequent recovery and normalizing. Finally, the Figures show significant expression of Ki67 on CD56+ NK cells (Figure 87A), CD16+ NK cells (Figure 87B), CD8+ T cells (CD45RA+) (Figure 87C), CD8+ T cells (CD45RA-) (Figure 87D), and CD4+ T cells (Figure 87E) indicating proliferative activity following treatment with XENP22821. Similar proliferative activity was observed following treatment with XENP20818, XENP22819, XENP22822, and XENP23343, demonstrating that most of the IL-15/Rα-Fc fusion proteins of the invention are active in cynomolgus monkeys.

XIII. Example 4: IL-15/Rα-Fc fusion proteins engineered with Xtend Fc

[00435] IL-15/Rα-Fc variants engineered for decreased potency as described above were further engineered with Xtend Fc (hereon referred to as "IL-15/Rα-XtendFc" fusion proteins) to further increase half-life by subcloning plasmids coding for IL-15 and/or IL-15Rα(sushi) into a pTT5 expression vector containing Fc fusion partners with M428L/N434S substitutions (see Figure 8, Backbone 11). Sequences for illustrative IL-15/Rα-XtendFc are depicted in Figures 94-96 (see also Table 5).

Table 5

XENP	Format	Mutation
24306	IL-15/Rα-heteroFc	D30N/E64Q/N65D
24341	IL-15/Rα-heteroFc	N1D/N65D
24301	IL-15/Rα-heteroFc	N4D/N65D
24383	ncIL-15/Rα-Fc	Q108E
24346	Bivalent IL-15/Rα-Fc	Q108E

A. Example 4A: In vitro activity of additional IL-15/Rα-Fc variants

[00436] Human PBMCs were incubated with the IL-15/Rα-XtendFc variants at the indicated concentrations for 3 days. Following incubation, PBMCs were stained with anti-CD3-FITC (OKT3), anti-CD4-PE (RPA-T4), anti-CD8-eFluor450 (SK-1), anti-CD45RA-PE/Cy7 (HI100), anti-CD16-PerCP/Cy5.5 (3G8), anti-CD25-APC/Fire750 (M-A251), and anti-Ki67-APC (Ki-67) to mark various cell populations and analyzed by FACS as generally described in Example 1D. Proliferation of CD8+ T cells, CD4+ T cells and NK cells following treatment as indicated by Ki67 expression are depicted in Figure 97.

[00437] As the Xtend variants were selected for investigating activity in cynomolgus monkeys, their ability to proliferate cynomolgus T cells was investigated. Cyno PBMCs were incubated with selected test articles at the indicated concentrations for 3 days. Following incubation, PBMCs were stained with anti-CD3-FITC (SP34), anti-CD4-PE/Cy7 (OKT4), anti-CD8-APC (RPA-T8), anti-CD45RA-APC/Fire750 (HI100), anti-CD16-BV605 (3G8), anti-CD25-BV421 (M-A251), and anti-Ki67-PerCP/Cy5.5 (Ki-67) to mark various cell populations and analyzed by FACS as generally described in Example 1D. Proliferation of CD8+ T cells, CD4+ T cells and NK cells following treatment as indicated by Ki67 expression are depicted in Figure 98.

B. Example 4B: In vivo activity of IL-15/Rα-XtendFc variants in a GVHD model

[00438] 10 million human PBMCs were engrafted into NSG mice via IV-OSP on Day -7 followed by dosing with the indicated test articles (0.3 mg/kg) on Day 0. Whole blood was collected on Day 4 and 7, and mice were sacrificed on Days 5-8 or 11 for their spleens to measure CD4+ T cell, CD8+ T cell, and CD45+ cell counts using FACS. Figures 99A-C respectively depict CD4+ T cell counts on Days 4 and 7 in whole blood and Day 8 in spleen. Figures 100A-C, respectively depict CD8+ T cell counts on Days 4 and 7 in whole blood and Day 8 in spleen. Figures 101A-C respectively depict CD4+ T cell counts on Days 4 and 7 in whole blood and Day 8 in spleen. Body weight of the mice were also measured on Day -8, -2, 1, 5, 8 and 11 as depicted in Figures 102A-102F. Each point represents one female NSG mouse.

C. Example 4C: In vivo activity of variant IL-15/Rα-XtendFc fusion proteins in cynomolgus monkeys

[00439] Monkeys (n=3) were administered a single intravenous (i.v.) dose of indicated test articles (Day 1) and blood was collected daily. CD8+ T cell, CD4+ T cell and NK cell counts in blood were assessed over time as depicted respectively in Figures 103A-C. Each point is an average of 3 cynomolgus monkeys. The data show that each of the variants were active in proliferating immune cells indicating that the IL-15/Rα-Fc fusion proteins of the invention could be useful as therapeutics for cancer in humans.

[00440] The examples set forth above are provided to give those of ordinary skill in the art a complete disclosure and description of how to make and use the embodiments of the compositions, systems and methods of the invention, and are not intended to limit the scope of what the inventors regard as their invention. Modifications of the above-described modes for carrying out the invention that are obvious to persons of skill in the art are intended to be within the scope of the following claims. All patents and publications mentioned in the specification are indicative of the levels of skill of those skilled in the art to which the invention pertains. All references cited in this disclosure are incorporated by reference to the same extent as if each reference had been incorporated by reference in its entirety individually.

[00441] All headings and section designations are used for clarity and reference purposes only and are not to be considered limiting in any way. For example, those of skill in the art will appreciate the usefulness of combining various aspects from different headings and sections as appropriate according to the spirit and scope of the invention described herein.

[00442] All references cited herein are hereby incorporated by reference herein in their entireties and for all purposes to the same extent as if each individual publication or patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety for all purposes.

[00443] Many modifications and variations of this application can be made without departing from its spirit and scope, as will be apparent to those skilled in the art. The specific embodiments and examples described herein are offered by way of example only, and the application is to be limited only by the terms of the appended claims, along with the full scope of equivalents to which the claims are entitled.

WHAT IS CLAIMED IS:

1. A heterodimeric protein comprising:

a) a first 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 first domain linker;

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

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 IL15 protein and said second protein domain comprises an IL15Rα protein.

2. The 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 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 heterodimeric protein according to any one of claims 1 to 3, wherein said 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 said IL15Rα protein has a polypeptide sequence selected from the group consisting of SEQ ID NO:3 (full-length human IL15Rα) and SEQ ID NO:4 (sushi domain of human IL15Rα).

5. The heterodimeric protein according to any one of claims 1 to 4, wherein said IL15 protein has one or more amino acid substitutions selected from the group consisting of N1D, N4D, D8N, D30N, D61N, E64Q, N65D, and Q108E.

6. The heterodimeric protein according to any one of claims 1 to 5, wherein said IL15 protein and said IL15Rα 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.

7. The heterodimeric protein according to any one of claims 1 to 6, wherein said heterodimeric protein comprises:

i) said first fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP15902) and said second fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP15908),

ii) said first fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP15902) and said second fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP15909),

iii) said first fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP16479) and said second fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP15908),

iv) said first fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP15902) and said second fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP16481),

v) said first fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP15902) and said second fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP16483),

vi) said first fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP16479) and said second fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP15909),

vii) said first fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP16479) and said second fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP16481),

viii) said first fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP16480) and said second fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP16482),

ix) said first fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP16480) and said second fusion protein h having as a polypeptide sequence of SEQ ID NO:XX (XENP15909),

x) said first fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP17064) and said second fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP17038),

xi) said first fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP17064) and said second fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP17040), or

xii) said first fusion protein having a polypeptide sequence of SEQ ID NO:XX (17062) and said second fusion protein having a polypeptide sequence of SEQ ID NO:XX (17044),

xiii) said first fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP17686) and said second fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP15908),

xiv) said first fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP17687) and said second fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP15908),

xv) said first fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP17688) and said second fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP15908),

xvi) said first fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP17689) and said second fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP15908),

xvii) said first fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP17690) and said second fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP15908),

xviii) said first fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP17691) and said second fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP15908),

xix) said first fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP17692) and said second fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP15908),

xx) said first fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP17693) and said second fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP15908),

xxi) said first fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP17694) and said second fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP15908),

xxii) said first fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP17695) and said second fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP15908),

xxiii) said first fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP17696) and said second fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP15908),

xxiv) said first fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP17697) and said second fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP15908),

xxv) said first fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP17698) and said second fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP15908),

xxvi) said first fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP17699) and said second fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP15908),

xxvii) said first fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP17701) and said second fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP15908),

xxviii) said first fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP17691) and said second fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP15908),

xxix) said first fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP17702) and said second fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP15908),

xxx) said first fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP17703) and said second fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP15908),

xxxi) said first fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP17704) and said second fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP15908),

xxxii) said first fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP17705) and said second fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP15908),

xxxiii) said first fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP18295) and said second fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP17761),

xxxiv) said first fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP18783) and said second fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP15908),

xxxv) said first fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP18784) and said second fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP15908),

xxxvi) said first fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP18786) and said second fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP15908),

xxxvii) said first fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP18788) and said second fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP15908),

xxxviii) said first fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP19242) and said second fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP16481), or

xxxix) said first fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP19243) and said second fusion protein having a polypeptide sequence of SEQ ID NO:XX (XENP16481).

8. The heterodimeric protein according to any one of claims 1 to 7, wherein said first protein domain is covalently attached to the N-terminus of said first Fc domain directly and without using said first domain linker and/or said second protein domain is covalently attached to the N-terminus of said second Fc domain directly and without using said second domain linker.

9. The heterodimeric protein according to any one of claims 1 to 6, wherein said heterodimeric protein is 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, 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 10.

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 IL15Rα protein and said second protein domain comprises an IL15 protein.

17. The heterodimeric protein according to claim 16, 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.

18. The heterodimeric protein according to claim 16 or 17, 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.

19. The heterodimeric protein according to any one of claims 16 to 18, wherein said 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 said IL15Rα protein has a polypeptide sequence selected from the group consisting of SEQ ID NO:3 (full-length human IL15Rα) and SEQ ID NO:4 (sushi domain of human IL15Rα).

20. The heterodimeric protein according to any one of claims 16 to 19, wherein said IL15 protein and said IL15Rα 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.

21. The heterodimeric protein according to any one of claims 16 to 20, wherein said first fusion protein has a polypeptide sequence of SEQ ID NO:XX (16478) and said Fc domain has a polypeptide sequence of SEQ ID NO:XX (8924).

22. The heterodimeric protein according to any one of claims 16 to 21, wherein said heterodimeric protein is XENP21478.

23. A nucleic acid composition encoding the fusion protein of any one of claims 16 to 22.

24. A nucleic acid composition encoding the second Fc domain of any one of claims 16 to 22.

25. An expression vector comprising the nucleic acid composition of claim 23.

26. An expression vector comprising the nucleic acid composition of claim 24.

27. The expression vector of claim 26, further comprising the nucleic acid composition of claim 23.

28. A host cell comprising one or two expression vectors of any one of claims 25 to 27.

29. A 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;

b) a second Fc domain; and

c) a second protein domain noncovalently attached to said first protein 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 IL15Rα and said second protein domain comprises an IL15 protein.

30. The heterodimeric protein according to claim 29, 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.

31. The heterodimeric protein according to claim 29 or 30, 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.

32. The heterodimeric protein according to any one of claims 29 to 31, wherein said 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 said IL15Rα protein has a polypeptide sequence selected from the group consisting of SEQ ID NO:3 (full-length human IL15Rα) and SEQ ID NO:4 (sushi domain of human IL15Rα).

33. The heterodimeric protein according to any one of claims 29 to 32, wherein said IL15 protein and said IL15Rα 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.

34. The heterodimeric protein according to any one of claims 29 to 33, wherein said fusion protein has a polypeptide sequence selected from the group consisting of SEQ ID NO:XX (16481), SEQ ID NO:XX (17034), SEQ ID NO:XX (17038), SEQ ID NO:XX (17036), SEQ ID NO:XX (17039), SEQ ID NO:XX (17040), SEQ ID NO:XX (17044), SEQ ID NO:XX (17041), SEQ ID NO:XX (17043), SEQ ID NO:XX (17045), SEQ ID NO:XX (17042), SEQ ID NO:XX (15908), and SEQ ID NO:XX (17603).

35. The heterodimeric protein according to any one of claims 29 to 34, wherein said second Fc domain has a polypeptide sequence of SEQ ID NO:XX (8793) or SEQ ID NO:XX (8927).

36. The heterodimeric protein according to any one of claims 29 to 35, wherein said second protein domain has a polypeptide sequence selected from the group consisting of SEQ ID NO:XX (16484), SEQ ID NO:XX (17074), SEQ ID NO:XX (17071), SEQ ID NO:XX (17072), SEQ ID NO:XX (17075), SEQ ID NO:XX (17070), SEQ ID NO:XX (17073), and SEQ ID NO:XX (17083).

37. The heterodimeric protein according to any one of claims 29 to 36, wherein said heterodimer protein comprises

i) said fusion protein having a polypeptide sequence of SEQ ID NO:XX (16481), said second Fc domain having a polypeptide sequence of SEQ ID NO:XX (8793), and a second protein domain having a polypeptide sequence of SEQ ID NO:XX (16484);

ii) said fusion protein having a polypeptide sequence of SEQ ID NO:XX (17034), said second Fc domain having a polypeptide sequence of SEQ ID NO:XX (8793), and a second protein domain having a polypeptide sequence of SEQ ID NO:XX (16484);

iii) said fusion protein having a polypeptide sequence of SEQ ID NO:XX (17038), said second Fc domain having a polypeptide sequence of SEQ ID NO:XX (8793), and a second protein domain having a polypeptide sequence of SEQ ID NO:XX (16484);

iv) said fusion protein having a polypeptide sequence of SEQ ID NO:XX (17036), said second Fc domain having a polypeptide sequence of SEQ ID NO:XX (8793), and a second protein domain having a polypeptide sequence of SEQ ID NO:XX (16484);

v) said fusion protein having a polypeptide sequence of SEQ ID NO:XX (17038), said second Fc domain having a polypeptide sequence of SEQ ID NO:XX (8793), and a second protein domain having a polypeptide sequence of SEQ ID NO:XX (17074);

vi) said fusion protein having a polypeptide sequence of SEQ ID NO:XX (17039), said second Fc domain having a polypeptide sequence of SEQ ID NO:XX (8793), and a second protein domain having a polypeptide sequence of SEQ ID NO:XX (17074);

vii) said fusion protein having a polypeptide sequence of SEQ ID NO:XX (17040), said second Fc domain having a polypeptide sequence of SEQ ID NO:XX (8793), and a second protein domain having a polypeptide sequence of SEQ ID NO:XX (17074);

viii) said fusion protein having a polypeptide sequence of SEQ ID NO:XX (17044), said second Fc domain having a polypeptide sequence of SEQ ID NO:XX (8793), and a second protein domain having a polypeptide sequence of SEQ ID NO:XX (17071);

ix) said fusion protein having a polypeptide sequence of SEQ ID NO:XX (17044), said second Fc domain having a polypeptide sequence of SEQ ID NO:XX (8793), and a second protein domain having a polypeptide sequence of SEQ ID NO:XX (17072);

x) said fusion protein having a polypeptide sequence of SEQ ID NO:XX (17075), said second Fc domain having a polypeptide sequence of SEQ ID NO:XX (8793), and a second protein domain having a polypeptide sequence of SEQ ID NO:XX (17041);

xi) said fusion protein having a polypeptide sequence of SEQ ID NO:XX (17043), said second Fc domain having a polypeptide sequence of SEQ ID NO:XX (8793), and a second protein domain having a polypeptide sequence of SEQ ID NO:XX (17070);

xii) said fusion protein having a polypeptide sequence of SEQ ID NO:XX (17045), said second Fc domain having a polypeptide sequence of SEQ ID NO:XX (8793), and a second protein domain having a polypeptide sequence of SEQ ID NO:XX (17073);

xiii) said fusion protein having a polypeptide sequence of SEQ ID NO:XX (17042), said second Fc domain having a polypeptide sequence of SEQ ID NO:XX (8793), and a second protein domain having a polypeptide sequence of SEQ ID NO:XX (17083); or

xiv) said fusion protein having a polypeptide sequence of SEQ ID NO:XX (15908), said second Fc domain having a polypeptide sequence of SEQ ID NO:XX (8793), and a second protein domain having a polypeptide sequence of SEQ ID NO:XX (16484).

38. The heterodimeric protein according to any one of claims 29 to 37, wherein said heterodimer protein is selected from the group consisting of XENP21479, XENP22357, XENP22354, XENP22355, XENP22356, XENP22357, XENP22358, XENP22359, XENP22360, XENP22361, XENP22362, XENP22363, XENP22364, XENP22365, XENP22366, XENP22637, XENP24349, and XENP24383.

39. A nucleic acid composition encoding the fusion protein of any one of claims 29 to 38.

40. A nucleic acid composition encoding the second Fc domain of any one of claims 29 to 37.

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

42. An expression vector comprising the nucleic acid composition of claim 40.

43. The expression vector of claim 42, further comprising the nucleic acid composition of claim 39.

44. The expression vector of any one of claims 41 to 43, further comprising a nucleic acid composition encoding said second protein domain.

45. A host cell comprising one or more expression vectors of any one of claims 41 to 44.

46. A heterodimeric protein comprising:

a) a first 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;

b) a second fusion protein comprising a second heavy chain comprising a second protein domain and a first second heavy chain comprising a second Fc domain, wherein said second protein domain is covalently attached to the C-terminus of said second Fc domain using a domain linker;

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

d) a fourth protein domain noncovalently attached to said second protein domain of said second 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, according to EU numbering andwherein said first protein domain and said second protein domain comprise an IL15Rα protein, and wherein said third protein domain and said fourth protein domain comprises an IL15 protein.

47. The heterodimeric protein according to claim 46, 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.

48. The heterodimeric protein according to claim 46 or 47, 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.

49. The heterodimeric protein according to any one of claims 46 to 48, wherein said 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 said IL15Rα protein has a polypeptide sequence selected from the group consisting of SEQ ID NO:3 (full-length human IL15Rα) and SEQ ID NO:4 (sushi domain of human IL15Rα).

50. The heterodimeric protein according to any one of claims 46 to 49, wherein said IL15 protein and said IL15Rα 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.

51. The heterodimeric protein according to any one of claims 46 to 50, wherein said heterodimer protein comprises:

i) said first fusion protein has a polypeptide sequence of SEQ ID NO:XX (17023) said second fusion protein has a polypeptide sequence of SEQ ID NO:XX (17023), said third protein domain has a polypeptide sequence of SEQ ID NO:XX (16484), and said fourth protein domain has a polypeptide sequence of SEQ ID NO:XX (16484) or

ii) said first fusion protein has a polypeptide sequence of SEQ ID NO:XX (17581), said second fusion protein has a polypeptide sequence of SEQ ID NO:XX (17581), said third protein domain has a polypeptide sequence of SEQ ID NO:XX (17074), and said fourth protein domain has a polypeptide sequence of SEQ ID NO:XX (17074).

52. The heterodimeric protein according to any one of claims 46 to 51, wherein said heterodimeric protein is XENP21978, XENP22634, XENP24342, and XENP24306.

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 53.

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

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

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,

according to EU numbering and wherein said first protein domain comprises an IL15Rα protein and said second protein domain comprises an IL15 protein.

62. The heterodimeric protein according to claim 61, 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.

63. The heterodimeric protein according to claim 61 or 62, wherein said first 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.

64. The heterodimeric protein according to any one of claims 61 to 63, wherein said 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 said IL15Rα protein has a polypeptide sequence selected from the group consisting of SEQ ID NO:3 (full-length human IL15Rα) and SEQ ID NO:4 (sushi domain of human IL15Rα).

65. The heterodimeric protein according to any one of claims 61 to 64, wherein said IL15 protein and said IL15Rα 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.

66. The heterodimeric protein according to any one of claims 61 to 65, wherein heterodimeric protein comprises

i) said first fusion protein having a polypeptide sequence of SEQ ID NO: XX (17603), said second Fc domain having a polypeptide sequence of SEQ ID NO: XX (8927), and said second protein domain having a polypeptide sequence of SEQ ID NO: XX (16484); or

ii) said first fusion protein having a polypeptide sequence of SEQ ID NO: XX (17605), said second Fc domain having a polypeptide sequence of SEQ ID NO: XX (8927), and said second protein domain having a polypeptide sequence of SEQ ID NO: XX (17074).

67. The heterodimeric protein according to any one of claims 61 to 66, wherein said heterodimeric protein is XENP22637 or XENP22639.

68. A nucleic acid composition encoding the first fusion protein of any one of claims 61 to 67.

69. A nucleic acid composition encoding the second Fc domain of any one of claims 61 to 67.

70. An expression vector comprising the nucleic acid composition of claim 68.

71. An expression vector comprising the nucleic acid composition of claim 69.

72. The expression vector of claim 71, further comprising the nucleic acid composition of claim 68.

73. The expression vector of any one of claims 70 to 72, further comprising a nucleic acid composition encoding said second protein domain.

74. A host cell comprising one or more expression vectors of any one of claims 70 to 73.

75. 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.

76. 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.

77. 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.

78. A host cell comprising the nucleic acid composition of claim 76.

79. A host cell comprising the expression vector composition of claim 77.

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. A method of treating cancer in a patient in need thereof comprising administering a therapeutically effective amount of the heterodimeric protein of claim 75 to said patient.