



(51) International Patent Classification:

C07K 14/54 (2006.01) A61K 38/20 (2006.01)

(21) International Application Number:

PCT/US2024/036745

(22) International Filing Date:

03 July 2024 (03.07.2024)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

63/511,974 05 July 2023 (05.07.2023) US
63/567,149 19 March 2024 (19.03.2024) US

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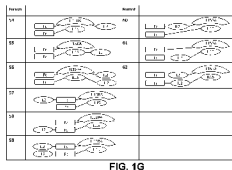
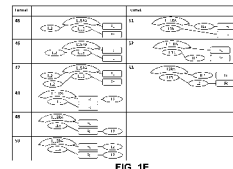
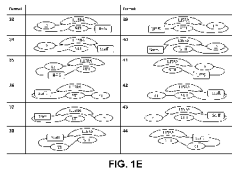
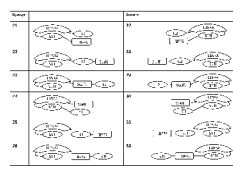
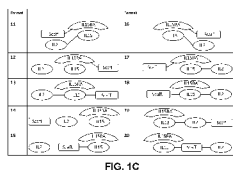
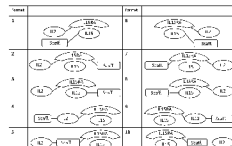
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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, MG, MK, MN, MU, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, CV,

(54) Title: CYTOKINE HYBRID PROTEINS AND METHODS RELATED THERETO



(57) Abstract: The present disclosure provides cytokine hybrid proteins such as IL2-IL15 cytokine hybrid proteins. The present disclosure further provides nucleic acids and vectors encoding such cytokine hybrid proteins and cells comprising such nucleic acids. Further provided are pharmaceutical compositions, therapeutic methods, and manufacturing methods relating to such cytokine hybrid proteins.

GH, GM, KE, LR, LS, MW, MZ, NA, RW, SC, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, ME, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

- *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
- *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

Published:

- *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*
- *with sequence listing part of description (Rule 5.2(a))*

CYTOKINE HYBRID PROTEINS AND METHODS RELATED THERETO**RELATED APPLICATIONS**

[001] The present application claims benefit of priority to US Provisional Application Nos.: 63/511,974, filed on July 5, 2024, and 63/567,149 filed March 19, 2024, the contents of each and all of which are incorporated by reference in its entirety.

SEQUENCE LISTING DISCLOSURE

[002] The contents of the electronic sequence listing (1143252_007013_SL.xml; Size: 86,838 bytes; and Date of Creation: June 24, 2024) is herein incorporated by reference in its entirety.

GOVERNMENT FUNDING

[003] This invention was made with government support under grant no. 1R21AI174202 awarded by NIAID. The government has certain rights in the invention.

BACKGROUND

[004] Interleukin-2 (IL2) was first identified as a T cell growth factor in 1976 (Malek. *Annu Rev Immunol.* 2008;26:453-479). Following its discovery, IL2 was tested for the treatment of various types of cancer, leading to its approval by the FDA for the treatment of metastatic renal cancer in 1992 and metastatic melanoma in 1998. IL2 was the first effective anti-cancer immunotherapy, resulting in durable complete responses in approximately 10% of patients (Rosenberg. *J Immunol.* 2014;192:5451-5458). However, due to its high toxicity and the need for frequent dosing, its use has been limited.

[005] IL2 exerts its biological effects by binding and signaling through IL2 receptors (IL2Rs), which may be composed of different combinations of IL2RA (also known as IL2R α or CD25), IL2RB (also known as IL2R β or common beta chain (β c), or CD122), and IL2RG (also known as common gamma chain (γ c) or CD132) (Smith. *Annu Rev Cell Biol.* 1989;5:397-425). IL2RA has a short intracellular tail and does not participate in the signaling process. Functional IL-2Rs may take a trimeric form IL2RA/B/G (also known as IL2R α / β / γ c) or a dimeric form IL2RB/G (also known as IL2R β / γ c). Compared to the affinity of IL2RA to IL2 (about 10 nM), the dimeric IL2RB/G has higher affinity (about 1 nM), and the trimeric IL2RA/B/G has even higher affinity (about 10 pM) (Ross SH, Cantrell DA. *Annu Rev Immunol.* 2018;36:411-433). Because the trimeric IL2RA/B/G is constitutively and predominantly expressed on regulatory T (Treg) cells, native IL2 in general preferentially expands and activates Treg cells over other T cell subsets (Malek. *Annu Rev Immunol.* 2008;26:453-479), which would limit efficacy for example in case of cancer therapy. Furthermore, the trimeric IL2RA/B/G is also expressed on endothelial cells and has been shown to contribute to the

development of vascular leakage syndrome, a prominent side effect of IL-2 (Krieg et al., *Proc Natl Acad Sci U S A*. 2010;107:11906-11911). Clinical use of IL2 is further challenged by its short half-life (about 7 minutes), which makes it difficult to circumvent toxicities (Lotze et al., *J Immunol*. 1985 Oct;135(4):2865-75; Yang et al., *J Clin Oncol*. 2003 Aug 15;21(16):3127-32.).

[006] The recent progress in the field of cancer immunotherapy has renewed interest in improving IL-2 therapy. Several approaches have been taken in an attempt to address the effects on Treg cells and/or toxicities. For example, complexing IL2 with an anti-IL2 antibody which blocks binding of IL2 to IL2RA and engineering IL2 to interrupt or reduce IL2RA binding have been proposed; nonetheless the efficacy was generally modest or in some cases even diminished compared to native IL2 (e.g., Lee et al., *Oncol Immunology*. 2019 Nov 4;9(1):1681869.; Sahin et al., *Nat Commun*. 2020;11:6440; Levin et al., *Nature*. 2012 Apr 26; 484(7395): 529–533.; Vazquez-Lombardi et al., *Nat Commun*. 2017;8:15373; Hashimoto et al., *Nature*. 2022 Oct;610(7930):173-181). Furthermore, while the combination of PD1 blockade and IL2 synergistically promotes expansion of antigen-specific CD8+ T cells, interestingly, IL2RA engagement appears indispensable in the synergy (Hashimoto et al., *Nature*. 2022 Oct;610(7930):173-181). In fact, the first IL2 variant modified to preferentially bind to the dimeric IL2RBG tested in the clinic, Bimpegaldesleukin, in combination with an anti-PD1 antibody, recently failed in phase III trials (Sznol and Rizvi. *J Immunother Cancer*. 2023 Jan;11(1):e006346).

[007] Therefore there is a need for the provision for an improved IL2-based therapy.

SUMMARY

[008] The present disclosure relates to IL2-IL15 cytokine hybrid proteins.

[009] One aspect of the present disclosure provides cytokine hybrid proteins.

[010] In some embodiments, a cytokine hybrid protein according to the present disclosure may comprise (a) an (interleukin 2) IL2 domain; and (b) an (interleukin 15) IL15 domain. In certain embodiments, the cytokine hybrid protein may not bind to human IL2 receptor subunit alpha (IL2RA) or displays reduced binding to human IL2RA (e.g., SEQ ID NO: 8, positions 1-219 of SEQ ID NO: 8, or positions 1-165 of SEQ ID NO: 8) relative to wildtype human IL2 (e.g., SEQ ID NO: 1).

[011] In certain embodiments, the amino acid sequence of the IL2 may comprise at least one amino acid substitution, insertion, or deletion (relative to SEQ ID NO: 1) which blocks or reduces binding to human IL2RA.

[012] In certain embodiments, the IL2 domain may comprise at least one amino acid modification, optionally a chemical modification or conjugation, which blocks or reduces binding to human IL2RA.

[013] In certain embodiments, the cytokine hybrid protein may further comprise a IL2RA domain which is bound to the IL2 domain. In some cases, the IL2 domain may comprise a wildtype human IL2 amino acid sequence, optionally SEQ ID NO: 1, or a variant thereof. In some cases, the IL2RA domain may comprise an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 98%, at least 99%, or at least 99.5% identity to SEQ ID NO: 8, optionally comprising the amino acid sequence of SEQ ID NO: 80 or 81.

[014] In certain embodiments, the cytokine hybrid protein may further comprise a IL15RA domain which is bound to the IL15 domain. In some cases, the IL15 domain may comprise a wildtype human IL15 amino acid sequence, optionally SEQ ID NO: 4, or a portion or variant thereof.

[015] In certain embodiments, the IL2 domain may comprise an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 98%, at least 99%, or at least 99.5% identity to SEQ ID NO: 1.

[016] In some cases, the IL2 domain may comprise an amino acid substitution, insertion, or deletion at one or more of amino acid positions 3, 35, 38, 41, 42, 43, 45, 61, 62, 65, 68, 69, 72, 80, 81, 85, 86, 92, and/or 125 relative to SEQ ID NO: 1.

[017] In some cases, the IL2 domain may comprise an amino acid substitution (relative to SEQ ID NO: 1) at: (i) positions 42 and 45; (ii) position 38; (iii) position 62; (iv) position 65; (v) position 72; (vi) positions 42 and 45 and one or more of positions 38, 62, 65, and/or 72; (vii) position 45; (viii) positions 43 and 45; (ix) positions 35, 38, 43, and 45; (x) positions 35, 38, and 72; (xi) positions 42, 45, and 72; (xii) positions 38, 42, 45, and 62; (xiii) positions 80, 81, 85, 86, and 92; or (xiv) positions 38, 43, and 61.

[018] In some cases, the IL2 domain may comprise one or more of the following amino acid substitutions or substitution sets (relative to SEQ ID NO: 1): (xv) F42A and Y45A; (xvi) R38A; (xvii) E62A; (xviii) P65A; (xix) L72A; (xx) R38A, F42A, and Y45A; (xxi) F42A, Y45A, and E62A; (xxii) F42A, Y45A, and P65A; (xxiii) F42A, Y45A, and L72A; (xxiv) R38A, F42A, Y45A, and E62A; (xxv) R38A, F42A, Y45A, and P65A; (xxvi) R38A, F42A, Y45A, and L72A; (xxvii) F42A, Y45A, E62A, and P65A; (xxviii) F42A, Y45A, E62A, and L72A; (xxix) F42A, Y45A, P65A, and L72A; (xxx) R38A, F42A, Y45A, E62A, and P65A; (xxxi) R38A, F42A, Y45A, E62A, and L72A; (xxxii) R38A, F42A, Y45A, P65A, and L72A; (xxxiii) F42A, Y45A, E62A, P65A, and L72A; (xxxiv) R38A, F42A, Y45A, E62A, P65A, and L72A; (xxxv) Y45S; (xxxvi) K43A and Y45A; (xxxvii) K35A, R38A, K43A, and Y45A; (xxxviii) K35A, R38A, and L72G; (xl) F42A, Y45A, and L72G; (xli) R38A, F42A, Y45A, and E62A; (xlii) L80F, R81D, L85V, I86V, and I92F; or (xliii) R38D, K43E, and E61R.

[019] In some cases, any of the IL2 domains described above, may further comprise a substitution at position 125, optionally C125S or C125A.

[020] In certain embodiments, the IL2 domain may comprise the amino acid sequence of SEQ ID NO: 10, optionally wherein: (i) X₅ and X₇ are A and A, respectively; (ii) X₃ is A; (iii) X₉ is A; (iv) X₁₀ is A; (v) X₁₃ is A; (vi) X₃, X₅, and X₇ are A, A, and A, respectively; (vii) X₅, X₇, and X₉ are A, A, and A, respectively; (viii) X₅, X₇, and X₁₀ are A, A, and A, respectively; (ix) X₅, X₇, and X₁₃ are A, A, and A, respectively; (x) X₃, X₅, X₇, and X₉ are A, A, A, and A, respectively; (xi) X₃, X₅, X₇, and X₁₀ are A, A, A, and A, respectively; (xii) X₃, X₅, X₇, and X₁₃ are A, A, A, and A, respectively; (xiii) X₅, X₇, X₉, and X₁₀ are A, A, A, and A, respectively; (xiv) X₅, X₇, X₉, and X₁₃ are A, A, A, and A, respectively; (xv) X₇, X₉, X₁₀, and X₁₃ are A, A, A, and A, respectively; (xvi) X₃, X₅, X₇, X₉, and X₁₀ are A, A, A, A, and A, respectively; (xvii) X₃, X₅, X₇, X₉, and X₁₃ are A, A, A, A, and A, respectively; (xviii) X₃, X₅, X₇, X₁₀, and X₁₃ are A, A, A, A, and A, respectively; (xix) X₅, X₇, X₉, X₁₀, and X₁₃ are A, A, A, A, and A, respectively; (xx) X₃, X₅, X₇, X₉, X₁₀, and X₁₃ are A, A, A, A, A, and A, respectively; (xxi) X₇ is S; (viii) X₆ and X₇ are A and A, respectively; (ix) X₂, X₃, X₆, and X₇ are A, A, A, and A, respectively; (x) X₂, X₃, and X₁₃ are A, A, and G, respectively; (xi) X₅, X₇, and X₁₃ are A, A, and G, respectively; (xii) X₃, X₅, X₇, and X₉ are A, A, A, and A, respectively; (xiii) X₁₄, X₁₅, X₁₆, X₁₇, and X₁₈ are F, D, V, V, and F, respectively; or (xiv) X₃, X₆, and X₈ are D, E, and R, respectively, optionally wherein X₁₃ is not C, further optionally wherein X₁₃ is S or A.

[021] In particular embodiments, the IL2 domain may comprise the amino acid sequence of SEQ ID NO: 11 or any one of SEQ ID NOS: 101-119.

[022] In some embodiments, the cytokine hybrid protein according to the present disclosure may comprise: (a) the IL2 domain according to any of those described above; (b) the IL15 domain; and (c) a/the IL15RA domain; and (d) optionally one or more scaffold domains.

[023] In certain embodiments, the IL15 domain may comprise an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 98%, at least 99%, or at least 99.5% identity to SEQ ID NO: 4. In some cases, the IL15 domain may comprise the amino acid sequence of SEQ ID NO: 40, further optionally wherein X₁, X₂, X₄, and/or X₆ is/are an amino acid which is not N and/or one or more of X₃, X₅, and/or X₇ is/are an amino acid which is not S or T. In particular cases, the IL15 domain may comprise the amino acid sequence of SEQ ID NO: 41.

[024] In certain embodiments, the IL15RA domain may comprise an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 98%, at least 99%, or at least 99.5% identity to SEQ ID NO: 2, positions 1-175 of SEQ ID NO: 2, or positions 1-66 of SEQ ID NO: 2. In particular cases, the IL15RA domain may comprise the amino acid sequence of SEQ ID NO: 21.

[025] In certain embodiments, each of the one or more scaffold domains may be or may comprise an albumin domain, optionally human albumin (HSA) domain. In some cases, the HSA domain may comprise an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 98%, at least 99%, or at least 99.5% identity to SEQ ID NO: 3. In particular cases, the HSA domain may comprise the amino acid sequence of SEQ ID NO: 31.

[026] In certain embodiments, each of the one or more scaffold domains may be or may comprise one or more immunoglobulin constant domains, optionally comprising a CH1 domain, a CH2 domain, and/or CH3 domain.

[027] In some cases, the immunoglobulin constant domain may comprise a CH1 domain of an IgG1, IgG2, IgG3, or IgG4 isotype. In some cases, the CH1 domain may comprise an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 98%, at least 99%, at least 99.5%, or 100% identity to SEQ ID NO: 61, 62, or 71.

[028] In some cases, the immunoglobulin constant domain may comprise a CH2 domain of an IgG1, IgG2, IgG3, or IgG4 isotype. In some cases, the CH2 domain may comprise an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 98%, at least 99%, at least 99.5%, or 100% identity to SEQ ID NO: 64 or 74.

[029] In some cases, the immunoglobulin constant domain may comprise a CH3 domain of an IgG1, IgG2, IgG3, or IgG4 isotype. In some cases, the CH3 domain may comprise an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 98%, at least 99%, at least 99.5%, or 100% identity to SEQ ID NO: 65, 66, 67, 68, or 75.

[030] In certain embodiments, each of the one or more scaffold domains may be or may comprise an immunoglobulin hinge. In some cases, the immunoglobulin hinge may be of an IgG1, IgG2, IgG3, or IgG4 isotype. In some cases, the immunoglobulin hinge may comprise an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 98%, at least 99%, at least 99.5%, or 100% identity to SEQ ID NO: 63 or 73.

[031] In certain embodiments, each of the one or more scaffold domains may be or may comprise an immunoglobulin fragment crystallizable (Fc) domain.

[032] In some cases, the Fc domain is of an IgG1, IgG2, IgG3, or IgG4 isotype. In some cases, the Fc domain may comprise the amino acid sequence of any of the immunoglobulin hinges described

herein or a partial sequence thereof, the amino acid sequence of any of the CH2 domains described herein, and the amino acid sequence of any of the CH3 domains described herein.

[033] In some cases, the Fc domain may comprise one or more amino acid substitutions which increases *in vivo* half-life and/or which reduces effector functions, optionally one or more of: a substitution at one or more of positions 234, 235, 236, 237, 265, 297 and/or 329; N297A; N297Q; L234A and L235A; L235E; L234F, L235E, and P331S; L234F, L235Q, and K322Q; A330S and P331S; L234A, L235A, P329G; L234A and G237A; L234A, L235A, and G237A; L234A, L235A, G237A, P238S, H268A, A330S, and P330S; L234A and L235E; G236R and L328R; L234A, L235A, and K322A; M252Y, S254T, and T256E; D265A and P329A; M428L and N434S; N434A; R435H.

[034] In some cases, the IL15 domain is bound to the IL15RA domain.

[035] In certain embodiments, the cytokine hybrid protein may further comprise one or more linkers between two domains, e.g., at least one set of two domains contained within a single polypeptide contained in the cytokine hybrid protein. In some cases, the at least one of the linker(s) may comprise one or more amino acids, optionally one, two, three, four, five, six, seven, eight, nine, ten, eleven, or twelve amino acids. In certain cases, the at least one of the linker(s) may consist of small amino acids consisting of G, S, and/or A. In certain cases, the at least one of the linker(s) may comprise an amino acid sequence which comprises or consists of the amino acid sequence selected from the group consisting of SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, G, GG, GGG, GS, SG, GGS, GSG, SGG, GSS, SGS, and SSG. In certain cases, the at least one of the linker(s) may comprise an amino acid sequence which comprises or consists of multiple repeats, optionally two, three, four, or five repeats, of the amino acid sequence selected from the group consisting of SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, G, GG, GGG, GS, SG, GGS, GSG, SGG, GSS, SGS, and SSG. In particular cases, the at least one of the linker(s) may comprise or consist of the amino acid sequence of SEQ ID NO: 58 or 59. When a cytokine hybrid protein comprises two or more linkers, all the linkers may have the same sequence or one or more of the linkers may have a different sequence.

[036] In particular embodiments, (a) the IL2 domain comprises the amino acid sequence of SEQ ID NO: 11; (b) the IL15 domain comprises the amino acid sequence of SEQ ID NO: 41; (c) the IL15RA domain comprises the amino acid sequence of SEQ ID NO: 21; and (d) the cytokine hybrid protein at least scaffold domain which comprises a HSA domain comprising the amino acid sequence of SEQ ID NO: 31.

[037] In some embodiments, a cytokine hybrid protein may comprise: (A) a first polypeptide comprising any of the IL15RA domains described herein; and (B) a second polypeptide comprising any of the IL15 domains described herein. In some cases, (i) the first polypeptide further comprises

any of the IL2 domains described herein; and/or (ii) the second polypeptide further comprises a IL2 domains described herein. In certain cases, (i) the first polypeptide further comprises the optional scaffold domain; and/or (ii) the second polypeptide further comprises the optional scaffold domain.

[038] In certain embodiments, the cytokine hybrid protein may comprise any of the formats depicted in **FIGS. 1B-1C and 1F-1G**. In particular embodiments, the cytokine hybrid protein may comprise Format 1.

[039] In some embodiments, a cytokine hybrid protein may comprise a polypeptide comprising any of the IL2 domains described herein, any of the IL15 domains described herein, and any of the IL15RA domains described herein and optionally the optional scaffold domain.

[040] In certain embodiments, the cytokine hybrid protein may comprise any of the formats depicted in **FIG. 1D-1E**.

[041] In certain embodiments, a cytokine hybrid protein may comprise: (A) a first polypeptide comprising an IL2 domain and an IL15RA domain, which comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 98%, at least 99%, or at least 99.5% identity to SEQ ID NO: 91; and (B) a second polypeptide comprising a HSA domain and an IL15 domain, which comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 98%, at least 99%, or at least 99.5% identity to SEQ ID NO: 92.

[042] In certain embodiments, a cytokine hybrid protein may comprise: (A) the first polypeptide comprising the amino acid sequence of SEQ ID NO: 91; and (B) the second polypeptide comprising the amino acid sequence of SEQ ID NO: 92.

[043] In particular embodiments, a cytokine hybrid protein may comprise: (a) an IL2 domain comprising the amino acid sequence of SEQ ID NO: 11; (b) an IL15 domain comprising the amino acid sequence of SEQ ID NO: 41; (c) an IL15RA domain comprising the amino acid sequence of SEQ ID NO: 21; and (d) a HSA domain (as a scaffold domain) comprising the amino acid sequence of SEQ ID NO: 31 in Format 1 as depicted in **FIG. 1B**.

[044] One aspect of the present disclosure provides nucleic acids and vectors encoding a cytokine hybrid protein.

[045] In some embodiments, a nucleic acid according to the present disclosure may encode any of the cytokine hybrid proteins described herein. In certain embodiments, the nucleic acid may comprise a first nucleic acid encoding any of the first polypeptides described herein and a second nucleic acid encoding any of the second polypeptides described herein.

[046] In some cases, the first nucleic acid may comprise a nucleic acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 98%, at least 99%, at least 99.5%, or 100% identity to SEQ ID NO: 93. In some cases, the second nucleic acid may comprise a nucleic acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 98%, at least 99%, at least 99.5%, or 100% identity to SEQ ID NO: 94.

[047] In some embodiments, a vector according to the present disclosure may encode any of the cytokine hybrid proteins described herein. In some embodiments, a vector according to the present disclosure may comprise any of the nucleic acids described herein.

[048] In certain embodiments, the vector may be or may comprise an expression vector. In certain embodiments, the vector may be or may comprise a plasmid, a viral vector (optionally adenoviral, lentiviral, or retroviral), a lipid-based vector, a self-replicating RNA vector, a virus-like particle, a polymer-based vector, and/or a nanoparticle, optionally a lipid-based nanoparticle.

[049] Another aspect of the present disclosure provides isolated, recombinant, and/or host cell comprising a nucleic acid according to the present disclosure and/or a vector according to the present disclosure.

[050] In some embodiments, a cell according to the present disclosure may be non-mammalian, optionally bacterial, yeast, fungal, protozoa, plant, or insect, bacterial. In some embodiments, a cell according to the present disclosure may be mammalian, optionally human, non-human primate, monkey, rabbit, rodent, hamster, rat, or mouse. In some embodiments, a cell according to the present disclosure may a population of cells comprising any of the isolated, recombinant, and/or host cell described herein.

[051] Another aspect of the present disclosure provides compositions.

[052] In some embodiments, a composition according to the present disclosure may comprise: (I) at least one of: (a) any of the cytokine hybrid proteins described herein; (b) any of the nucleic acids described herein; (c) any of the vectors described herein; and/or (d) any of the isolated, recombinant, and/or host cells as described herein or any of the populations of cells comprising such cells as described herein; and (II) a pharmaceutically acceptable carrier.

[053] A further aspect of the present disclosure provides methods of treating a disease, disorder, or condition in a subject.

[054] In some embodiments, a method according to the present disclosure may comprising administering to the subject an effective amount of at least one of: (a) any of the cytokine hybrid proteins described herein; (b) any of the nucleic acids described herein; (c) any of the vectors described herein; (d) any of the isolated, recombinant, and/or host cells as described herein or any

of the populations of cells comprising such cells as described herein; and/or (e) any of the compositions described herein.

[055] In certain embodiments, the subject may be a mammal, optionally a human, a non-human primate, a monkey, a horse, a cow, a sheep, a goat, a pig, a dog, a cat, a rabbit, a rodent, a hamster, a rat, or a mouse. In certain embodiments, the subject may be a non-mammalian vertebrate, optionally a bird, fish, an amphibian, or a reptile.

[056] In certain embodiments, the method may further comprise administering to the subject an additional agent, optionally an adjuvant or a therapeutic agent.

[057] In certain embodiments, the disease, disorder, or condition comprises cancer, an infectious disease, or another disease.

[058] In particular embodiments, the cancer is a solid cancer. In some cases, the cancer is optionally chosen from: one or more of mesothelioma, malignant pleural mesothelioma, non-small cell lung cancer, small cell lung cancer, squamous cell lung cancer, large cell lung cancer, pancreatic cancer, pancreatic ductal adenocarcinoma, esophageal adenocarcinoma, breast cancer, glioblastoma, ovarian cancer, colorectal cancer, prostate cancer, cervical cancer, skin cancer, melanoma, renal cancer, liver cancer, brain cancer, thymoma, sarcoma, carcinoma, uterine cancer, kidney cancer, gastrointestinal cancer, urothelial cancer, pharynx cancer, head and neck cancer, rectal cancer, esophagus cancer, or bladder cancer, or a metastasis thereof.

[059] In particular embodiments, the cancer is a liquid cancer. In some cases, the cancer is optionally chosen from: chronic lymphocytic leukemia (CLL), mantle cell lymphoma (MCL), multiple myeloma, acute lymphoid leukemia (ALL), Hodgkin lymphoma, B-cell acute lymphoid leukemia (BALL), T-cell acute lymphoid leukemia (TALL), small lymphocytic leukemia (SLL), B cell prolymphocytic leukemia, blastic plasmacytoid dendritic cell neoplasm, Burkitt's lymphoma, diffuse large B cell lymphoma (DLBCL), DLBCL associated with chronic inflammation, chronic myeloid leukemia, myeloproliferative neoplasms, follicular lymphoma, pediatric follicular lymphoma, hairy cell leukemia, small cell- or a large cell-follicular lymphoma, malignant lymphoproliferative conditions, MALT lymphoma (extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue), Marginal zone lymphoma, myelodysplasia, myelodysplastic syndrome, non-Hodgkin lymphoma, plasmablastic lymphoma, plasmacytoid dendritic cell neoplasm, Waldenstrom macroglobulinemia, splenic marginal zone lymphoma, splenic lymphoma/leukemia, splenic diffuse red pulp small B-cell lymphoma, hairy cell leukemia-variant, lymphoplasmacytic lymphoma, a heavy chain disease, plasma cell myeloma, solitary plasmacytoma of bone, extraosseous plasmacytoma, nodal marginal zone lymphoma, pediatric nodal marginal zone lymphoma, primary cutaneous follicle center lymphoma, lymphomatoid granulomatosis, primary mediastinal (thymic) large B-cell

lymphoma, intravascular large B-cell lymphoma, ALK+large B-cell lymphoma, large B-cell lymphoma arising in HHV8-associated multicentric Castleman disease, primary effusion lymphoma, B-cell lymphoma, acute myeloid leukemia (AML), or unclassifiable lymphoma.

[060] In particular embodiments, the infectious disease may be a viral, bacterial, fungal, yeast, protozoan, prion or parasitic disease.

[061] In some cases, the viral disease may be human immunodeficiency virus (HIV), hepatitis virus (optionally hepatitis A, B, or C virus), human papillomavirus (HPV), herpes simplex virus (HSV) (optionally HSV-1 or HSV-2), enterovirus, human cytomegalovirus, adenovirus, rhinovirus, Pox virus, Influenza virus, coronavirus (optionally MERS-CoV, SARS-CoV, or SARS-CoV-2, or common human coronavirus), norovirus, West Nile Virus, Zika virus, poliovirus, Ebola virus, or dengue virus (DENV) infection.

[062] In some cases, the bacterial disease may be *Salmonella*, *Escherichia coli*, *Mycobacterium tuberculosis*, *methicillin-resistant staphylococcus aureus (MRSA)*, *Clostridium difficile*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Helicobacter pylori*, *Neisseria gonorrhoeae*, *Vibrio vulnificus*.

[063] In some cases, the fungal disease may be *Aspergillosis*, *Candida*, *Candida auris*, *Cryptococcus neoformans*, *Pneumocystis jirovecii*, *Mucoromycetes*, *Talaromyces*, ringworm, *Blastomyces*, *Coccidioides*, *Cryptococcus gattii*, *Histoplasma*, *Paracoccidioides*, or *Sporothrix* infection.

[064] A further aspect of the present disclosure provides manufacturing methods, such as methods of manufacturing any of the cytokine hybrid proteins described herein or methods of manufacturing any of the isolated, recombinant, and/or host cell described herein or the population of cells comprising any of the isolated, recombinant, and/or host cell described herein.

[065] In some embodiments, a method of manufacturing a cytokine hybrid protein may comprise: (a) culturing cells comprising a nucleic acid encoding the cytokine hybrid protein (e.g., any of those described herein) in a condition that allows for expression of said cytokine hybrid protein, and (b) harvesting and purifying the cytokine hybrid protein from the cell culture from (a).

[066] In some embodiments, a method of manufacturing an isolated, recombinant, and/or host cell comprising a nucleic acid encoding a cytokine hybrid protein (e.g., any of those described herein) or a population of cells comprising any of such cells may comprise introducing a nucleic acid encoding the cytokine hybrid protein or the vector comprising such a nucleic acid into one or more cells. In some cases, the introducing occurs *in vitro*. In some cases, the introducing occurs *ex vivo*. In some cases, the introducing occurs *in vivo*.

[067] Any of the cytokine hybrid proteins described herein, any of the nucleic acids described herein, any of the vectors described herein, any of the isolated, recombinant, and/or host cells

described herein, any of the populations of cells described herein, and/or any of the compositions described herein may be for use in medicine and/or for use in treating a disease, disorder, or condition, which for example may be any of the diseases, disorders, or conditions described herein.

[068] Any of the cytokine hybrid proteins described herein, any of the nucleic acids described herein, any of the vectors described herein, any of the isolated, recombinant, and/or host cells described herein, any of the populations of cells described herein, and/or any of the compositions described herein may be used for the manufacture of a medicament, and in some cases a medicament for treatment of a disease, disorder, or condition, which for example may be any of the diseases, disorders, or conditions described herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[069] **FIG. 1A** provides an exemplary IL15-IL15RA complex structure. IL15 and IL15RA bind to each other very tightly at a K_D of about 10 pM. For designing a cytokine hybrid protein, in some embodiments, another cytokine (e.g., IL2) or another polypeptide (e.g., HSA) may be fused to either the N-terminus (shown as “A”) and/or the C-terminus (shown as “B”) of IL15RA and/or the N-terminus (shown as “C”) and/or the C-terminus (shown as “D”) of IL15.

[0001] **FIGS. 1B-1G** provide schematics of exemplary and non-limiting formats a IL2-IL15 cytokine hybrid protein (“IL2-IL15”) according to the present disclosure may take. In the schematics, the following rules applies unless otherwise indicated: **(1)** Each domain is presented as an oval, a rectangle, or a curved rectangle with a text therein showing the domain name (e.g., IL2 (oval), IL15 (oval), Scaffold shown as “Scaff.” (rectangle), IL15RA (curved rectangle), etc); **(2)** a set of multiple domains connected with each other with or without a linker (shown as a solid line) represents a polypeptide; **(3)** the direction of domains within a polypeptide is according to the direction of the text showing domain names, from the N-terminus to the C-terminus; **(4)** even when the FIGS explicitly show a linker, the linker may or may not be present; **(5)** a covalent or nonvalent bond (e.g., a disulfide bond, a hydrogen bond) may exist between polypeptides and/or within a polypeptide, even when such a bond is not explicitly shown; **(6)** IL15 (oval) and IL15RA (curved rectangle) domains are capable of binding to each other; **(7)** even when Scaffold (rectangle) is shown, Scaffold domain may be present or absent; **(8)** Scaffold domain may be or may comprise any appropriate sequence and size, e.g., those capable of increasing serum half-life (e.g., albumin such as HSA, one or more immunoglobulin constant domains, or Fc region (in some FIGS depicted as “Fc” domain)); and **(9)** individual domains may comprise a wildtype sequence or may comprise one or more amino acid substitutions relative to a wildtype sequence. For example, IL2-IL15 in Format 1 has (i) a first

polypeptide comprising an IL2 domain and an IL15RA domain in a direction from the N-terminus to the C-terminus and (ii) a second polypeptide comprising an optional scaffold domain and an IL15 domain in a direction from the N-terminus to the C-terminus.

[070] **FIG. 2** provides a schematic of IL2-IL2RA, comprising a circularly permuted IL2 (cpIL2) domain, an IL2RA domain, and a HSA domain, used as a benchmark in Examples. The rules described above for **FIGS. 1B-1C** apply, except that in **(1)** Each domain is presented as an oval, a rectangle, or a curved rectangle with a text therein showing the domain name (e.g., cpIL2 (oval), HSA (rectangle), and IL2RA (curved rectangle)) and in **(6)** cpIL2 (oval) and IL2RA (curved rectangle) domains are capable of binding to each other.

[071] **FIG. 3** provides exemplary SDS-PAGE results of IL2-IL15 and IL2-IL2RA proteins as produced and purified according to Example 1. Lane M: Protein Marker (top to bottom, 250, 150, 100, 75, 50, 37, 25, 20, 15 and 10 kDa); Lane 1: IL2-IL2RA; Lane 2: IL2-IL15.

[072] **FIG. 4** provides exemplary ELISA results showing binding of IL2-IL15 (triangle) or IL2-IL2RA (square) to different concentrations (x axis) of IL2RA (left) or IL2RB (right), as analyzed according to Example 2. The two headed arrow indicates the fold-difference between the binding EC50 values of IL2-IL15 and IL2-IL2RA.

[073] **FIG. 5** provides exemplary flow cytometry results showing STAT5 signaling levels (MFI values, Y axis) observed in Treg (left) and CD8+ Teff (right) cells when cultured with different concentrations (X axis) of IL2-IL15 (triangle) or IL2-IL2RA (square), as analyzed according to Example 3. The two headed arrows indicate the fold-difference between the EC50 values of IL2-IL15 and IL2-IL2RA.

[074] **FIG. 6** provides exemplary body weight changes in mice injected with IL2-IL15 (triangle) or IL2-IL2RA (square), as described in Example 4. The red arrows indicate days injections occurred.

[075] **Fig. 7A-D** contains an *in vivo* comparison of IL215 and IL2-2R α in normal mice. **Panel A** shows *in vivo* pharmacokinetics of 0.5 mg/kg IL215 after intraperitoneal (i.p.) injection. **Panel B** shows the toxicity after repeated dosing assessed by the change in body weight. **Panel C** compares wet lung weight, an indicator of pulmonary edema after repeated dosing. The arrows indicate cytokine administration. Statistical analysis was performed using Welch's ANOVA analysis. *: p<0.05; n.s.: not significant

[076] **Fig. 8A-E** assesses the pharmacodynamics of IL2-IL15 in naive C57BL/6 mice. **Panel A** shows the spleen weight, **Panel B** shows T and NK cell numbers, **Panel C** shows the CD8:Treg ratio, **Panel D** shows PD1 and TIM3 expression on CD8 T-cells, and **Panel E** shows Granzyme B and IFN- γ expression on CD8 T-cells in mice after IL2-IL15 administration. The differences in cell number were analyzed using one-way ANOVA followed by either Tukey's or Kruskal Wallis multiple comparison tests. *: p<0.05; **: p<0.01; ***: p<0.001.

[077] **FIG. 9A** provides exemplary changes in tumor sizes post tumor inoculation (left) and exemplary tumor weights on Day 18 (right) in mice untreated (circle) or injected with IL2-IL15 (triangle) or IL2-IL2RA (square), as described in Example 6. Statistical differences were analyzed using one-way ANOVA and Tukey's multiple comparison test.

[078] **FIG. 9B** provides exemplary numbers of CD3⁺, CD8⁺, CD4⁺, and Treg cells per mg of tumor and CD8:Treg ratios in the tumors harvested from mice untreated (circle) or injected with IL2-IL15 (triangle) or IL2-IL2RA (square), as described in Example 6.

[079] **Fig. 10A-G** further shows the anticancer effect of IL2-IL15 or IL2-IL2RA in the syngeneic B16F10 melanoma model. **Panel A** contains a schematic depicting protein treatment in tumor-bearing mice. **Panels B & C respectively** show tumor volume (**B**) and tumor weight (**C**). **Panels D-G** contain the results of the tumor-infiltrating lymphocytes (TIL) analysis. The absolute T-cell counts per mg of tumor are in **Panel D**, the percentage of different populations of T-cells in **Panel E**, the CD8 and Treg ratios in **Panel F**, and the state of CD8 T-cells in **Panel G**. Differences between curves and bars were analyzed using one-way ANOVA followed by Tukey's multiple comparison test.

DETAILED DESCRIPTION

Definitions

[080] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs.

[081] Although various embodiments and examples of the present invention have been described referring to certain molecules, compositions, methods, or protocols, it is to be understood that the present invention is not limited to the particular molecules, compositions, methods, or protocols described herein, as these may vary. It is also to be understood that the terminology used in the description is for the purpose of describing the particular versions or embodiments only and is not intended to limit the scope of the present invention which will be limited only by the appended claims.

[082] It should be understood that, unless clearly indicated otherwise, in any methods disclosed or claimed herein that comprise more than one step, the order of the steps to be performed is not restricted by the order of the steps cited.

[083] Throughout this disclosure, numerical features are presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of any embodiments. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges

as well as individual numerical values within that range to the tenth of the unit of the lower limit unless the context clearly dictates otherwise. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual values within that range, for example, 1.1, 2, 2.3, 5, and 5.9. This applies regardless of the breadth of the range. The upper and lower limits of these intervening ranges may independently be included in the smaller ranges, and are also encompassed within the disclosure, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the disclosure, unless the context clearly dictates otherwise.

[084] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

[085] It must also be noted that, unless the context clearly dictates otherwise, the singular forms “a,” “an,” and “the” as used herein and in the appended claims include plural reference. Thus, the reference to “a cell” refers to one or more cells and equivalents thereof known to those skilled in the art, and so forth. Similarly, the reference to “a nucleic acid” refers to one or more nucleic acid molecules and equivalents thereof known to those skilled in the art, and so forth. Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by a person of skilled in the art.

[086] As used herein, the term “about” or “approximately” when used in reference to a particular recited numerical value, means that the value may vary from the recited value by no more than 10%. For example, as used herein, the expression “about 100” includes 90 and 110 and all values in between (e.g., 91, 92, 93, 99, 99.1, 99.2, 99.3, 99.4, 100, 100.8, 100.9, 101, 106, 107, 108, 109, etc.).

[087] It is understood that aspects and embodiments of the disclosure described herein include “comprising,” “consisting,” and “consisting essentially of” aspects and embodiments. Transitional phrases such as “comprising,” “including,” “having,” “containing,” “involving,” “composed of,” and the like are to be understood to be open-ended, namely, to mean including but not limited to. Only the transitional phrases “consisting of” and “consisting essentially of” shall be closed or semi-closed transitional phrases, respectively.

[088] It will be further understood that all transitional terms such as “comprises,” “comprising,” “including,” “having,” “containing,” “involving,” “composed of,” and the like, when used in this specification, are to be understood to be open-ended, namely, to specify the presence of stated features, integers, steps, operations, elements, and/or components, but not to preclude the

presence or addition of one or more other features, integers, steps, operations, elements, components, and/or groups thereof. Only the transitional phrases “consists of” or “consisting of” shall be closed transitional phrases. The semi-closed transitional phrase “consists essentially of” or “consisting essentially of” shall be understood to specify the presence of stated features, integers, steps, operations, elements, and/or components and to allow the presence or addition of one or more other features, integers, steps, operations, elements, components, and/or groups thereof as long as they do not materially affect the basic characteristics of stated features, integers, steps, operations, elements, and/or components. For example, in some embodiments, “cells consisting essentially of T cells” may encompass a population of cells about 95% or more, about 96% or more, about 97% or more, about 98% or more, about 99% or more of which are T cells.

[089] As used herein, the term “and/or” includes any and all combinations of one or more of the associated listed items.

[090] A standard form of “antibody” or “immunoglobulin (Ig)” molecules may comprise two heavy chains and two immunoglobulin light chains or multiple units each comprising two pairs of heavy and light chains interconnected by disulfide bonds. Antibodies may be of one of the five major classes, IgA, IgD, IgE, IgG, and IgM and may be further classified based on the subclasses (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called α , δ , ϵ , γ , and μ , respectively. Each heavy chain is comprised of: a heavy chain variable domain (VH); and a heavy chain constant region (CH), which is typically comprised of a CH1 domain, a hinge, a CH2 domain and a CH3 domain.

[091] The numbering of amino acid residues in the antibody constant region may be performed by the EU-index or EU numbering system, as described in Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991). The EU numbering system is used in the present specification unless otherwise specified.

[092] According to IMGT (the international ImMunoGeneTics information system for immunoglobulins or antibodies, T cell receptors, MH, immunoglobulin superfamily IgSF and MhSF), the CH1 domain is the amino acid positions (or simply referred to as “positions” herein) 118-215 (EU numbering) and the hinge region is the amino acid positions 216-230 (EU numbering). The term “CH1 domain” is used in a broad sense herein to encompass any naturally occurring, corresponding heavy chain constant domain and/or region allotypes and variants thereof, which may comprise fewer or more amino acids and/or amino acid modification(s) and to refer to a heavy chain region comprising at least 80% of the heavy chain positions 118-215 (EU numbering)) and in some instances also comprising a portion of the hinge region (a portion of heavy chain positions 216-230 (EU numbering)) is included (e.g., up to position 218 or 220). Human IgG1 CH1 domain reference

sequences are provided herein as SEQ ID NOS: 61 and 62. A human IgG4 CH1 domain reference sequence is provided herein as SEQ ID NO: 71. These CH1 domain reference sequences are intended to be exemplary as Applicant intends for “CH1 domain” sequences to include any naturally occurring CH1 domain allotype or allelic variant.

[093] According to IMGT, the hinge is the amino acid positions 216-230 (EU numbering). The term “hinge” is used in a broad sense herein to refer to a heavy chain region comprising at least at least 60% of the hinge positions 216-230 (EU numbering). Human IgG1 and IgG4 hinge reference sequences, corresponding to the amino acid positions 231-340 according to EU numbering, are provided herein as SEQ ID NOS: 63 and 73, respectively, which are exemplary amino acid sequence of a wildtype (WT) hinge. These hinge reference sequences are intended to be exemplary as Applicant intends for “hinge” sequences to include any naturally occurring hinge allotype or allelic variant.

[094] According to IMGT, the CH2 domain is the amino acid positions (or simply referred to as “positions” herein) 231-340 (EU numbering). The term “CH2 domain” is used in a broad sense herein to refer to a heavy chain region comprising at least at least 80% of the heavy chain positions 231-340 (EU numbering)). Human IgG1 and IgG4 CH2 domain reference sequences, corresponding to the amino acid positions 231-340 according to EU numbering, are provided herein as SEQ ID NOS: 64 and 74, respectively, which are exemplary amino acid sequences of a wild-type (WT) CH2 domain. These CH2 domain reference sequences are intended to be exemplary as Applicant intends for “CH2 domain” sequences to include any naturally occurring CH2 domain allotype or allelic variant.

[095] According to IMGT, the CH3 domain is the amino acid positions (or simply referred to as “positions” herein) 341-446 (EU numbering). The term “CH3 domain” is used in a broad sense herein to refer to a heavy chain region comprising at least seven consecutive amino acid positions of the heavy chain positions 341-446 (EU numbering)). Human IgG1 CH3 domain reference sequences are provided herein as SEQ ID NOS: 65-68. A human IgG4 CH1 domain reference sequence is provided herein as SEQ ID NO: 75. These CH3 domain reference sequences are intended to be exemplary as Applicant intends for “CH3 domain” sequences to include any naturally occurring CH3 domain allotype or allelic variant. In some embodiments, a CH3 domain may further comprise lysine at the C terminus (i.e., K447, according to EU numbering).

[096] A “Fc region” is a C-terminal region of an immunoglobulin heavy chain that contains at least a portion of the constant region, including native sequence Fc regions and variant Fc regions. A human IgG heavy chain Fc region can extend from Asp221, Thr223, or Thr225 (in case of IgG1) or Tyr (one residue after K218, in case of IgG4), to the carboxyl-terminus of the heavy chain. However, the C-terminal lysine (Lys447) of the Fc region may or may not be present. Unless otherwise specified

herein, numbering of amino acid residues in the Fc region or constant region is according to the EU numbering system, as described in Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md., 1991.

[097] The phrase “effector function” of an antibody refers to biological activities attributable to the Fc region of an antibody, which varies by antibody class or isotype. Exemplary effector functions include: complement (e.g., C1q) binding and complement dependent cytotoxicity (CDC); Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (e.g., B cell receptor); and B cell activation.

[098] “Cancer” refers to a physiological condition in mammals characterized by unregulated cell growth. Examples of cancer include, but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia or lymphoid malignancies; with more particular examples including squamous cell cancer (e.g., epithelial squamous cell cancer), lung cancer including small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung and squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer including gastrointestinal cancer and gastrointestinal stromal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, cancer of the urinary tract, hepatoma, breast cancer, colon cancer, rectal cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, anal carcinoma, penile carcinoma, melanoma, superficial spreading melanoma, lentigo malignant melanoma, acral lentiginous melanomas, nodular melanomas, multiple myeloma and B-cell lymphoma (including low grade/follicular non-Hodgkin's lymphoma (NHL); small lymphocytic (SL) NHL; intermediate grade/follicular NHL; intermediate grade diffuse NHL; high grade immunoblastic NHL; high grade lymphoblastic NHL; high grade small non-cleaved cell NHL; bulky disease NHL; mantle cell lymphoma; AIDS-related lymphoma; and Waldenström's Macroglobulinemia); chronic lymphocytic leukemia (CLL); acute lymphoblastic leukemia (ALL); hairy cell leukemia; chronic myeloblastic leukemia; and post-transplant lymphoproliferative disorder (PTLD), as well as abnormal vascular proliferation associated with phacomatoses, edema (such as that associated with brain tumors), Meigs' syndrome, brain, as well as head and neck cancer, and associated metastases. In certain embodiments, cancers that are amenable to treatment by cytokine hybrid proteins of the disclosure include breast cancer, colorectal cancer, rectal cancer, non-small cell lung cancer, glioblastoma, non-Hodgkin's lymphoma (NHL), renal cell cancer, prostate cancer, liver cancer, pancreatic cancer, soft-tissue sarcoma, Kaposi's sarcoma, carcinoid carcinoma, head and neck cancer, ovarian cancer, mesothelioma, and multiple myeloma. In some embodiments, the cancer is selected from: small cell lung cancer, glioblastoma, neuroblastomas, melanoma, breast carcinoma,

gastric cancer, colorectal cancer (CRC), and hepatocellular carcinoma. Yet, in some embodiments, the cancer is selected from: non-small cell lung cancer, colorectal cancer, glioblastoma and breast carcinoma, including metastatic forms of those cancers. In other embodiments, the cancer is selected from a class of mature B-Cell cancers excluding Hodgkin's Lymphoma but including germinal-center B-cell-like (GCB) DLBCL, activated B-cell-like (ABC) DLBCL, follicular lymphoma (FL), mantle cell lymphoma (MCL), acute myeloid leukemia (AML), chronic lymphoid leukemia (CLL), marginal zone lymphoma (MZL), small lymphocytic leukemia (SLL), lymphoplasmacytic lymphoma (LL), Waldenstrom macroglobulinemia (WM), central nervous system lymphoma (CNSL), Burkitt's lymphoma (BL), B-cell prolymphocytic leukemia, Splenic marginal zone lymphoma, Hairy cell leukemia, Splenic lymphoma/leukemia, unclassifiable, Splenic diffuse red pulp small B-cell lymphoma, Hairy cell leukemia variant, Waldenström macroglobulinemia, Heavy chain diseases, a Heavy chain disease, γ Heavy chain disease, μ Heavy chain disease, Plasma cell myeloma, Solitary plasmacytoma of bone, Extravascular plasmacytoma, Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma), Nodal marginal zone lymphoma, Pediatric nodal marginal zone lymphoma, Pediatric follicular lymphoma, Primary cutaneous follicle centre lymphoma, T-cell/histiocyte rich large B-cell lymphoma, Primary DLBCL of the CNS, Primary cutaneous DLBCL, leg type, EBV-positive DLBCL of the elderly, DLBCL associated with chronic inflammation, Lymphomatoid granulomatosis, Primary mediastinal (thymic) large B-cell lymphoma, Intravascular large B-cell lymphoma, ALK-positive large B-cell lymphoma, Plasmablastic lymphoma, Large B-cell lymphoma arising in HHV8-associated multicentric Castleman disease, Primary effusion lymphoma: B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma, and B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and classical Hodgkin lymphoma.

[099] A "conservative amino acid substitution" is one in which an amino acid residue is substituted by another amino acid residue having a side chain (R group) with similar chemical properties (e.g., charge or hydrophobicity). In general, a conservative amino acid substitution will not substantially change the functional properties of a protein. In cases where two or more amino acid sequences differ from each other by conservative substitutions, the percent or degree of similarity may be adjusted upwards to correct for the conservative nature of the substitution. Means for making this adjustment are well known to those of skill in the art. (See, e.g., Pearson (1994) *Methods Mol. Biol.* 24: 307- 331). Examples of groups of amino acids that have side chains with similar chemical properties include 1) aliphatic side chains: glycine, alanine, valine, leucine and isoleucine; 2) aliphatic- hydroxyl side chains: serine and threonine; 3) amide-containing side chains: asparagine and glutamine; 4) aromatic side chains: phenylalanine, tyrosine, and tryptophan; 5) basic side chains:

lysine, arginine, and histidine; 6) acidic side chains: aspartate and glutamate, and 7) sulfur-containing side chains: cysteine and methionine. In some embodiments, conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, glutamate-aspartate, and asparagine-glutamine. Alternatively, in some embodiments, a conservative replacement comprises any change having a positive value in the PAM250 log-likelihood matrix disclosed in Gonnet et al. (1992) *Science* 256: 1443-45. In some embodiments, a "moderately conservative" replacement comprises any change having a nonnegative value in a PAM250 log-likelihood matrix.

[100] The term "cytokines" as used herein refers to a broad category of small proteins that are involved in cell signaling. Generally, their release has some effect on the behavior of cells around them. Cytokines may be involved in autocrine signaling, paracrine signaling, and/or endocrine signaling as immunomodulating agents. Cytokines may include chemokines, interferons, interleukins, lymphokines, and tumor necrosis factors. Cytokines are produced by a broad range of cells, including but not limited to immune cells like macrophages, B lymphocytes, T lymphocytes and mast cells, as well as endothelial cells, fibroblasts, epithelial cells, and various stromal cells. "Chemokines" are a family of cytokines generally involved in mediating chemotaxis.

[101] The phrase "cytokine hybrid protein" as used herein refers to any protein which contains at least one domain derived from one cytokine and another domain derived from another cytokine. For example, a protein comprising an IL2 domain (a domain derived from IL2) and an IL15 domain (a domain derived from IL15) may be referred to as "IL2-IL15 cytokine hybrid protein", "IL2-IL15 hybrid protein", "IL2-IL15 hybrid", "IL2-IL15", or the like. The domain derived from cytokines may be present in the same polypeptide or in separate polypeptides of the cytokine hybrid protein.

[102] The term "EC50" refers to the "half maximal effective concentration", which value measures the effectiveness of compound (e.g. an IL2-IL15 cytokine hybrid protein) towards a biological or biochemical utility. This quantitative measure indicates the quantity or concentration required for a particular compound to elicit a given biological process to half of the maximal response.

[103] An "effective amount" of a cell disclosed herein or a composition (e.g., pharmaceutical composition) described herein, is at least the minimum amount required to achieve the desired therapeutic or prophylactic result, e.g., a measurable improvement (e.g., in a symptom, severity, grade, or progression) of or prevention of a particular disease, disorder, or condition, e.g., a cell proliferative disorder, e.g., cancer, preferably with minimal or no toxic or detrimental effects. An effective amount may vary according to inter alia disease state, age, sex, and weight of the patient, and the ability of the active ingredient (e.g. cell) to elicit a desired response in the individual and, in some instances, by co-administering one or more additional therapeutic agents. For therapeutic use,

beneficial or desired results include clinical results such as decreasing one or more symptoms resulting from the disease, increasing the quality of life of those suffering from the disease, decreasing the dose of other medications required to treat the disease, enhancing effect of another medication such as via targeting, delaying the progression of the disease, and/or prolonging survival. In the case of cancer or tumor, an effective amount of the drug may have the effect in reducing the number of cancer cells; reducing the tumor size; inhibiting (i.e., slow to some extent or desirably stop) cancer cell infiltration into peripheral organs; inhibit (i.e., slow to some extent and desirably stop) tumor metastasis; inhibiting to some extent tumor growth; and/or relieving to some extent one or more of the symptoms associated with the disorder. An effective amount can be administered in one or more administrations. As is understood in the clinical context, an effective amount of a drug, compound, or pharmaceutical composition may or may not be achieved in conjunction with another drug, compound, or pharmaceutical composition. Thus, an "effective amount" may be considered in the context of administering one or more therapeutic agents, and a single agent may be considered to be given in an effective amount if, in conjunction with one or more other agents, a desirable result may be or is achieved. An "effective amount" may be ascertainable by one skilled in the art using known techniques (see, for example, Lloyd (1999) *The Art, Science and Technology of Pharmaceutical Compounding*).

[104] The terms "enteral," "enterally," "oral," "orally," "non-parenteral," "non-parenterally," and the like, refer to administration of a compound or composition to an individual by a route or mode along the alimentary canal. Examples of "oral" routes of administration of a composition include, without limitation, swallowing liquid or solid forms of a composition from the mouth, administration of a composition through a nasojunal or gastrostomy tube, intraduodenal administration of a composition, and rectal administration, e.g., using suppositories for the lower intestinal tract of the alimentary canal.

[105] The term "format" as used herein in relation to cytokine hybrid proteins refers to the structure of a cytokine hybrid protein having all the domains contained in a referenced cytokine hybrid protein structure, wherein the domains are placed in essentially the same orientation as in the referenced cytokine hybrid protein structure. Other parts such as a linker, a signal peptide, a tag (e.g., 6His tag (SEQ ID NO: 120), FLAG tag, etc) may be added or removed as appropriate. For example, by "Format 1" it is meant that all the domains contained in the structure shown in "Format 1" of **FIG. 1B** are present in the same orientation as shown in "Format 1" of **FIG. 1B**. That is, an IL2-IL15 cytokine hybrid protein ("IL2-IL15") of Format 1 comprises a first polypeptide comprising an IL2 domain and an IL15RA domain in the direction from the N-terminus to the C-terminus and a second polypeptide comprising a scaffold domain and an IL15 domain in the direction from the N-terminus

to the C-terminus, wherein the IL15 domain and the IL15RA domain may be paired or bound with each other. Each domain may or may not contain modifications (e.g., amino acid sequence changes, glycosylation, etc.) relative to the corresponding domain or part of a wild-type sequence.

[106] “Human albumin”, also known as “HSA”, the most abundant protein in human blood. HSA regulates blood plasma colloid osmotic pressure and acts as a carrier protein for a wide range of endogenous molecules including hormones, fatty acids, and metabolites, as well as exogenous drugs. HSA is often used in protein- or polypeptide-based therapeutics for its role in increasing *in vivo* half-life. In humans, albumin is encoded by the albumin (gene symbol ALB) gene on chromosome 4, with gene location 4q13.3 (NCBI). In some embodiments, HSA may for example have the canonical amino acid sequence of SEQ ID NO: 3 (according to NCBI Reference Sequence: NP_000468.1 (without the signal peptide and propeptide)). In some embodiments, HSA may encompass such a HSA sequence, another HSA isoform, and/or and their variants comprising the equivalent residues from a non-human species, e.g., mouse, rodent, monkey, ape and the like.

[107] “IL2”, also known as “interleukin 2”, “IL-2”, “TCGF”, or “lymphokine”, is a member of the IL2 cytokine subfamily which includes IL4, IL7, IL9, IL15, IL21, erythropoietin, and thrombopoietin. IL2 may be produced by various immune cells including activated CD4+ and CD8+ T cells and is important for the proliferation of T and B cells. In humans, IL2 is encoded by the interleukin 2 (gene symbol IL2) gene on chromosome 4, with gene location 4q27 (NCBI). In some embodiments, human IL2 may for example have the canonical amino acid sequence of SEQ ID NO: 1 (according to NCBI Reference Sequence: NP_000577.2 (without the signal peptide)). In some embodiments, human IL2 may encompass such a human IL2 sequence, another IL2 isoform, and/or and their variants comprising the equivalent residues from a non-human species, e.g., mouse, rodent, monkey, ape and the like.

[108] “IL2RA”, also known as “interleukin 2 receptor subunit alpha”, “CD25”, “IL2R α ”, “IL-2RA”, “IL-2R α ”, “p55”, “IMD41”, “TCGFR”, or “IDDM10”, binds to IL2 with low affinity (with K_D about 10 nM) but, together with the common beta (β c) receptor subunit (also known as “IL2RB”, “IL2R β ”, or CD122) and the common gamma chain (γ c) receptor subunit (also known as “IL2RG”, “IL2R γ ” or CD132), constitutes the high-affinity (with K_D of about 10 pM), trimeric IL2A/B/G receptor. In humans, IL2RA is encoded by the interleukin 2 receptor subunit alpha (gene symbol IL2RA) gene on chromosome 10, with gene location 10p15.1 (NCBI). In some embodiments, human IL2RA may for example have the canonical amino acid sequence of SEQ ID NO: 8 (according to NCBI Reference Sequence: NP_000408.1 (without the signal peptide)), in which positions 1-219 correspond to the extracellular domain, positions 220-238 correspond to the transmembrane domain, and positions 239-251 correspond to the intracellular domain. In some embodiments, human IL2RA may

encompass such a human IL2RA sequence, another IL2RA isoform, and/or and their variants comprising the equivalent residues from a non-human species, e.g., mouse, rodent, monkey, ape and the like.

[109] “IL15”, also known as “interleukin 15” or “IL-15”, is a cytokine that regulates T and natural killer cell activation and proliferation. Both IL15 and IL2 bind to common beta (β c) and common gamma chain (γ c) receptor subunits and share and/or cooperate in several biological activities. For example, the number of CD8⁺ memory cells may be controlled by a balance between IL15 and IL2. IL15 induces the activation of JAK kinases, as well as the phosphorylation and activation of transcription activators STAT3, STAT5, and STAT6. In humans, IL15 is encoded by the interleukin 15 (gene symbol IL15) gene on chromosome 4, with gene location 4q31.21 (NCBI). In some embodiments, human IL15 may for example have the canonical amino acid sequence of SEQ ID NO: 4 (according to NCBI Reference Sequence: NP_000576.1 (without the signal peptide and propeptide)). In some embodiments, human IL15 may encompass such a human IL15 sequence, another IL15 isoform, and/or and their variants comprising the equivalent residues from a non-human species, e.g., mouse, rodent, monkey, ape and the like.

[110] “IL15RA”, also known as “interleukin 15 receptor subunit alpha”, “CD215”, “IL15R α ”, “IL-15RA”, “IL-15R α ”, binds to IL15 at high affinity (with K_D of about 10 pM) and, together with the common beta (β c) receptor subunit (also known as “IL2RB”, “IL2R β ”, or CD122) and the common gamma chain (γ c) receptor subunit (also known as “IL2RG”, “IL2R γ ” or CD132), constitutes the trimeric IL15 receptor. In humans, IL15RA is encoded by the interleukin 15 receptor subunit alpha (gene symbol IL15RA) gene on chromosome 10, with gene location 10p15.1 (NCBI). In some embodiments, human IL15RA may for example have the canonical amino acid sequence of SEQ ID NO: 2 (according to GenBank: AAI21142.1 (without the signal peptide)), in which positions 1-66 correspond to the sushi domain (SEQ ID NO: 21), positions 1-175 correspond to the extracellular domain, positions 176-198 correspond to the transmembrane domain, and positions 199-237 correspond to the intracellular domain. In some embodiments, human IL15RA may encompass such a human IL15RA sequence, another IL15RA isoform, and/or and their variants comprising the equivalent residues from a non-human species, e.g., mouse, rodent, monkey, ape and the like.

[111] An “isolated” biological component (such as an isolated protein, nucleic acid, vector, or cell) refers to a component that has been substantially separated or purified away from its environment or other biological components in the cell of the organism in which the component naturally occurs, for instance, other chromosomal and extra-chromosomal DNA and RNA, proteins, and organelles. Nucleic acids and proteins may be “isolated” include nucleic acids and proteins purified by standard purification methods. The term also embraces nucleic acids and proteins prepared by recombinant

technology as well as chemical synthesis. An isolated nucleic acid or protein can exist in a substantially purified form, or can exist in a non-native environment such as, for example, in a host cell.

[112] The term "linker" refers to a construct of variable length connecting two or more domains or portions of a polypeptide or connecting two or more polypeptides. In some cases, a linker is used to confer flexibility, improved spatial organization, proximity, etc and in such a case may be referred to as a flexible linker. Exemplary linkers may comprise one or more amino acids, optionally between 1-50 amino acids, such as one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, or twenty amino acids. In some embodiments, the linker may predominantly or entirely consist of G, S, and/or A amino acid residues. In some embodiments, the linker may comprise an amino acid sequence which comprises or consists of the amino acid sequence selected from the group consisting of GGGGS (SEQ ID NO: 51, which may also be called "G4S"), GGGS (SEQ ID NO: 52, which may also be called "G3S"), GGGGS (SEQ ID NO: 53, which may also be called "G5S"), G, GG, GGG, GS, SG, GGS (which may also be called "G2S"), GSG, SGG, GSS, SGS, and SSG. In some embodiments, the linker may comprise an amino acid sequence which comprises or consists of multiple repeats (e.g., two, three, four, five, or more repeats) of the amino acid sequence selected from the group consisting of G, GS, SG, GGS, GSG, SGG, GSS, SGS, SSG, and any combinations thereof. When the linker comprises or consists of multiple repeats of G5S (SEQ ID NO: 53), G4S (SEQ ID NO: 51), G3S (SEQ ID NO: 52), G2S, GS, or G, the linker may optionally be called a (G5S)_n linker (SEQ ID NO: 53), a (G4S)_n linker (SEQ ID NO: 51), a (G3S)_n linker (SEQ ID NO: 52), a (G2S)_n linker, a (GS)_n linker, or a (G)_n linker, respectively (n is a natural number, optionally selected from 1-20, e.g., 2, 3, 4, 5, etc). In particular embodiments, the linker may comprise two or three repeats of SEQ ID NO: 51, i.e., have the sequence of GGGGSGGGGS (SEQ ID NO: 58) or GGGGSGGGGSGGGGS (SEQ ID NO: 59), respectively, and may optionally be called a (G4S)₂ linker (SEQ ID NO: 58) or a (G4S)₃ linker (SEQ ID NO: 59), respectively.

[113] The term "mammal" refers to any mammal, including, but not limited to, mammals of the order Rodentia, such as mice, rats, and hamsters, and mammals of the order Logomorpha, such as rabbits. The mammals may be from the order Carnivora, including Felines (cats) and Canines (dogs). The mammals may be from the order Artiodactyla, including Bovines (cows) and Swines (pigs) or of the order Perissodactyla, including Equines (horses). The mammals may be of the order Primates, Ceboidea, or Simiiformes (monkeys) or of the order Anthropoidea (humans and apes).

[114] The term "nucleic acid" or "polynucleotide" refers to RNA or DNA that is linear or branched, single or double stranded, or a hybrid thereof. The term also encompasses RNA/DNA hybrids. The following are non-limiting examples of polynucleotides: a gene or gene fragment, exons, introns,

mRNA, tRNA, rRNA, ribozymes, cDNA, recombinant polynucleotides, branched polynucleotides, plasmids, vectors, isolated DNA of any sequence, isolated RNA of any sequence, nucleic acid probes and primers. A nucleic acid may comprise modified nucleotides, such as methylated nucleotides and nucleotide analogs, uracil, other sugars and linking groups such as fluororibose and thiolate, and nucleotide branches. The sequence of nucleotides may be further modified after polymerization, such as by conjugation, with a labeling component. Other types of modifications included in this definition are caps, substitution of one or more of the naturally occurring nucleotides with an analog, and introduction of means for attaching the polynucleotide to proteins, metal ions, labeling components, other polynucleotides or solid support. The polynucleotides can be obtained by chemical synthesis, recombinantly, or derived from a microorganism.

[115] The term "parenteral" or "parenterally" as used herein includes any route of administration of a compound or composition, characterized by physical breaching of a tissue of a subject and administration of the pharmaceutical composition through the breach in the tissue, thus generally resulting in the direct administration into the blood stream, into muscle, or into an internal organ. Parenteral administration thus includes, but is not limited to, administration of a pharmaceutical composition by injection of the composition, by application of the composition through a surgical incision, by application of the composition through a tissue-penetrating non-surgical wound, and the like. In particular, parenteral administration is contemplated to include, but is not limited to, subcutaneous, intraperitoneal, intramuscular, intrasternal, intravenous, intraarterial, intrathecal, intraventricular, intraurethral, intracranial, intrasynovial injection or infusions; and kidney dialytic infusion techniques. In a preferred embodiment, parenteral administration of the compositions of the present invention comprises subcutaneous or intraperitoneal administration.

[116] A "pharmaceutical composition" refers to a preparation in such form as to permit the biological activity of an active ingredient contained therein, such as a cell described herein, to be effective and which preferably contains no additional components which are unacceptably toxic to a subject to which the composition would be administered.

[117] A "pharmaceutical carrier", as used herein, includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic, and absorption delaying agents that are physiologically compatible. A pharmaceutically acceptable carrier includes, but is not limited to, a buffer, excipient, stabilizer, or preservative. In one embodiment, the carrier is suitable for parenteral, intravenous, intraperitoneal, intramuscular, or sublingual administration. Pharmaceutically acceptable carriers include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. The use of such media and agents for pharmaceutically active substances is well known in the art. Except

insofar as any conventional media or agent is incompatible with the active compound, use thereof in the pharmaceutical compositions of the present disclosure is contemplated. Supplementary active compounds can also be incorporated into the compositions. In some embodiments, the carrier may be a liquid, in which an active therapeutic agent is formulated. The excipient generally does not provide any pharmacological activity to the formulation, though it may provide chemical and/or biological stability, and release characteristics. Exemplary formulations can be found, for example, in Remington's Pharmaceutical Sciences, Gennaro, A. editor, 19th edition, Philadelphia, PA: Williams and Wilkins (1995), which is incorporated by reference.

[118] As used herein, the term "polypeptide" refers to polymers of amino acids of any length. The terms also encompass an amino acid polymer that has been modified; for example, to include disulfide bond formation, glycosylation, lipidation, phosphorylation, or conjugation with a labeling component.

[119] As used herein, the term "protein" refers to a compound comprised of one or more polypeptides. When a protein comprises two or more polypeptides, the polypeptides may covalently joined (e.g., disulfide bond) to or noncovalently paired (e.g., hydrogen bond) with each other to form a complex.

[120] The term "recombinant" generally refers to any protein, polypeptide, or cell expressing a gene of interest that is produced by genetic engineering methods. Therefore, the term "recombinant" as used with respect to a protein or polypeptide, means a protein or polypeptide produced by expression of a recombinant polynucleotide. "Recombinant," as applied to a polynucleotide means that the polynucleotide is the product of various combinations of cloning, restriction or ligation steps, and other procedures that result in a construct that is distinct from a polynucleotide found in nature.

[121] The term "host cell" refers to cells into which an exogenous nucleic acid has been introduced, including the progeny of such cells. Host cells include transformants and transformed cells, which include the primary transformed cell and progeny derived therefrom without regard to the number of passages. In some embodiments, a cell according to the present disclosure may be host cell.

[122] The term "recombinant cell" or "host cell" refers to cells into which an exogenous nucleic acid sequence has been introduced, including the progeny of such cells. Such cells include transformants and transformed cells, which include the primary transformed cell and progeny derived therefrom without regard to the number of passages.

[123] A polynucleotide or polypeptide has a certain percent "sequence identity" to another polynucleotide or polypeptide, meaning that, when aligned, that percentage of bases or amino acids

are the same when comparing the two sequences. The terms "identical" or "identity" when used in the context of two or more nucleic acids or polypeptide sequences, refer to the number or percentage of residues that are the same in a sequence of interest and a reference sequence. The percentage can be calculated by optimally aligning the sequence of interest to the reference sequence; comparing the two sequences over the entire length of the reference sequence; determining the number of positions at which the identical amino acid residue or nucleic acid base occurs in both sequences to yield the number of matched positions; dividing the number of matched positions by the total number of positions in the reference sequence adjusted by adding the number of gap positions introduced into the reference sequence in generating the alignment; and multiplying the result by 100 to yield the percentage of sequence identity. When comparing DNA and RNA, thymine (T) and uracil (U) can be considered equivalent. Sequence identity may be determined by using the stand-alone executable BLAST engine program for blasting two sequences (bl2seq), which can be retrieved from the National Center for Biotechnology Information (NCBI) ftp site or over the worldwide web at ncbi.nlm.nih.gov/BLAST/, using the default parameters (Tatusova and Madden, *FEMS Microbiol Lett.*, 1999, 174, 247-250; which is incorporated herein by reference in its entirety).

[124] The term "subject" as used herein may be any living organisms, preferably a mammal. In some embodiments, the subject is a primate such as a human. In some embodiments, the primate is a monkey or an ape. The subject can be male or female and can be any suitable age, including infant, juvenile, adolescent, adult, and geriatric subjects. In some examples, the patient or subject is a validated animal model for disease and/or for assessing toxic outcomes. The subject may also be referred to as "patient" in the art. The subject may have a disease or may be healthy.

[125] The term "transfected," "transformed," or "transduced" refers to a process by which exogenous nucleic acid is transferred or introduced into the host cell. A "transfected" or "transformed" or "transduced" cell is one which has been transfected, transformed or transduced with exogenous nucleic acid. The cell includes the primary subject cell and its progeny.

[126] As used herein, the term "treat," "treatment," or "treating" generally refers to the clinical procedure for reducing or ameliorating the progression, severity, and/or duration of a disease or of a condition, or for ameliorating one or more conditions or symptoms (preferably, one or more discernible ones) of a disease. The type of disease or condition to be treated may be, for example, but are not limited to, cancer and cancer-associated diseases and conditions. In specific embodiments, the effect of the "treatment" may be evaluated by the amelioration of at least one measurable physical parameter of a disease, resulting from the administration of one or more cells and/or composition according to the present disclosure. The parameter may be, for example, gene

expression profiles, the mass of disease-affected tissues, inflammation-associated markers, cancer-associated markers, the number or frequency of disease-associated cells, tumor/cancer burden, the presence or absence of certain cytokines or chemokines or other disease-associated molecules, the presence or absence of a certain cell type e.g., a certain immune cells such as T cells or a specific subset thereof, and may not necessarily be discernible by the patient. In other embodiments "treat", "treatment," or "treating" may result in the inhibition of the progression of a disease, either physically by, e.g., stabilization of a discernible symptom, physiologically by, e.g., stabilization of a physical parameter, or both. In other embodiments the terms "treat", "treatment" and "treating" refer to the reduction or stabilization of cancerous tissue or cells. Additionally, the terms "treat," and "prevent" as well as words stemming therefrom, as used herein, do not necessarily imply 100% or complete cure or prevention. Rather, there are varying degrees of treatment effects or prevention effects of which one of ordinary skill in the art recognizes as having a potential benefit or therapeutic effect. In this respect, the inventive methods can provide any amount of any level of treatment or prevention effects of a disease in a mammal. Furthermore, the treatment or prevention provided by the inventive method can include treatment or prevention of one or more conditions or symptoms of the disease being treated or prevented. Also, for purposes herein, "prevention" can encompass delaying the onset of the disease, or a symptom or condition thereof.

[127] The term "tumor" as used herein, refers to all neoplastic cell growth and proliferation, whether malignant or benign, and all pre-cancerous and cancerous cells and tissues. The terms "cancer", "cancerous", "cell proliferative disorder", "proliferative disorder" and "tumor" are not mutually exclusive as referred to herein.

[128] A "vector" is a compound or a composition of matter which comprises an isolated nucleic acid and which can be used to deliver the isolated nucleic acid to the interior of a cell. Numerous vectors are known in the art including, but not limited to, linear polynucleotides, polynucleotides associated with ionic or amphiphilic compounds, plasmids, viruses, and virus-like particles (VLPs). Thus, the term "vector" includes an autonomously replicating plasmid, a self-replicating RNA, or a viral particle. The term should also be construed to include non-plasmid and non-viral compounds which facilitate transfer of nucleic acid into cells, such as, for example, polylysine compounds, liposomes, and the like. Examples of viral vectors include, but are not limited to, adenoviral vectors, adeno-associated virus vectors, retroviral vectors, lentiviral vectors, and the like.

[129] An "expression vector" is a vector, e.g., plasmid, minicircle, viral vector, liposome, and the like as discussed herein or as known in the art, comprising a region which encodes a gene product of interest, and is used for effecting the expression of the gene product in an intended target cell. An expression vector also comprises control elements, e.g., promoters, enhancers, UTRs, miRNA

targeting sequences, etc., operatively linked to the encoding region to facilitate expression of the gene product in the target. The combination of control elements and a gene or genes to which they are operably linked for expression is sometimes referred to as an "expression cassette," a large number of which are known and available in the art or can be readily constructed from components that are available in the art.

[130] A "promoter" as used herein encompasses a DNA sequence that directs the binding of RNA polymerase and thereby promotes RNA synthesis, i.e., a minimal sequence sufficient to direct transcription. Promoters and corresponding protein or polypeptide expression may be ubiquitous, meaning strongly active in a wide range of cells, tissues and species or cell-type specific, tissue-specific, or species specific. Promoters may be "constitutive," meaning continually active, or "inducible," meaning the promoter can be activated or deactivated by the presence or absence of biotic or abiotic factors. Also included in the nucleic acid constructs or vectors of the present disclosure are enhancer sequences that may or may not be contiguous with the promoter sequence. Enhancer sequences influence promoter-dependent gene expression and may be located in the 5' or 3' regions of the native gene.

[131] "Operatively linked" or "operably linked" refers to a juxtaposition of genetic elements, wherein the elements are in a relationship permitting them to operate in the expected manner. For instance, a promoter is operatively linked to a coding region if the promoter helps initiate transcription of the coding sequence. There may be intervening residues between the promoter and coding region so long as this functional relationship is maintained.

[132] The term "wildtype" or "native" as used herein refers to a nucleotide sequence, e.g., gene, or gene product, e.g., RNA or protein, that is present in a wild-type cell, tissue, organ or organism. The term "variant" as used herein in relation to a polynucleotide or polypeptide refers to a mutant of a reference polynucleotide or polypeptide sequence, for example a native or wild-type polynucleotide or polypeptide sequence, i.e., having less than 100% sequence identity with the reference polynucleotide or polypeptide sequence. Put another way, a variant polypeptide or polynucleotide comprises at least one amino acid difference (e.g., amino acid substitution, amino acid insertion, and amino acid deletion) or at least one nucleic acid different (e.g., base substitution, base insertion, and base deletion), respectively, relative to a reference polypeptide or polynucleotide sequence, e.g., a native or wild-type, polypeptide or polynucleotide sequence. For example, a variant polypeptide or polynucleotide may be a polypeptide or polynucleotide having a sequence identity of 50% or more, 60% or more, or 70% or more to a full-length native or wild-type, polypeptide or polynucleotide sequence, e.g., an identity of 75% or 80% or more, such as 85%, 90%, or 95% or more, for example, 98% or 99% identity with the full-length native or wild-type,

polypeptide or polynucleotide sequence. Variants may also include variant fragments of a reference, e.g., native, sequence sharing a sequence identity of 70% or more with a fragment of the reference, e.g., native, sequence, e.g., an identity of 75% or 80% or more, such as 85%, 90%, or 95% or more, for example, 98% or 99% identity to the native or wild-type sequence.

Cytokine hybrid proteins

[133] In one aspect the present disclosure provides cytokine hybrid proteins, particularly, IL2-IL15 cytokine hybrid proteins ("IL2-IL15"), i.e., proteins comprising at least (i) one domain derived from IL2 and (ii) another domain derived from IL15. The cytokine hybrid protein according to the present disclosure may comprise an IL2 domain and an IL15 domain.

[134] Various IL2 variants (e.g., mutants and chemical conjugates) which do not bind or have reduced binding to IL2RA to avoid preferential activation of Treg cells (which express the trimeric IL2RA/B/G rather than the dimeric IL2RB/G) have been developed, e.g., as anti-cancer therapeutics, but the outcome has been largely disappointing. One potential explanation may include what is often referred to as IL2RA dependency. That is, TCR and IL2 signaling promotes IL2RA expression in T cells and thereby provides a selective advantage to IL2RA-expressing T cells via a positive feedback mechanism during T cell activation processes. IL2R and IL15R share both the beta and gamma subunits. Without wishing to be bound by theory, designing cytokine hybrid proteins by combining IL2 and IL15 may provide further increased avidity to the preferred IL2 receptor (dimeric IL2RB/G) and may address at least part of the IL2RA dependency.

IL2 domain

[135] In some embodiments, the IL2 domain may not bind to human IL2RA (e.g., SEQ ID NO: 8 or the extracellular domain thereof) or may bind to human IL2RA but with reduced affinity compared to wildtype human IL2 (e.g., SEQ ID NO: 1).

[136] In certain embodiments, the IL2 domain may comprise one or more amino acid substitutions, insertions, and/or deletions relative to a wildtype IL2 (e.g., SEQ ID NO: 1) and/or one or more amino acid modifications (e.g., phosphorylation, methylation, acetylation, amidation, formation of pyrrolidone carboxylic acid, isomerization, hydroxylation, sulfation, flavin-binding, cysteine oxidation, and/or nitrosylation, and/or conjugation to another molecule such as a PEG), which reduce or abrogate binding IL2RA. The one or more amino acid substitutions, insertions, and/or deletions may be at any appropriate amino acid positions, and such positions may for example be determined or selected structurally, such as based on crystal structure, and/or using mutagenesis. In some cases, one or more amino acid positions in the IL2-IL2RA binding surface may be altered. In some cases, a position of interest or a combination of positions of interest in the IL2

domain may be substituted and binding to IL2RA may be tested to determine which position(s) to be altered.

[137] In certain embodiments, the IL2 domain may comprise at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 98%, at least 99%, or at least 99.5% identity to SEQ ID NO: 1. In some cases, the IL2 domain may comprise an amino acid substitution, insertion, or deletion at one or more of amino acid positions 3, 35, 38, 41, 42, 43, 45, 61, 62, 65, 68, 69, 72, 80, 81, 85, 86, 92, and/or 125. In particular cases, the IL2 domain may comprise an amino acid substitution, insertion, or deletion at one or more of amino acid positions 38, 42, 45, 62, 65, and/or 72. In some instances, the substitution(s) may be or may comprise a non-conservative amino acid substitution(s). In some instances, the substitution(s) may be or may comprise a substitution to an aliphatic amino acid. In certain instances, the substitution(s) may be or may comprise a substitution to alanine, glycine, isoleucine, leucine, proline, or valine. In particular instances, the substitution(s) may be or may comprise a substitution to alanine or glycine.

[138] In particular embodiments, positions 42 and/or 45 may be substituted. In some cases, positions 42 and/or 45 may be substituted to a non-conservative amino acid. In certain cases, positions 42 and/or 45 may be substituted to alanine. In particular cases, the IL2 domain comprises F42A and Y45A. In some cases, the IL2 domain may further comprises one or more amino acid substitutions. In certain cases, the one or more amino acid substitutions may be at positions selected from positions 3, 35, 38, 41, 43, 61, 62, 65, 68, 69, 72, 80, 81, 85, 86, 92, and/or 125 (SEQ ID NO: 10). In certain cases, the one or more amino acid substitutions may be at positions selected from positions 38, 62, 65, 72, and/or 125. In certain cases, the IL2 domain may comprises amino acid substitutions at positions 42, 45, and 125. Without wishing to be bound by theory, substitution at position 125 from cysteine to another amino acid (e.g., alanine, serine, etc) may reduce or prevent dimerization and/or aggregation of IL2 domains. In particular cases, the IL2 domain may comprise the amino acid sequence of SEQ ID NO: 11.

[139] In some particular embodiments, the IL2 domain may comprise a substitution(s) at any of the following positions or position combinations: 42; 45; 42 and 45; 43 and 45; 35, 38, 43, and 45; 35, 38, and 72; 42, 45, and 72; 38, 42, 45, and 62; 35; 65; 80, 81, 85, 86, and 92; 38, 43, and 61. In some cases, the IL2 domain may further comprise a substitution(s) at position(s) 3 and/or 125. In certain cases, the IL2 domain may comprise any of the following substitutions or substitution combinations: F42A and Y45A; F42A; Y45A; Y45S; K43A and Y45A; K35A, R38A, K43A, and Y45A; K35A, R38A, and L72G; F42A, Y45A, and L72G; R38A, F42A, Y45A, and E62A; L80F, R81D, L85V, I86V, and I92F; or R38D, K43E, and E61R. In some cases, the IL2 domain may further comprise T3A and/or C125A (or C125S).

[140] In particular embodiments, positions 38, 62, 65, and/or 72 may be substituted. In some cases, positions 38, 62, 65, and/or 72 may be substituted to a non-conservative amino acid. In certain cases, positions 38, 62, 65, and/or 72 may be substituted to alanine. In particular cases, the IL2 domain may comprise R38A. In particular cases, the IL2 domain may comprise E62A. In particular cases, the IL2 domain may comprise P65A. In particular cases, the IL2 domain may comprise L72A. Any one or more of these substitutions may, in some cases, be combined with a substitution at position(s) 42 and/or 45. In particular cases, the IL2 domain may comprise the amino acid sequence according to any of SEQ ID NOS: 101-119.

[141] In any of the embodiments above, in some cases, the IL2 domain may comprise one or more amino acid substitutions, insertions, and/or deletions, for example, to prevent aggregation. In certain cases, the cysteine residue at position 125 may be substituted to another amino acid. In particular cases, the cysteine residue at position 125 may be substituted to alanine or serine.

[142] In any of the embodiments above, in some cases, the IL2 domain may comprise one or more amino acid substitutions, insertions, and/or deletions, for example, to alter (e.g., remove or add) potential glycosylation. In certain cases, threonine or serine may be substituted to an amino acid other than threonine or serine to remove a potential *O*-glycosylation site. In certain cases, arginine may be substituted to an amino acid other than arginine to remove a potential *N*-glycosylation site. In certain cases, the threonine residue at position 3 may be substituted to another amino acid (e.g., an amino acid other than threonine or serine). In particular cases, the threonine residue at position 3 may be substituted to alanine.

[143] In certain embodiments, the cytokine hybrid protein may comprise an IL2RA domain bound to the IL2 domain, such that binding of the IL2 domain to an IL2 receptor or IL2RA (which is not part of the cytokine hybrid protein) is reduced or blocked. The IL2RA domain may comprise a wildtype IL2RA sequence (SEQ ID NO: 8 or the extracellular domain thereof (e.g., positions 1-165 (i.e., SEQ ID NO: 80)) or a segment thereof sufficient for binding to the IL2 domain) or a variant IL2RA sequence, which may comprise one or more amino acid substitutions, insertions, and/or deletions relative to SEQ ID NO: 8. In some cases, the IL2RA domain and the IL2 domain may be present in the same polypeptide or present in separate polypeptides.

[144] In some cases, a potential *N*-glycosylation site (Asn residue) and/or *O*-glycosylation site (Ser or Thr residue) in the IL2RA domain may be altered to prevent glycosylation. In certain cases, N49 and/or N68 in SEQ ID NO: 8 or 80 may be altered (e.g., as in SEQ ID NO: 81).

[145] In some cases, the IL2 and/or IL2RA domains may be modified (e.g., one or more amino acid substitutions) to promote binding or tighter binding between IL2 and/or IL2RA. In certain cases, one or more cysteine residues may be introduced (e.g., by substitution) to the IL2 and/or IL2RA domains.

In particular cases, the cytokine hybrid protein may comprise one or more disulfide bonds between the IL2 domain and the IL2RA domain. Exemplary IL2 and/or IL2RA substitutions include but are not limited to those described in WO2021120350A1. In particular cases, the IL2 domain may comprise P65C and IL2RA domain may comprise K38C.

[146] In certain cases, the cytokine hybrid protein may comprise a circularly permuted IL2 domain and an IL2RA domain. In particular cases, the circularly permuted IL2 domain may comprise an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 98%, at least 99%, or at least 99.5% identity to SEQ ID NO: 12. In particular cases, the IL2RA domain may comprise an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 98%, at least 99%, or at least 99.5% identity to SEQ ID NO: 81. In particular cases, the circularly permuted IL2 domain may comprise SEQ ID NO: 12 and the IL2RA domain may comprise SEQ ID NO: 81.

IL15 domain

[147] In certain embodiments, the cytokine hybrid protein may comprise an IL15RA domain bound to the IL15 domain, such that binding of the IL15 domain to an IL15 receptor or IL15RA (which is not part of the cytokine hybrid protein) is reduced or blocked.

[148] In some cases, the IL15 domain may comprise one or more amino acid substitutions, insertions, and/or deletions relative to a wildtype IL15 (e.g., SEQ ID NO: 4), for example to alter (e.g., remove or add) potential glycosylation. In certain cases, threonine or serine may be substituted to an amino acid other than threonine or serine to remove a potential O-glycosylation site. In certain cases, arginine may be substituted to an amino acid other than arginine to remove a potential N-glycosylation site. In particular cases, the IL15 domain may have the amino acid sequence of SEQ ID NO: 40, wherein one or more of the X residues may be altered to another amino acid which does not allow for O-glycosylation and/or N-glycosylation. In some instances, S73 and/or T81 may be substituted to an amino acid other than serine or threonine, for example to alanine or glycine. In certain instances, the IL15 domain may comprise S73A and T81. In particular instances the IL15 domain may comprise the amino acid sequence of SEQ ID NO: 41 or an amino acid sequence at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 98%, at least 99%, or at least 99.5% identity to SEQ ID NO: 41.

[149] In some cases, the IL15 and/or IL15RA domains may be modified (e.g., one or more amino acid substitutions) to promote tighter binding between IL15 and/or IL15RA. In certain cases, one or more cysteine residues may be introduced (e.g., by substitution) to the IL15 and/or IL15RA domains.

In particular cases, the cytokine hybrid protein may comprise one or more disulfide bonds between the IL15 domain and the IL15RA domain.

IL15RA domain

[150] In some embodiments, the cytokine hybrid proteins according to the present disclosure may comprise an IL15RA domain. The IL15 domain may be bound or may be capable of binding to the IL15RA domain. Without wishing to be bound by theory, wildtype human IL15 (e.g., SEQ ID NO: 4) and wildtype human IL15RA (e.g., SEQ ID NO: 2) tightly bind to each other (about 10 pM) and thus inclusion of an IL15-IL15RA complex formed by an IL15 domain and an IL15RA domain (e.g., (the extracellular domain of SEQ ID NO: 2, i.e., position 1-175 of SEQ ID NO: 2, or particularly the sushi domain, corresponding to positions 1-66 of SEQ ID NO: 2, i.e., SEQ ID NO: 21)) may be used as a platform for forming an IL2-IL15 cytokine hybrid protein.

[151] In certain embodiments, the IL15RA domain may comprise an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 98%, at least 99%, or at least 99.5% identity to SEQ ID NO: 2, or a segment thereof, such as positions 1-175 or 1-66 of SEQ ID NO: 2.

[152] In particular embodiments, the IL15RA domain may comprise the amino acid sequence of SEQ ID NO: 21.

Scaffold domain

[153] In some embodiments, the cytokine hybrid protein may further comprise a scaffold domain. Without wishing to be bound by theory, the scaffold domain may bring the cytokine hybrid protein to a desired size (which may for example slow *in vivo* clearance), and/or allow the cytokine protein to have a prolonged *in vivo* half-life, an increased stability, an improved production yield and/or purification feasibility, a desired polarity and/or net charge, and/or desired conformation.

[154] In certain embodiments, the scaffold domain may comprise an albumin domain, such as a domain derived from human albumin (also known as human serum albumin or HSA) or a HSA domain. Wildtype human albumin (e.g., SEQ ID NO: 3) is known to have an *in vivo* half-life of about 3 weeks, which is much longer than that of typical peptides, which is often as short as about 2-30 minutes due to the clearance by the kidneys.

[155] Therefore, in some cases, the scaffold domain may comprise an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 98%, at least 99%, or at least 99.5% identity to SEQ ID NO: 3.

[156] In certain cases, a threonine or serine in the HSA domain may be substituted to an amino acid other than threonine or serine to remove a potential *O*-glycosylation site. In certain cases,

arginine in the HSA domain may be substituted to an amino acid other than arginine to remove a potential *N*-glycosylation site.

[157] In certain cases, a cysteine residue in the HSA domain may be substituted to another amino acid to prevent an intermolecular or intramolecular disulfide bond formation and/or to prevent aggregation. In some instances, the cysteine residue at position 34 may be substituted to another amino acid (e.g., serine, alanine, glycine). In certain instances, the HSA domain may comprise C34S. In particular instances, the HSA domain may comprise the amino acid sequence of SEQ ID NO: 31.

[158] In certain embodiments, the scaffold domain may comprise an immunoglobulin constant region sequence or part thereof (e.g., a CH1 domain, a hinge, a CH2 domain, a CH3 domain, a Fc region, etc). In case of human IgG1, the Fc region corresponds to positions Asp221 (or Thr223, or Thr225) to Lys447 (or Gly446), according to EU numbering. In case of IgG4, the Fc region corresponds to Tyr (one residue after K218) to Lys447 (or Gly446), according to the EU numbering. In mammalian bodies, antibodies are recycled and maintained in circulation by binding to neonatal Fc receptors (FcRn).

[159] Therefore, in some cases, the scaffold domain may comprise an Fc domain comprising an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 98%, at least 99%, or at least 99.5% identity to the Fc domain of a human IgG, e.g., IgG1, IgG4, IgG2, and/or IgG2.

[160] In certain cases, the Fc domain may comprise one or more amino acid substitutions which increases *in vivo* half-life (e.g., increased binding to FcRn) and/or which reduces effector functions (e.g., by reduced binding to Fc receptors such as FcγR or complements such as C1q). Without wishing to be bound by theory, reduced effector functions in some instances may provide reduced side effects. Any appropriate substitution(s) at any appropriate position(s) (e.g., Wang et al., *Protein Cell*. 2018 Jan;9(1):63-73.; Liu et al., *Antibodies (Basel)*. 2020 Nov 17;9(4):64.) may be incorporated to the Fc domain of a cytokine hybrid protein according to the present disclosure.

Formats

[161] The cytokine hybrid proteins according to the present disclosure comprising at least an IL2 domain and an IL15 domain as described herein may take or may comprise any appropriate formats.

[162] In some embodiments, the cytokine hybrid protein may comprise the IL2 domain and the IL15 domain in separate polypeptides. In certain embodiments, the cytokine hybrid protein may comprise an IL15RA domain and/or an IL2RA domain, which may individually be comprised in the polypeptide comprising the IL2 domain and/or in the polypeptide comprising the IL15 domain. In certain embodiments, the cytokine hybrid protein may comprise a scaffold domain, which may be

comprised in the polypeptide comprising the IL2 domain and/or in the polypeptide comprising the IL15 domain.

[163] In some embodiments, the cytokine hybrid protein may comprise the IL2 domain and the IL15 domain in the same polypeptide. In certain embodiments, the cytokine hybrid protein may comprise a single polypeptide comprising the IL2 domain and the IL15 domain. In some cases, the polypeptide may further comprise an IL15RA domain and/or an IL2RA domain. In some cases, the polypeptide may further comprise a scaffold domain (e.g., a HSA domain or a Fc domain). In certain embodiments, the cytokine hybrid protein may comprise at least two polypeptides, wherein at least one of the polypeptides comprises the IL2 domain and the IL15 domain. In some cases, the cytokine hybrid protein may comprise an IL15RA domain and/or an IL2RA domain, which may individually be comprised in the polypeptide comprising the IL2 domain and the IL15 domain or in another/the other polypeptide. In some cases, the cytokine hybrid protein may comprise a scaffold domain, which may individually be comprised in the polypeptide comprising the IL2 domain and the IL15 domain or in another/the other polypeptide.

[164] In some embodiments, the domains contained in a polypeptide of a cytokine hybrid protein may be joined directly or indirectly via a linker. In certain embodiments, the linker may be a peptide linker, such as a flexible peptide linker. In certain embodiments, the linker may comprise about 1-50 amino acids and may comprise an amino acid sequence comprising or consisting of one or more small amino acids, such as glycine (G), serine (S), and/or alanine (A). In certain embodiments, the linker may comprise a linker unit amino acid sequence selected from the group consisting of SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, G, GG, GGG, GS, SG, GGS, GSG, SGG, GSS, SGS, and SSG or multiple repeats of any of such linker unit amino acid sequences. In particular embodiments, the linker may comprise a (G4S)₂ linker (SEQ ID NO: 58) or a (G4S)₃ linker (SEQ ID NO: 59).

[165] In some embodiments, the cytokine hybrid protein may comprise any of the formats depicted in **FIGS. 1B-1G**.

[166] In certain embodiments, the cytokine hybrid protein may take or comprise Format 1 as depicted in **FIG. 1B** and may comprise a first polypeptide comprising an IL2 domain and an IL15RA domain in the direction from the N-terminus to the C-terminus and a second polypeptide comprising a scaffold domain and an IL15 domain in the direction from the N-terminus to the C-terminus.

[167] In certain embodiments, the cytokine hybrid protein may take or comprise Format 3 as depicted in **FIG. 1B** and may comprise a first polypeptide comprising an IL2 domain and an IL15RA domain in the direction from the N-terminus to the C-terminus and a second polypeptide comprising an IL15 domain and a scaffold domain in the direction from the N-terminus to the C-terminus.

[168] In certain embodiments, the cytokine hybrid protein may take or comprise Format 6 as depicted in **FIG. 1B** and may comprise a first polypeptide comprising an IL15RA domain and an IL2 domain in the direction from the N-terminus to the C-terminus and a second polypeptide comprising an IL15 domain and a scaffold domain in the direction from the N-terminus to the C-terminus.

[169] In certain embodiments, the cytokine hybrid protein may take or comprise Format 8 as depicted in **FIG. 1B** and may comprise a first polypeptide comprising an IL15RA domain and an IL2 domain in the direction from the N-terminus to the C-terminus and a second polypeptide comprising a scaffold domain and an IL15 domain in the direction from the N-terminus to the C-terminus.

[170] In certain embodiments, the cytokine hybrid protein may take or comprise Format 11 as depicted in **FIG. 1C** and may comprise a first polypeptide comprising a scaffold domain and an IL15RA domain in the direction from the N-terminus to the C-terminus and a second polypeptide comprising an IL2 domain and an IL15 domain in the direction from the N-terminus to the C-terminus.

[171] In certain embodiments, the cytokine hybrid protein may take or comprise Format 12 as depicted in **FIG. 1C** and may comprise a first polypeptide comprising an IL15RA domain and a scaffold domain in the direction from the N-terminus to the C-terminus and a second polypeptide comprising an IL2 domain and an IL15 domain in the direction from the N-terminus to the C-terminus.

[172] In certain embodiments, the cytokine hybrid protein may take or comprise Format 16 as depicted in **FIG. 1C** and may comprise a first polypeptide comprising an IL15RA domain and a scaffold domain in the direction from the N-terminus to the C-terminus and a second polypeptide comprising an IL15 domain and an IL2 domain in the direction from the N-terminus to the C-terminus.

[173] In certain embodiments, the cytokine hybrid protein may take or comprise Format 17 as depicted in **FIG. 1C** and may comprise a first polypeptide comprising a scaffold domain and an IL15RA domain in the direction from the N-terminus to the C-terminus and a second polypeptide comprising an IL15 domain and an IL2 domain in the direction from the N-terminus to the C-terminus.

[174] In any of the formats, in some cases, the scaffold domain may be or may comprise a HSA domain, e.g., any of the HSA domains described herein. In any of the formats, in some cases, the scaffold domain may be or may comprise a Fc domain, e.g., any of the Fc domains described herein.

[175] In certain embodiments, the cytokine hybrid protein may comprise two or more IL2 domain, two or more IL15 domains, two or more IL15RA domain, and/or two or more scaffold domains.

[176] In certain embodiments, the cytokine hybrid protein may comprise two Fc domains (e.g., as two scaffold domains). In some cases, the two Fc domains may be paired with each other, e.g., via one or more disulfide bonds. In certain cases, the cytokine hybrid protein may comprise any one of Formats 45-62 as depicted in **FIGS. 1F-1G**.

[177] In any of the formats described herein, when the cytokine hybrid protein comprises two or more polypeptides, the polypeptides may be covalently joined (e.g., via a disulfide bond) with each other and/or noncovalently paired with each other.

[178] In particular embodiments, the cytokine hybrid protein may comprise an IL2 domain of SEQ ID NO: 11, an IL15 domain of SEQ ID NO: 21, an IL15RA domain of SEQ ID NO: 21, and a scaffold domain comprising SEQ ID NO: 31 in any of Formats 1-44 as depicted in **FIGS. 1B-1E**. In some cases, the cytokine hybrid protein may comprise any of Formats 1, 3, 6, 8, 11, 12, 16, and 17. In certain cases, the cytokine hybrid protein may comprise Format 1. In particular cases, the domains are joined by a linker (e.g., a GS linker such as SEQ ID NO: 51, 58, or 59) within each polypeptide.

[179] In particular embodiments, the cytokine hybrid protein may comprise an IL2 domain of SEQ ID NO: 11, an IL15 domain of SEQ ID NO: 21, an IL15RA domain of SEQ ID NO: 21, and two Fc domains in any of Formats 45-62 as depicted in **FIGS. 1F-1G**.

Nucleic acids and vectors

[180] In one aspect the present disclosure provides nucleic acids (or polynucleotides) and vectors encoding any of the cytokine hybrid proteins described above.

[181] A nucleic acid according to the present disclosure may comprise one nucleic acid (or polynucleotides) molecule or two or more nucleic acid (or polynucleotides) molecules. A vector according to the present disclosure may comprise one vector or two or more vectors.

[182] In some embodiments, the cytokine hybrid protein encoded by a nucleic acid or a vector according to the present disclosure may comprise at least two polypeptides (a first polypeptide and a second polypeptide), for example having any of the formats shown in **FIGS. 1B-1C and 1F-1G**. In certain embodiments, the cytokine hybrid protein encoded by a nucleic acid or a vector according to the present disclosure may comprise at least (i) a first polypeptide comprising an IL2 domain and an IL15RA domain in the direction from the N-terminus to the C-terminus and (ii) a second polypeptide comprising a scaffold domain (e.g., a HSA domain, a Fc domain, etc) and an IL15 domain in the direction from the N-terminus to the C-terminus, having Format 1 shown in **FIG. 1B**. In particular embodiments, the first polypeptide may comprise an amino acid sequence comprising or consisting of SEQ ID NO: 91 (or an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 98%, at least 99%, or

at least 99.5% identity thereto) and the second polypeptide may comprise an amino acid sequence comprising SEQ ID NO : 92 (or an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 98%, at least 99%, or at least 99.5% identity thereto).

[183] In any of the embodiments of nucleic acids above, in some cases, the nucleic acid may comprise a first polynucleotide comprising a nucleic acid sequence comprising or consisting of SEQ ID NO: 93 (or an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 98%, at least 99%, or at least 99.5% identity thereto) and a second polypeptide comprising an amino acid sequence comprising SEQ ID NO: 94 (or an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 98%, at least 99%, or at least 99.5% identity thereto).

[184] In some cases, the first and second polynucleotides may be comprised in separate nucleic acid molecules. In some cases, a vector according to the present disclosure may comprise a single vector comprising the first and second polynucleotides. In certain cases, a vector according to the present disclosure may comprise two separate vectors, a first vector comprising the first polynucleotide and a second vector comprising the second polynucleotide.

[185] In some cases, the first and second polynucleotides may be comprised in the same nucleic acid molecule. In some cases, a vector according to the present disclosure may comprise the nucleic acid molecule comprising the first and second polynucleotides. In certain cases, the first polypeptide and the second polypeptide are encoded in the same polynucleotide strand. In some instances the first polypeptide and the second polypeptide are encoded in the same direction, under the same promoter or separate promoters. In some instances the first polypeptide and the second polypeptide are encoded in the opposite directions. In certain cases, the first polypeptide and the second polypeptide are encoded in the opposite polynucleotide strands.

[186] In some embodiments, the cytokine hybrid protein encoded by a nucleic acid or a vector according to the present disclosure may comprise at least two polypeptides (a first polypeptide and a second polypeptide), for example having any of the formats shown in **FIGS. 1B-1C and 1F-1G**. In certain embodiments, the cytokine hybrid protein encoded by a nucleic acid or a vector according to the present disclosure may comprise at least (i) a first polypeptide comprising an IL2 domain and an IL15RA domain in the direction from the N-terminus to the C-terminus and (ii) a second polypeptide comprising a scaffold domain (e.g., a HSA domain, a Fc domain, etc) and an IL15 domain in the direction from the N-terminus to the C-terminus, having Format 1 shown in **FIG. 1B**. In particular embodiments, the first polypeptide may comprise an amino acid sequence comprising or consisting

of SEQ ID NO: 91 (or an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 98%, at least 99%, or at least 99.5% identity thereto) and the second polypeptide may comprise an amino acid sequence comprising SEQ ID NO : 92 (or an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 98%, at least 99%, or at least 99.5% identity thereto).

Cells

[187] In one aspect the present disclosure provides cells which express any of the cytokine hybrid proteins described herein, and/or comprise or transformed/transfected/transduced with any of the nucleic acids and/or any of the vectors described herein.

[188] For expressing a cytokine hybrid protein, any appropriate cells may be used. For example, cells may be: (i) prokaryotic cells, such as gram-negative bacteria and gram-positive bacteria; or (ii) eukaryotic cells, such as yeast, filamentous fungi, protozoa, insect cells, plant cells, and mammalian cells (reviewed in Frenzel A. et al. *Front Immunol.* 2013; 4: 217. Published online 2013 Jul 29. doi: 10.3389/fimmu.2013.00217).

[189] Specific examples of gram-negative bacteria that are suited for production of cytokine hybrid proteins include, but are not limited to, *E. coli*, *Proteus mirabilis*, and *Pseudomonas putida*. Specific examples of gram-positive bacteria include, but are not limited to, *Bacillus brevis*, *Bacillus subtilis*, *Bacillus megaterium*, *Lacto-bacilluszeae/casei*, and *Lactobacillus paracasei*. Specific examples of yeast bacteria that are suited for production of cytokine hybrid proteins include, but are not limited to, *Pichia pastoris*, *Saccharomyces cerevisiae*, *Hansenula polymorpha*, *Schizosaccharomyces pombe*, *Schwanniomyces occidentalis*, *Kluyveromyces lactis*, and *Yarrowia lipolytica*. Specific examples of filamentous fungi that are suited for production of cytokine hybrid proteins include, but are not limited to, the genera *Trichoderma* and *Aspergillus*, *A. niger* (subgenus *A. awamori*), *Aspergillus oryzae*, and *Chrysosporium lucknowense*. Specific examples of protozoa that are suited for production of cytokine hybrid proteins include, but are not limited to, *Leishmania tarentolae*. Specific examples of insect cells that are suited for production of cytokine hybrid proteins include, but are not limited to, insect cell lines like Sf-9 and Sf-21 or SfSWT-1 "Mimic™" cells of *Spodoptera frugiperda*, , DS2 cells of *Drosophila melanogaster*, High Five cells (BTI-TN-5B1-4) of *Trichopulsia ni*, or Schneider2 (S2) cells of *D. melanogaster*. They can be efficiently transfected with insect-specific viruses from the family of *Baculoviridae*, particularly the *Autographa californica* nuclear polyhedrosis virus (AcNPV). Specific examples of mammalian cells that are suited for production of cytokine hybrid proteins include, but are not limited to, Chinese hamster ovary (CHO)

cells, the human embryonic retinal cell line Per.C6 [Crucell, Leiden, Netherlands], CHO-derived cell lines such as K1-, DukXB11-, Lec13, and DG44- cell lines, mouse myeloma cells such as SP 2/0, YB 2/0, and NS0 cells, GS-NSO, hybridoma cells, baby hamster kidney (BHK) cells, and the human embryonic kidney cell line HEK293, HEK293T, HEK293E, and human neuronal precursor cell line AGE1.HN (Probiogen, Berlin, Germany). Specific examples of plant cells that are suited for production of cytokine hybrid proteins include but are not limited to BY2 or NT1 cells of *N. tabacum*, Bengal, Donjin, or Taipie cells of *Oryza sativa*, and cells of *Hordeum vulgare*.

[190] Alternatively, genetically modified organisms such as transgenic plants and transgenic animals may be used. Exemplary plants that may be used include, but are not limited to, tobacco, maize, duckweed, *Chlamydomonas reinhardtii*, *Nicotiana tabacum*, *Nicotianabenthamiana*, and *Nicotiana glauca*. Exemplary animals that may be used include, but are not limited to mouse, rat, and chicken.

Compositions

[191] In one aspect the present disclosure provides compositions comprising: (I) at least one of: (a) any of the cytokine hybrid proteins described herein; (b) any of the nucleic acids described herein; (c) any of the vectors described herein; and/or (d) any of the isolated, recombinant, and/or host cells or a population of cells comprising any of the isolated, recombinant, and/or host cells; and (II) a pharmaceutically acceptable carrier. Such a composition may also be referred to as a pharmaceutical composition.

Carrier

[192] Pharmaceutically acceptable carriers are generally nontoxic to recipients at the dosages and concentrations employed, and include, but are not limited to: 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 dextrans; 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 polyethylene glycol (PEG). Exemplary pharmaceutically acceptable carriers herein further include interstitial drug dispersion agents such

as soluble neutral-active hyaluronidase glycoproteins (sHASEGP), for example, human soluble PH-20 hyaluronidase glycoproteins, such as rHuPH20 (HYLENEX®, Baxter International, Inc.). Certain exemplary sHASEGPs and methods of use, including rHuPH20, are described in US Patent Publication Nos. 2005/0260186 and 2006/0104968.

[193] Active ingredients may be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980).

[194] In some embodiments, a pharmaceutical composition according to the present disclosure may be used for any of the *in vivo* and/or treatment methods described herein.

Additional agents

[195] In some embodiments, the pharmaceutical composition may be used alone without an additional agent.

[196] In certain embodiments, the additional agent(s) may be or may comprise a chemotherapeutic agent, gene therapy agent, DNA therapy agent, viral therapy agent, RNA therapy agent, immunotherapy agent, nanotherapy agent, monoclonal antibody, or a combination of the foregoing. In certain embodiments, the additional therapeutic agent(s) may be or may comprise an adjuvant or neoadjuvant. In certain embodiments, the additional therapeutic agent(s) may be or may comprise a small molecule enzymatic inhibitor or anti-metastatic agent.

[197] In certain embodiments, the additional therapeutic agent(s) may be or may comprise a side-effect limiting agents (e.g., agents intended to lessen the occurrence and/or severity of side effects of treatment, such as anti-nausea agents, including but not limited to a neurokinin-1 receptor antagonist (NK1 RA), serotonin receptor antagonist (5-HT₃ RA), dexamethasone, olanzapine, and palonosetron, etc.).

[198] Non-limiting exemplary additional agents may include a chemotherapy agent, an antibody-drug conjugate (ADC), an immunotherapy agent, and/or a biological modifier.

[199] In certain embodiments, chemotherapy agents may be selected from alkylating agents, antimetabolites, plant alkaloids, and anti-cancer antibiotics, further optionally one or more selected from cyclophosphamide, cisplatin, carboplatin, oxaliplatin, etoposide, irinotecan, lurbinectedin, paclitaxel, docetaxel, cabazitaxel, altretamine, capecitabine, gemcitabine, ifosfamide, melphalan, pemetrexed, topotecan, vinorelbine, mitoxantrone, ixabepilone, eribulin, estramustine, vinblastine, vincristine, 5-fluorouracil (5-FU), doxorubicin, epirubicin, dactinomycin, or a derivative thereof. In

certain embodiments, chemotherapy agents may be selected from cyclophosphamide, doxorubicin, vincristine, and prednisolone (CHOP).

[200] In certain embodiments, ADC may be selected from an anti-CD79b antibody drug conjugate (such as anti-CD79b-MC-vc-PAB-MMAE or the anti-CD79b antibody drug conjugate described in any one of U.S. Pat. No. 8,088,378 and/or US 2014/0030280, or polatuzumab vedotin), an anti-CD19 antibody drug conjugate, an anti-CD22 antibody drug conjugate, an anti-CD45 antibody drug conjugate, and an anti-CD32 drug conjugate. A biological modifier may be selected from a BCL-2 inhibitor (such as GDC-0199/ABT-199), lenalidomide (REVLIMID®), a PI3K-delta inhibitor (such as idelalisib (ZYDELIG®)), a PD-1 axis binding antagonist, an agonist, e.g., agonist antibody, directed against an activating co-stimulatory molecule, e.g., CD40, CD226, CD28, OX40 (e.g., AgonOX), GITR, CD137 (also known as TNFRSF9, 4-1 BB, or ILA), CD27 (e.g., CDX-1127), HVEM, or CD127, an antagonist, e.g., antagonist antibody, directed against an inhibitory co-stimulatory molecule, e.g., CTLA-4 (also known as CD152), PD-1, TIM-3, BTLA, VISTA, LAG-3, B7-H3, B7-H4, IDO (e.g., 1-methyl-D-tryptophan (also known as 1-D-MT)), TIGIT, MICA/B, GITR (e.g., TRX518) or arginase, ipilimumab (also known as MDX-010, MDX-101, or YERVOY®), tremelimumab (also known as ticilimumab or CP-675,206, urelumab (also known as BMS-663513), MGA271, an antagonist directed against a TGF beta, e.g., metelimumab (also known as CAT-192), fresolimumab (also known as GC1008), LY2157299k, and an adoptive transfer of a T cell (e.g., a cytotoxic T cell or CTL) expressing a chimeric antigen receptor (CAR), e.g., adoptive transfer of a T cell comprising a dominant-negative TGF beta receptor, e.g., a dominant-negative TGF beta type II receptor.

[201] In certain embodiments, chemotherapy agents may be or may comprise an immune checkpoint inhibitor and/or a growth factor or growth factor receptor inhibitor, optionally an inhibitor of PD-L1, PD-1, CTLA-4, VISTA, EGF, EGFR, VEGF, and/or VEGFR, or an antibody or antigen-binding fragment against PD-L1, PD-1, CTLA-4, VISTA, EGF, EGFR, VEGF, and/or VEGFR, or an antibody or antigen-binding fragment against a cancer antigen.

[202] In certain embodiments, the additional agent(s) may be or may comprise a chemotherapeutic agent, cytotoxic agent, an anti-hormonal agent, growth inhibitory agent, cytotoxic agent, agent used in radiation therapy, anti-angiogenesis agent, apoptotic agent, anti-tubulin agent, or other agent, such as a epidermal growth factor receptor (EGFR) antagonist (e.g., a tyrosine kinase inhibitor), HER1/EGFR inhibitor (e.g., erlotinib (TARCEVA™)), platelet derived growth factor inhibitor (e.g., GLEEVEC™ (Imatinib Mesylate)), a COX-2 inhibitor (e.g., celecoxib), interferon, cytokine, antibody other than the anti-CD3 antibody of the disclosure, such as an antibody that bind to one or more of the following targets ErbB2, ErbB3, ErbB4, PDGFR-beta, BlyS, APRIL, BCMA VEGF, or VEGF receptor(s), TRAIL/Apo2, PD-1, PD-L1, PD-L2, or another bioactive or organic chemical

agent. In certain embodiments, the additional therapeutic agent(s) may be or may comprise a glucocorticoid, optionally dexamethasone.

Treatment methods and uses

[203] In another aspect the present disclosure provides therapeutic methods, such as methods of treating a disease, disorder, or condition in a subject and methods of stimulating an immune response such as T cell response in a subject.

[204] In some embodiments, such a method may comprise administering to the subject an effective amount of: at least one of: (a) any of the cytokine hybrid proteins described herein; (b) any of the nucleic acids described herein; (c) any of the vectors described herein; (d) any of the isolated, recombinant, and/or host cells or a population of cells comprising any of the isolated, recombinant, and/or host cells; and/or (e) any of the compositions described herein.

[205] In any of the aspects and embodiments herein including methods and uses, in some cases, the subject may be a mammal and, in particular, a human.

[206] In any of the aspects and embodiments herein including methods and uses, in some cases, the subject may have or may have a risk of developing a disease, a disorder, or a condition.

[207] In any of the aspects and embodiments herein including methods and uses, in some cases, the disease, disorder, or condition may be any appropriate disease, disorder, or condition including but not limited to those described herein. In some embodiments, the disease, disorder, or condition may be a proliferative disorder, cancer, an oncological disorder, a neurological disorder, and/or an infectious disease. In certain embodiments, the disease, disorder, or condition may be cancer. In particular embodiments, the disease, disorder, or condition may be skin cancer, such as melanoma.

[208] *Administration route and dosing*

[209] In any of the aspects and embodiments of methods described herein, an effective amount of such a cytokine hybrid protein (and optionally any additional agent), a nucleic acid or vector encoding, or a cell comprising such or a pharmaceutical composition comprising such may be administered by any suitable means, including parenteral, intrapulmonary, and intranasal, and, if desired for local treatment, intralesional administration. Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous administration. In certain embodiments, the administration is subcutaneous administration, which may exhibit a less toxic response in the subject compared to intravenous injection. Dosing can be by any suitable route (e.g., injections, such as intravenous or subcutaneous injections) and may depend in part on whether the administration is brief or chronic. Various dosing schedules including but not limited to single or multiple

administrations over various time-points, bolus administration, and pulse infusion are contemplated herein.

[210] Cytokine hybrid proteins of the disclosure would be formulated (e.g., as a pharmaceutical composition), dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disorder being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the agent, the method of administration, the scheduling of administration, and other factors known to medical practitioners. The cytokine hybrid protein need not, but may optionally be, formulated (e.g., as a pharmaceutical composition) with one or more agents currently used to prevent or treat the disorder in question. The effective amount of such other agents may depend on the amount of the cytokine hybrid protein present in the composition, the type of disorder or treatment, and other factors discussed above. These are generally used in the same dosages and with administration routes as described herein, or about from 1 to 99% of the dosages described herein, or in any dosage and by any route that is empirically/clinically determined to be appropriate.

[211] For the prevention or treatment of a disease, a disorder, or a condition, the appropriate dosage of a cytokine hybrid protein of the disclosure (when used alone or in combination with one or more other additional therapeutic agents) will depend on the type of disease to be treated, the severity and course of the disease, whether the cytokine hybrid protein is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the cytokine hybrid protein, and the discretion of the attending physician. In certain embodiments, the cytokine hybrid protein may be suitably administered to the patient at one time or over a series of treatments.

[212] As a general proposition, an effective amount (e.g., therapeutically effective amount) of the cytokine hybrid protein administered to humans may be in the range of about 0.01 to about 100 mg/kg of patient body weight whether by one or more administrations. In some embodiments, a cytokine hybrid protein may be administered at about 0.01 to about 45 mg/kg, about 0.01 to about 40 mg/kg, about 0.01 to about 35 mg/kg, about 0.01 to about 30 mg/kg, about 0.01 to about 25 mg/kg, about 0.01 to about 20 mg/kg, about 0.01 to about 15 mg/kg, about 0.01 to about 10 mg/kg, about 0.01 to about 5 mg/kg, or about 0.01 to about 1 mg/kg daily, for example. In one embodiment, a cytokine hybrid protein described herein is administered to a human at a dose of about 100 mg, about 200 mg, about 300 mg, about 400 mg, about 500 mg, about 600 mg, about 700 mg, about 800 mg, about 900 mg, about 1000 mg, about 1100 mg, about 1200 mg, about 1300 mg or about 1400 mg on day 1 of 21-day cycles. The dose may be administered as a single dose or as multiple doses (e.g., 2 or 3 doses), such as infusions. For repeated administrations over several days

or longer, depending on the condition, the treatment would generally be sustained until a desired suppression of disease symptoms occurs. One exemplary dosage of the cytokine hybrid protein would be in the range from about 0.05 mg/kg to about 10 mg/kg. Thus, one or more doses of about 0.5 mg/kg, 2.0 mg/kg, 4.0 mg/kg, or 10 mg/kg (or any combination thereof) may be administered to the patient. Such doses may be administered intermittently, for example, every week or every three weeks (e.g., such that the patient receives from about two to about twenty, or, for example, about six doses). An initial higher loading dose, followed by one or more lower doses, may be administered. The progress of this therapy is easily monitored by conventional techniques and assays.

[213] *Monotherapy and combination therapy*

[214] In any of the aspects and embodiments of methods and uses described herein, an effective amount of such a cytokine hybrid protein may be administered alone to the subject.

[215] In some embodiments, an effective amount of such a cytokine hybrid protein may be administered in combination with at least one additional agent to the subject. The additional agent(s) (e.g., therapeutic agents and/or adjuvants) may be or may comprise any appropriate agents including but not limited to those described herein. In certain embodiments, the additional agent(s) may be contained in a pharmaceutical composition together with a cytokine hybrid protein according to the present disclosure. In certain embodiments, the additional agent(s) may not be contained in the pharmaceutical composition comprising a cytokine hybrid protein according to the present disclosure but may be administered together (e.g., simultaneously) with the cytokine hybrid protein. In certain embodiments, the additional agent(s) may be administered separately from (e.g., prior to or following the administration of) the cytokine hybrid protein. In some cases, administration of the cytokine hybrid protein and administration of the additional agent(s) may occur within about one month, or within about one, two or three weeks, or within about one, two, three, four, five, or six days, of each other.

[216] In some embodiments, an effective amount of such a cytokine hybrid protein may be administered in combination with at least one additional therapy to the subject. The additional therapy(ies) may be or may comprise radiation therapy (e.g., gamma irradiation), surgery, bone marrow transplantation, chemotherapy, or any combination of the foregoing. In certain embodiments, the additional therapy(ies) may be administered together (e.g., simultaneously) with the cytokine hybrid protein. In certain embodiments, the additional therapy(ies) may be administered separately from (e.g., prior to or following the administration of) the cytokine hybrid protein. In some cases, administration of the cytokine hybrid protein and administration of the

additional therapy(ies) may occur within about one month, or within about one, two or three weeks, or within about one, two, three, four, five, or six days, of each other.

[217] Any cytokine hybrid proteins of the disclosure may be used in combination therapies described herein, such as in combination with radiation therapy.

Manufacturing methods

[218] In a further aspect, the present disclosure provides manufacturing methods, such as methods of manufacturing any of the cytokine hybrid proteins described herein and methods of manufacturing any of the isolated, recombinant, and/or host cells or any of the populations of cells comprising isolated, recombinant, and/or host cells according to the present disclosure.

[219] Any of the cytokine hybrid proteins described herein may be produced or manufactured using any appropriate methods, including recombinant methods, e.g., *in vitro*, *ex vivo*, or *in vivo*.

[220] In some embodiments, a method of manufacturing a cytokine hybrid protein according to the present disclosure may comprise culturing a cell according to the present disclosure in a condition that allows for expression of the cytokine hybrid protein and harvesting and purifying the cytokine hybrid protein from the cell culture.

[221] In some embodiments, a method of manufacturing a cell or a population of cells according to the present disclosure may introducing a nucleic acid and/or a vector according to the present disclosure into one or more cells. Such a method may be performed *in vitro*, *ex vivo*, or *in vivo*.

[222] Physical methods for introducing a nucleic acid into a cell include calcium phosphate precipitation, lipofection, particle bombardment, microinjection, electroporation, and the like. Methods for producing cells comprising vectors and/or exogenous nucleic acids are well-known in the art. See, for example, Sambrook et al. (2001, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, New York). A preferred method for the introduction of a polynucleotide into a host cell is calcium phosphate transfection.

[223] Biological methods for introducing a nucleic acid of interest into a cell include the use of DNA and RNA vectors. In certain embodiments, nucleic acid encoding the cytokine hybrid protein may be isolated and inserted into one or more vectors (e.g., viral vectors, plasmids, and the like) for further cloning and/or expression in an isolated, recombinant, and/or cell. Such nucleic acids may be readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the cytokine hybrid protein). Viral vectors, and especially retroviral vectors, have become the most widely used method for inserting genes into mammalian, e.g., human cells. Other viral vectors can be derived from lentivirus, poxviruses, herpes

simplex virus I, adenoviruses and adeno-associated viruses, and the like. See, for example, U.S. Pat. Nos. 5,350,674 and 5,585,362.

[224] Chemical means for introducing a nucleic acid into a cell include colloidal dispersion systems, such as macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. An exemplary colloidal system for use as a delivery vehicle *in vitro* and *in vivo* is a liposome (e.g., an artificial membrane vesicle).

[225] In the case where a non-viral delivery system is utilized, an exemplary delivery vehicle is a liposome. The use of lipid formulations is contemplated for the introduction of the nucleic acids into a host cell (*in vitro*, *ex vivo* or *in vivo*). In some cases, the nucleic acid may be associated with a lipid. The nucleic acid associated with a lipid may be encapsulated in the aqueous interior of a liposome, interspersed within the lipid bilayer of a liposome, attached to a liposome via a linking molecule that is associated with both the liposome and the oligonucleotide, entrapped in a liposome, complexed with a liposome, dispersed in a solution containing a lipid, mixed with a lipid, combined with a lipid, contained as a suspension in a lipid, contained or complexed with a micelle, or otherwise associated with a lipid. Lipid, lipid/DNA or lipid/expression vector associated compositions are not limited to any particular structure in solution. For example, they may be present in a bilayer structure, as micelles, or with a "collapsed" structure. They may also simply be interspersed in a solution, possibly forming aggregates that are not uniform in size or shape. Lipids are fatty substances which may be naturally occurring or synthetic lipids. For example, lipids include the fatty droplets that naturally occur in the cytoplasm as well as the class of compounds which contain long-chain aliphatic hydrocarbons and their derivatives, such as fatty acids, alcohols, amines, amino alcohols, and aldehydes.

[226] Examples are provided below to illustrate embodiments of the present disclosure. These examples are not meant to constrain the present invention to any particular application or theory of operation.

EXAMPLES

Example 1: Protein production and purification

Methods

[227] IL2-IL15 of Format 1 (see **FIG. 1B**), a hybrid protein comprised of a first polypeptide ("IL2-IL-15RA chain") (SEQ ID NO: 91) comprising a variant human IL2 (SEQ ID NO: 11) comprising F42A and Y45A to abolish binding to IL2RA and C125S to increase stability and homogeneity (Wang et al.,

Science. 2005;310:1159-1163; Rickert et al., *Science*. 2005;308:1477-1480) fused via a linker (SEQ ID NO: 58) to human IL15RA Sushi domain (SEQ ID NO: 21, which is amino acid positions 1-66 of SEQ ID NO: 2) and a second polypeptide ("HSA-IL15 chain") (SEQ ID NO: 92) comprising variant human serum albumin (SEQ ID NO: 31) comprising C34S to increase homogeneity and stability (Zhao et al., *Eur J Pharm Biopharm*. 2009;72:405-411.) fused via a linker (SEQ ID NO: 58) to variant human IL15 (SEQ ID NO: 41) comprising S73A and T81A to eliminate the *O*-glycosylation sites (Thaysen-Andersen et al., *Glycoconj*. 2016;J 33:417-433), was expressed in *Pichia pastoris* GS115 (Invitrogen).

[228] IL2-IL2RA, a polypeptide (SEQ ID NO: 90) comprising a circular permutated human IL2 (SEQ ID NO: 12) via a linker (SEQ ID NO: 54) to variant human IL2RA (SEQ ID NO: 81) comprising N49S and N68Q to eliminate the *N*-glycosylation sites, further fused via a linker (SEQ ID NO: 51) to a variant human serum albumin (SEQ ID NO: 31) comprising C34S to increase homogeneity and stability (Zhao et al., *Eur J Pharm Biopharm*. 2009;72:405-411.), used as a benchmark protein (mimicking namvaleukin) in Examples, was also expressed in *Pichia pastoris* GS115 (Invitrogen).

[229] Briefly, the gene encoding IL2-IL15 was synthesized according to preferred codon usage in *Pichia pastoris* (Zhao et al., *Appl Environ Microbiol*. 2014; 80:2746-2753). To co-express the two chains of IL2-IL15, the IL2-IL-15RA chain gene was cloned into pPIC9 and the HSA-IL15 chain gene was cloned into pPICZ α (Zhang et al., *Biotechnol*. 2006;Prog 22:1090-1095). The gene encoding IL2-IL2RA was synthesized based on the sequence of ALKS 4230 (Lopes et al., *J Immunother Cancer*. 2020 Apr;8(1):e000673) and cloned into pPIC9 vector.

[230] Recombinant *Pichia pastoris* was cultured in a 2.5L bioreactor (Applikon Biotechnology) and the secreted protein was purified to homogeneity by sequential chromatographic purification of immobilized metal affinity chromatography (IMAC) (Ni Sepharose FF, GE Healthcare), hydrophobic interaction chromatography (HIC) (Phenyl HP, GE Healthcare), size exclusion chromatography (SEC) (G-25 Fine, GE Healthcare) and ion exchange chromatography (IEC) (SP Sepharose FF, GE Healthcare) (Zhao et al., *Appl Microbiol Biotechnol*. 2008;81:235-241). 1 μ g of purified proteins were analyzed by SDS-PAGE.

Results

[231] SDS-PAGE results are shown in **FIG. 3**. IL2-IL15 and IL2-IL2RA were successfully produced and purified.

Example 2: Receptor binding

Methods

[232] The affinity of IL2-IL15 and IL2-IL2RA to IL2RA and IL2RB was measured by ELISA. Briefly, flat Nunc-Immuno 96-well plates (Thermo Fisher Scientific) were coated overnight at 4 °C with 1 μ g/well

of purified IL2-IL15 or IL2-IL2RA (obtained in Example 1) in PBS. The plates were then washed with 0.1% Tween 20 (RPI) and blocked for 1 hour at room temperature (RT) with 1% BSA (RPI) in 0.1% Tween 20. After washing, serially diluted IL2RA (with Fc tag) and IL2RB (with Fc tag) (Acro Biosystems) were added to plates in blocking solution and incubated for 1 h at RT. Plates were then washed and incubated with horseradish peroxidase (HRP) conjugated goat anti-human IgG Fc secondary antibody (Thermo Fisher Scientific) for 1 h at RT in the dark. Following a final wash, plates were developed using 1-Step™ Ultra TMB-ELISA Substrate Solution (Thermo Fisher Scientific) for 5–10 minutes until the reaction was stopped by the addition of 1.8 M H₂SO₄. The plates were read at 450 nm absorbance using a microplate reader.

Results

[233] Exemplary results are shown in **FIG. 4**. As shown in the graphs, neither IL2-IL15 nor IL2-IL2RA showed detectable binding to IL2RA, while both IL2-IL15 and IL2-IL2RA bound to IL2RB. Compared to IL2-IL2RA, IL2-IL15 showed about 3.5 folds higher binding to IL2RB.

Example 3: T cell activation based on STAT 5 signaling

Methods

[234] The ability of IL2-IL15 and IL2-IL2RA to induce signaling in trimeric IL2RA/B/G-expressing Treg cells and dimeric IL2RB/G-expressing CD8⁺ effector T (Teff) cells were compared by measuring the intracellular phosphorylated STAT5 (pSTAT5) levels.

[235] Human peripheral blood mononuclear cells (PBMCs) were freshly isolated from platelet cones by centrifugation over Histopaque. Washed cells were either untreated or stimulated by dilutions of recombinant IL2-IL15 or IL2-IL2RA (obtained in Example 1) in BD™ Staining Buffer, for 30 mins at 37°C, concurrently staining with anti-human CD127-AF647 (Cat. No. 558598). The cells were fixed using BD™ Cytfix Fixation Buffer, and permeabilized using BD™ Phosflow Perm Buffer III, as per BD protocols. The fixed cells were further stained with anti-human STAT5-AF488 (Cat No. 612598), anti-human CD4-PerCP Cy5.5 (Cat. No. 560650), anti-human CD8-APC Cy7 (Cat. No. 557760), anti-human CD25-BV421 (Cat. No. 562442) for 30 minutes on ice. Samples were analyzed by flow cytometry on a BioRad Cell analyzer ZE5. Fluorescence compensation settings were established using single-color BD™ CompBead control samples and the software compensation procedure. FloJo Software was utilized for cell sub-population definition, as described in Protocol III of the BD Phosflow™ Protocols for Human PBMCs.

[236] Conventional CD4 T cells were defined as CD3⁺ CD4⁺ CD8⁺ effector T cells were defined as CD3⁺ CD8⁺. Tregs were defined as CD4⁺ CD25⁺ CD127^{low}. The levels of STAT5 signaling in the Treg and

CD8 effector cell populations were compared based on the median fluorescence intensity (MFI) values.

Results

[237] Exemplary results are shown in **FIG. 5**. As shown in the graphs, IL2-IL15 induced STAT5 signaling more efficiently in both Treg and CD8⁺ Teff cells compared to IL2-IL2RA. While the signal induction efficiency in Treg cells was about x26 higher with IL2-IL15 compared to IL2-IL2RA, the signal induction efficiency in CD8⁺ Teff cells was about x200 higher with IL2-IL15 compared to IL2-IL2RA. I.e., the signal induction was more skewed to CD8⁺ Teff cells over Treg cells by about 5.7-fold in IL2-IL15 compared to IL2-IL2RA.

Example 4: Pharmacokinetics (PK) and *in vivo* toxicity in Normal Mice

Methods

[238] To evaluate potential toxicity, five groups of healthy, female C57BL/6 mice (n=4 per group) received intraperitoneal (i.p.) injections once every other day for a total of 6 doses with vehicle PBS, 20 µg IL2-IL2RA in PBS, or 10 µg IL2-IL15 in PBS (IL2-IL15 and IL2-IL2RA as obtained in Example 1). Changes in the mouse body weight were recorded.

[239] Also, the pharmacokinetics of IL215 was determined on serum samples collected at various times after i.p. cytokine injections by sandwich ELISA using IL-2-specific detection and IL-15-specific capture antibodies.

Results

[240] Exemplary body weight changes are shown in **FIG. 6**. As shown in the graph, after the 3rd and 4th doses, the mice experienced mild (< 20%) and transient body weight loss with both IL2-IL15 and IL2-IL2RA but fully recovered at the end of the treatment. No mortality was observed during the observation period.

[241] Additional results are in **Figure 7A-D**. **Panel A** shows the *in vivo* pharmacokinetics of 0.5 mg/kg IL215 after intraperitoneal (i.p.) injection. **Panel B** compares toxicity after repeated dosing based on changes in body weight. **Panel C** compares the lung wet weight, an indicator of pulmonary edema after repeated dosing. The arrows indicate cytokine administration. Statistical analysis was performed using Welch's ANOVA analysis. *: p<0.05; n.s.: not significant.

[242] These results show that maximum IL215 serum concentration was achieved at 2 h after injection. IL215 was then cleared from the circulation slowly, with a half-life (T_{1/2}) of about 8 h. Twenty-four h after administration, IL215 serum concentration remained above 0.1 µg/ml, which represents the EC₅₀ of its cellular signaling activity (**Fig. 7A**). The PK of IL215 is in line with other IL-2 and HSA fusion proteins, indicating its high stability in circulation.

As noted toxicity was further assessed by monitoring changes in the body weight of naive C57BL/6 mice following cytokine administration. The results showed that 2 mg/kg of IL2-2R α and 1 mg/kg of IL215 resulted in about 15% weight loss following 3 doses given every other day. Continued dosing however did not result in further weight loss. Instead, mice were able to regain weight at the end of 6 treatment doses. For 1 mg/kg of IL2-2R α and 0.5 mg/kg IL215 doses, we observed modest (about 5%) transient weight loss (**Fig. 7B**). In line with the body weight results, 1 mg/kg of IL2-2R α and 0.5 mg/kg IL215 also led to similar pulmonary edema as indicated by the increase of lung wet weight (**Fig. 7C**). Taken together, the toxicity evaluation indicated that IL215 is about twice as toxic as IL2-2R α . Our subsequent *in vivo* comparisons were conducted with doses with comparably minimal toxicity (1 mg/kg of IL2-2R α and 0.5 mg/kg IL215).

Example 5: Pharmacodynamics (PD) in normal mice

[243] After establishing toxicity profiles, we compared the PD of IL2-2R α and IL215 after administering 4 doses of IL2-2R α or IL215 to naive mice (**Fig. 8A-E**). Both IL2-2R α and IL215 led to increased spleen weight as compared to untreated control spleens, but IL215 resulted in greater weight gain, suggesting that IL215 is more effective in expanding lymphocytes (**Fig. 8A**). A close inspection of T-cell subpopulations revealed that IL215 expanded CD8 T-cells more effectively than IL2-2R α but had little effect on Treg cells (**Fig. 8B**). In contrast, IL2-2R α expanded both CD8 T-cells and Treg cells. The overall effect is a much greater CD8/Treg ratio for the IL215-treated group (**Fig. 8B, C**). T-cell inhibitory receptors (PD-1 and TIM-3) can mark activated and exhausted T-cells. IL2-2R α and IL215 preferentially expanded non-exhausted T-cells (**Fig. 8D**). Moreover, IL215 increased granzyme B expression by CD8 T-cells compared to untreated and IL2-2R α treated CD8 T-cells. In contrast, IL2-2R α increased CD8 T-cell expression of IFN γ compared to other treatments (**Fig. 8E**).

Example 6: *In vivo* efficacy in syngeneic melanoma cancer model and tumor-infiltrating lymphocytes (TIL) analysis

Methods

[244] *Mouse B16F10 melanoma model and treatment*

[245] On Day 0, 3×10^5 B16F10 cells in 50 μ L of HBSS were injected intradermally (i.d.) into the left flank of shaved C57BL/6 mice. Tumors were established by Day 7. Starting on Day 7, mice were treated i.p. every other day with vehicle PBS, 20 μ g IL2-IL2RA in PBS, or 10 μ g IL2-IL15 in PBS (IL2-IL15 and IL2-IL2RA as obtained in Example 1) for a total of 6 doses. Tumor volumes were calculated by multiplying the tumor length, width, and depth every other day, and mice reaching the maximum

tumor burden (15 mm in diameter) or exhibiting moribund signs were euthanized. Each *in vivo* experiment was independently repeated at least twice.

[246] *Tumor weights and digestion*

[247] Tumors were removed on Day 18 and tumor weights were measured. Tumors were then filtered through a wet 70- μ m cell strainer (Falcon) and cells were resuspended in PBS or RPMI media at 4°C prior to further processing.

[248] *Isolation of tumor-infiltrating leukocytes*

[249] To isolate the tumor-infiltrating leukocytes, cell from digested tumors were resuspended in 80% percoll (Cytiva 17-0891) and underlayered in 40% percoll to create a separation gradient. Samples were spun at 2000 rpm for 20 minutes with no break. Buffy coat layer between 40% and 80% percoll was harvested and washed in RPMI before further processing.

[250] *Flow cytometry of tumor-infiltrating lymphocytes*

[251] Tumor-infiltrating leukocytes were isolated and analyzed by flow cytometry following staining with antibodies specific for CD3 (17A2, BioLegend 100204), CD4 (RM4-5, BioLegend 100509 or GK1.5, BD Biosciences 563790), CD8 β (H35-17.2, BD Biosciences 751609; 53-5.8; BioLegend 140415), CD25 (PC61, BD Biosciences 563061), Foxp3 (MF-14, BioLegend 126403), LIVE/DEAD Fixable Blue Dead Cell Stain (Invitrogen L34962). Surface proteins were stained in staining buffer (PBS, 2% FBS, 1 mmol/L EDTA) on ice for 20 minutes and washed twice in staining buffer. Intracellular proteins were stained by incubating surface-stained cells in Fix/Perm buffer for 45 minutes on ice, followed by two washes in Perm/Wash buffer (Cytek FoxP3/Transcription Factor Staining Buffer Kit TNB-0607-KIT), incubation with anti-Foxp3 for 45 minutes on ice, two washes in Perm/Wash buffer and then resuspended in staining buffer prior to analysis. Samples were analyzed on an Aurora Spectral Cytometer (Cytek) using SpectroFlo® Version 3.03. Data analysis was performed using FlowJo® software v10.8.1. Conventional CD4 T cells were defined as CD3⁺ CD4⁺. CD8 effector T cells were defined as CD3⁺ CD8⁺. Tregs were defined as CD4⁺ CD25⁺ Foxp3⁺. Both the cell counts/mg of tumor and CD8:Treg ratios were compared.

Results

[252] Exemplary changes in tumor sizes post tumor inoculation and exemplary tumor weights on Day 18 are shown in **FIG. 9A** (left and right, respectively). As shown in the graphs, IL2-IL15 potently inhibited tumor growth. The tumor weight on Day 18 in the IL2-IL15 group was significantly smaller compared to that in the IL2-IL2RA or untreated group. In contrast IL2-IL2RA showed no tumor inhibitory effects (essentially the same as untreated).

[253] Exemplary comparison of the number of CD3⁺, CD8⁺, CD4⁺, and Treg cells per mg of tumor and CD8:Treg ratios in the tumors between treatment groups are shown in **FIG. 9B** (left and right,

respectively). As shown in the graphs, treatment with IL2-IL15 dramatically increased CD3+ cells, particularly CD8+ cells, and increased the CD8:Treg ratio in the tumor. In contrast, IL2-IL2RA showed no effects (essentially the same as untreated).

[254] **Fig. 10A-G** further shows the anticancer effect of IL2-IL15 in the syngeneic B16F10 melanoma model. **Panel A** contains a schematic depicting protein treatment in tumor-bearing mice. **Panel B & C** respectively show tumor volume and tumor weight after treatment. **Panels D-G** contain the tumor-infiltrating lymphocyte (TIL) analysis. The absolute T-cell counts per mg of tumor are in **Panel D**, the percentage of different populations of T-cells in **Panel E**, the CD8 and Treg ratios in **Panel F**, and the state of CD8 T-cells in **Panel G**. Differences between curves and bars were analyzed using one-way ANOVA followed by Tukey's multiple comparison test.

[255] The results show that while IL215 showed potent tumor growth inhibition activity, IL2-2R α elicited lesser activity compared to untreated mice (**Fig. 10B**). Based on tumor weight, the tumor growth inhibition (TGI) was about 67% for IL215 treated tumors on day 18 post tumor inoculation (**Fig. 10C**).

[256] More specifically our results showed that IL215 caused a substantial expansion of total numbers and proportions of tumor-infiltrating T-cells (**Fig 10D, E**). Moreover, IL215 preferentially expanded CD8 T-cells over Treg and the overall effect is a 7.4x increase of CD8:Treg ratio (**Fig. 10E**). IL215 also preferentially expanded PD-1 TIM-3 double-negative T-cells over exhausted PD-1 TIM-3 double-positive T-cells (**Fig. 10F**).

APPENDIX

Table 1: IL2 sequences

SEQ ID NO:	Name	Sequence	Note
1	wildtype human IL2	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT	NCBI Reference Sequence: NP_000577.2 without signal peptide
10	variant human IL2	APX ₁ SSSTKKTQLQLEHLLLDLQMILNGINNYKNPX ₂ LTX ₃ MLX ₄ X ₅ X ₆ FX ₇ MPKKATELKHLQCLEX ₈ X ₉ LKX ₁₀ LEX ₁₁ X ₁₂ LNX ₁₃ AQSKNFHX ₁₄ X ₁₅ PRDX ₁₆ X ₁₇ SNINVX ₁₈ VLELKGSETTFMCEYADETATIVEFLNRWITFX ₁₉ QSIISTLT	Each X may be any amino acid. Optionally, X positions (T3, K35, R38, T41, F42, K43, Y45, E61, E62, P65, E68, V69, L72, L80, R81, L85, I86, I92, C125) may individually be substituted relative to SEQ ID NO: 1.
11	variant human IL2 (F42A, Y45A, C125S)	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTAKFAMPKKATELKHLQCLEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFSQSIISTLT	
12	circular permuted human IL2	SKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLTGGSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEELKPLEEVLNLAQ	
101	variant human IL2 (R38A, C125S)	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMPKKATELKHLQCLEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFSQSIISTLT	
102	variant human IL2 (E62A, C125S)	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFSQSIISTLT	
103	variant human IL2 (P65A, C125S)	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEELKALEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFSQSIISTLT	
104	variant human IL2 (L72A, C125S)	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEELKPLEEVLNAAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFSQSIISTLT	
105	variant human IL2 (R38A, F42A, Y45A, C125S)	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTAMLTAKFAMPKKATELKHLQCLEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFSQSIISTLT	
106	variant human IL2 (F42A, Y45A, E62A, C125S)	APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTAKFAMPKKATELKHLQCLEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFSQSIISTLT	

SEQ ID NO:	Name	Sequence	Note
107	variant human IL2 (F42A, Y45A, P65A, C125S)	APTSSSTKKTQLQLEHLLDLQMILNGINNYKNPKLTRMLTAKFAMPKKATELKH LQCLEEEELKALEEVLNLAQSKNFHLRPRDLISININVIVLELKGSETTFMCEYADETA TIVEFLNRWITFSQSIISTLT	
108	variant human IL2 (F42A, Y45A, L72A, C125S)	APTSSSTKKTQLQLEHLLDLQMILNGINNYKNPKLTRMLTAKFAMPKKATELKH LQCLEEEELKPLEEVLNAAQSKNFHLRPRDLISININVIVLELKGSETTFMCEYADETA TIVEFLNRWITFSQSIISTLT	
109	variant human IL2 (R38A, F42A, Y45A, E62A, C125S)	APTSSSTKKTQLQLEHLLDLQMILNGINNYKNPKLTAMLTAKFAMPKKATELKH LQCLEEALKPLEEVLNLAQSKNFHLRPRDLISININVIVLELKGSETTFMCEYADETA TIVEFLNRWITFSQSIISTLT	
110	variant human IL2 (R38A, F42A, Y45A, P65A, C125S)	APTSSSTKKTQLQLEHLLDLQMILNGINNYKNPKLTAMLTAKFAMPKKATELKH LQCLEEEELKALEEVLNLAQSKNFHLRPRDLISININVIVLELKGSETTFMCEYADETA TIVEFLNRWITFSQSIISTLT	
111	variant human IL2 (R38A, F42A, Y45A, L72A, C125S)	APTSSSTKKTQLQLEHLLDLQMILNGINNYKNPKLTAMLTAKFAMPKKATELKH LQCLEEEELKPLEEVLNAAQSKNFHLRPRDLISININVIVLELKGSETTFMCEYADETA TIVEFLNRWITFSQSIISTLT	
112	variant human IL2 (F42A, Y45A, E62A, P65A, C125S)	APTSSSTKKTQLQLEHLLDLQMILNGINNYKNPKLTRMLTAKFAMPKKATELKH LQCLEEALKALEEVLNLAQSKNFHLRPRDLISININVIVLELKGSETTFMCEYADETA TIVEFLNRWITFSQSIISTLT	
113	variant human IL2 (F42A, Y45A, E62A, L72A, C125S)	APTSSSTKKTQLQLEHLLDLQMILNGINNYKNPKLTRMLTAKFAMPKKATELKH LQCLEEALKPLEEVLNAAQSKNFHLRPRDLISININVIVLELKGSETTFMCEYADETA TIVEFLNRWITFSQSIISTLT	
114	variant human IL2 (F42A, Y45A, P65A, L72A, C125S)	APTSSSTKKTQLQLEHLLDLQMILNGINNYKNPKLTRMLTAKFAMPKKATELKH LQCLEEEELKALEEVLNAAQSKNFHLRPRDLISININVIVLELKGSETTFMCEYADETA TIVEFLNRWITFSQSIISTLT	
115	variant human IL2 (R38A, F42A, Y45A, E62A, P65A, C125S)	APTSSSTKKTQLQLEHLLDLQMILNGINNYKNPKLTAMLTAKFAMPKKATELKH LQCLEEALKALEEVLNLAQSKNFHLRPRDLISININVIVLELKGSETTFMCEYADETA TIVEFLNRWITFSQSIISTLT	
116	variant human IL2 (R38A, F42A, Y45A, E62A, L72A, C125S)	APTSSSTKKTQLQLEHLLDLQMILNGINNYKNPKLTAMLTAKFAMPKKATELKH LQCLEEALKPLEEVLNAAQSKNFHLRPRDLISININVIVLELKGSETTFMCEYADETA TIVEFLNRWITFSQSIISTLT	
117	variant human IL2 (R38A, F42A, Y45A, P65A, L72A, C125S)	APTSSSTKKTQLQLEHLLDLQMILNGINNYKNPKLTAMLTAKFAMPKKATELKH LQCLEEEELKALEEVLNAAQSKNFHLRPRDLISININVIVLELKGSETTFMCEYADETA TIVEFLNRWITFSQSIISTLT	
118	variant human IL2 (F42A, Y45A, E62A, P65A, L72A, C125S)	APTSSSTKKTQLQLEHLLDLQMILNGINNYKNPKLTRMLTAKFAMPKKATELKH LQCLEEALKALEEVLNAAQSKNFHLRPRDLISININVIVLELKGSETTFMCEYADETA TIVEFLNRWITFSQSIISTLT	

SEQ ID NO:	Name	Sequence	Note
119	variant human IL2 (R38A, F42A, Y45A, E62A, P65A, L72A, C125S)	APTSSSTKKTQLQLEHLLDLQMILNGINNYKNPKLTAMLTAKFAMPKKATELKH LQCLEEALKALEEVLNAAQSKNFHLRPRDLISININVIVLELKGSETTFMCEYADETA TIVEFLNRWITFSQSIISTLT	

Table 2: IL15RA sequences

SEQ ID NO:	Name	Sequence	Note
2	wildtype human IL15RA	ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAH WTTPSLKCIRD PALVHQRPAAPPSTVTTAGVTPQPESLSPSGKEPAASSPSSNNTA ATTAAIVPGSQLMPSPSTGTTEISSHESSHGTPTSQTAKNWELTASASHQPPG VYPQGHSDTTVAISTSTVLLCGLSAVSLACYLKSRQTPPLASVEMEAMEALPVT WGTSSRDEDELENCSHHL	GenBank: AAI21142.1 without signal peptide. Positions 1-66 = Sushi domain; positions 1-175 = extracellular domain; positions 176-198 = transmembrane domain; and positions 199-237 = intracellular domain.
21	human IL15RA sushi domain	ITCPPPMSVEHADIWVKSYSLYSRERYICNSGFKRKAGTSSLTECVLNKATNVAH WTTPSLKCIRD	

Table 3: albumin sequences

SEQ ID NO:	Name	Sequence	Note
3	wildtype human albumin (HSA)	DAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQCFEDHVKLVNEVTEFAKTCVADES AENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNECFLQHKDDNPRLVRPEV DVMCTAFHDNEETFLKKYLYEIAARRHPYFYAPELLFFAKRYKAAFTGCCQAADKAACLLPKL DELRDEGKASSAKQRLKCASLQKFGERAFAKAWAVARLSQRFPAEFAEVSKLVTDLTKVH TECCHGDLLECADDRADLAKYICENQDSISSKLKECKEPLLEKSHCIAEVENDEMPADLPS LAADFVESKDVCKNYAEAKDVFLGMFLYFYARRHPDYSVVLRLAKTYETTLEKCCAAAD PHECYAKVFDEFKPLVEEPQNLKQNCLEFELGEYKFQNALLVRYTKKVPQVSTPTLVEVS RNLGKVGSKCKHPEAKRMPCAEDYLSVVLNQLCVLHEKTPVSDRVTKCCTESLVNRRPC FSALEVDETYVPKEFNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAVMD DFAAFVEKCKKADDKETCFEEGKKLVAASQAALGL	NCBI Reference Sequence: NP_000468.1 without signal peptide and propeptide.

SEQ ID NO:	Name	Sequence	Note
31	variant HSA (C34S)	DAHKSEVAHRFKDLGGEENFKALVLIAFAQYLQQSPFEDHVKLNEVTEFAKTCVADESAE NCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNECFLQHKDDNPNLRLVRPEV DVMCTAFHDNEETFLLKKYLYEARRHPYFYAPELFFAKRYKAAFTECCQAADKAACLLPKL DELRDEGKASSAKQRLKCASLQKFGERAFKAWAVARLSQRFPAEFAEVSKLVTDLTKVH TECCHGDLLECADDRADLAKYICENQDSISSKLKECCEKPLEKSHCIAEVENDEMPADLPS LAADFVESKDVCNKYAEAKDVLGMFLYFYARRHPDYSVLLRLAKTYETTLKCCAAAD PHECYAKVFDEFKPLVEEPQNLKQNCLEFELQGEYKFQNALLVRYTKKVPQVSTPTLVEVS RNLGKVGSKCCKHPEAKRMPCAEDYLSVVLNQLCVLHEKTPVSDRVTKCCTESLVNRRPC FSALEVDETYVPKEFNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQLKAVMD DFAAFVEKCKCKADDKETCFAEEGKKLVAASQAALGL	

Table 4: IL15 sequences

SEQ ID NO:	Name	Sequence	Note
4	wildtype human IL15	NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTA MKCFLELQVISLESGDASIHDTVENLIILANNSLSSNGN VTESGCKECEEELEEKNIKEFLQSFVHIVQMFINTS	NCBI Reference Sequence: NP_000576.1 without signal peptide and propeptide
40	variant human IL15	X ₁ NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTA MKCFLELQVISLESGDASIHDTVENLIILAX ₂ NX ₃ LSSNG X ₄ VX ₅ ESGCKECEEELEEKNIKEFLQSFVHIVQMFIX ₆ TX ₇	Each X may be any amino acid. Optionally, X positions (N1, N71, S73, N79, T81, N112, S114) may individually be substituted relative to SEQ ID NO: 1. For example, N may be substituted to a non-N amino acid, and T or S may be substituted to a non-T non-S amino acid.
41	variant human IL15 (S73A, T81A)	NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTA MKCFLELQVISLESGDASIHDTVENLIILANNSLSSNGN VAESGCKECEEELEEKNIKEFLQSFVHIVQMFINTS	

Table 5: linker sequences

SEQ ID NO:	Name	Sequence
51	G4S linker unit	GGGGS
52	G3S linker unit	GGGS
53	G5S linker unit	GGGGGS
54	GSGGGS linker unit	GSGGGS
55	GGSGS linker unit	GGSGS
56	GSGSG linker unit	GSGSG
58	(G4S) ₂ linker	GGGGSGGGGS
59	(G4S) ₃ linker	GGGGSGGGGSGGGGS

Table 6: Immunoglobulin constant region sequences

SEQ ID NO:	Name	Sequence	Note
61	Human IgG1 CH1 domain reference 1	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVPSSSLGTQTYICNVNHKPSNTKV DKKVEPKSC	
62	Human IgG1 CH1 domain reference 2	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVPSSSLGTQTYICNVNHKPSNTKV DKRVEPKSC	
63	Human IgG1 hinge reference	EPKSCDKTHTCPPCP	
64	Human IgG1 CH2 domain reference	APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK	
65	Human IgG1 CH3 domain reference 1	GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG	May further contain lysine (K) at the C-terminus
66	Human IgG1 CH3 domain reference 2	GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG	May further contain lysine (K) at the C-terminus
67	Human IgG1 CH3 domain reference 3	GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPPVLDSDGSFFLYSKLTVDKSRWQQGNIFSCSVMHEALHNHYTQKSLSLSPG	May further contain lysine (K) at the C-terminus
68	Human IgG1 CH3 domain reference 4	GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEGLHNHYTQKSLSLSPG	May further contain lysine (K) at the C-terminus
71	Human IgG4 CH1 domain reference 1	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVPSSSLGTQTYTCNVDHKPSNTKV DKRV	
73	Human IgG4 hinge reference	ESKYGPPCPSCP	
74	Human IgG4 CH2 domain reference	APEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAK	
75	Human IgG4 CH3 domain reference	GQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSPG	May further contain lysine (K) at the C-terminus

Table 7: IL2RA sequences

SEQ ID NO:	Name	Sequence	Note
8	wildtype human IL2RA	ELCDDDPPEIPHATFKAMAYKEGTM LNCECKRGFRRIK SGSLYMLCTGNSSHSSWDNQCQCTSSATRN TTKQVTP QPEEQKERKTTEMQSPMQPVDQASLP GHCREPPPWE NEATERIYHFVVGQMVYYQCVQGYRALHRGPAESVCK MTHGKTRWTQPQLICTGEMETSQFPGEEKPQASPEGR PESETSCLVTTTDFQIQTEMAATMETSIFTTEYQVAVAG CVFLLISVLLLSGLTWQRRQRKSRRTI	NCBI Reference Sequence: NP_000408.1 without signal peptide. Positions 1-219 = extracellular domain; positions 220-238 = transmembrane domain; and positions 239-251 = intracellular domain.
80	variant human IL2RA (positions 1-165)	ELCDDDPPEIPHATFKAMAYKEGTM LNCECKRGFRRIK SGSLYMLCTGX ₂ SSHSSWDNQCQCTSSATRX ₂ TTKQVT PQPEEQKERKTTEMQSPMQPVDQASLP GHCREPPPW ENEATERIYHFVVGQMVYYQCVQGYRALHRGPAESVC KMTHGKTRWTQPQLICTG	Each X may be any amino acid. Optionally, X positions (N49 and N68) may individually be substituted relative to SEQ ID NO: 8.
81	variant human IL2RA (positions 1-165)	ELCDDDPPEIPHATFKAMAYKEGTM LNCECKRGFRRIK SGSLYMLCTGSSSSHSSWDNQCQCTSSATRQT TTKQVTP QPEEQKERKTTEMQSPMQPVDQASLP GHCREPPPWE NEATERIYHFVVGQMVYYQCVQGYRALHRGPAESVCK MTHGKTRWTQPQLICTG	

Table 8: Cytokine hybrid protein and control protein sequences (amino acid and nucleic acid sequences)

SEQ ID NO:	Name	Sequence	Note
90	IL2-IL2RA	SKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLTGGSSS TKKTQLQLEHLLDLQMLNGINNYKNPKLTRMLTFKFYMPKKATELKHLQCLEELKPLE EVLNLAQSGGGSELCDDEPPEIPHATFKAMAYKEGTMLNCECKRGFRRIKSGSLYMLC TGSSSHSSWDNQCQCTSSATRQTTKQVTPQPEEQKERKTTEMQSPMQPVDQASLPGH CREPPPWENEATERIYHFVVGQMVVYQCVQGYRALHRGPAESVCKMTHGKTRWTQP QLICTGGGGGSDAHKSEVAHRFKDLGEENFKALVLI AFAQYLQQSPFEDHV KLVNEVTEF AKTCVADESAENCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNECFLQHKDD NPNLRLVRPEVDVMCTAFHDNEETFLKKYLYEIARRHPYFYAPELLFFAKRYKAAFECC QAADKAACLLPKLDEL RDEGKASSAQRLKASLQKFGERAFAKAWAVARLSQRFPKAEF AEVSKLVTDLT KVHTECCHGDLLECADDRADLAKYICENQDSISSKLKECCEKPLLEKSHCI AEVENDEMPADLPSLAADFVESKDVCKNYAEAKDVFLGMFLY EYARRHPDYSV VLLLRL AKTYETTL EKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNC ELFQ LGEYKFQNAL LV R YTKKVPQVSTPTLVEVSRNLGKVGSKCKHPEAKRMPCAEDYLSVVLNQLCVLHEKTPVS DRVTKCCTESLVNRRPCFSALEVDETYVPKEFNAETFTFHADICTLSEKERQIKKQTALVEL VKHKPKATKEQLKAVMDDFAAFVEKCKCADDKETCF AE EGKLV AASQAALGL	A tag may be added to the N- or C-terminus (e.g., 6His tag (SEQ ID NO: 120) at the N-terminus)
91	IL2-IL15RA chain of IL2-IL15 (Format 1)	APTSSSTKKTQLQLEHLLDLQMLNGINNYKNPKLTRMLTAKFAMPKKATELKHLQCLE EELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWIT FSQSIISTLTGGGGSGGGGSITCPPPMSVEHADIWVKSYSLSRERYICNSGFKRKAGTSSL TECVLNKATNVAHWTTPSLKCIRD	A tag may be added to the N- or C-terminus (e.g., 6His tag (SEQ ID NO: 120) at the N-terminus)
92	HSA-IL15 chain of IL2-IL15 (Format 1)	DAHKSEVAHRFKDLGEENFKALVLI AFAQYLQQSPFEDHV KLVNEVTEFAKTCVADESAE NCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNECFLQHKDDNPNLRLVRPE VDVMCTAFHDNEETFLKKYLYEIARRHPYFYAPELLFFAKRYKAAFECCQAADKAACLLP KLDEL RDEGKASSAQRLKASLQKFGERAFAKAWAVARLSQRFPKAEFAEVSKLVTDLT KVHTECCHGDLLECADDRADLAKYICENQDSISSKLKECCEKPLLEKSHCIAEVENDEMPAD LPSLAADFVESKDVCKNYAEAKDVFLGMFLY EYARRHPDYSV VLLLRLAKTYETTL EKCCA AADPHECYAKVFDEFKPLVEEPQNLIKQNC ELFQ LGEYKFQNAL LV RYTKKVPQVSTPT LVEVSRNLGKVGSKCKHPEAKRMPCAEDYLSVVLNQLCVLHEKTPVSDRVTKCCTESLV NRRPCFSALEVDETYVPKEFNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKATKEQL KAVMDDFAAFVEKCKCADDKETCF AE EGKLV AASQAALGLGGGGSGGGGSNWNVI SDLKKIEDLIQSMHIDATLYTESDVHPCKVTAMKCFLELQVISLESGDASIHTVENLIIL ANNALSSNGNVAESGCKECEEEKNIFEQLQSFVHIVQMFINTS	A tag may be added to the N- or C-terminus (e.g., 6His tag (SEQ ID NO: 120))

SEQ ID NO:	Name	Sequence	Note
93	IL2-IL15RA chain of IL2-IL15 (Format 1)	GCCCCAACCTCCTCCTCCACCAAGAAGACCCAATTGCAACTGGAGCACTTGCTGTTG GACTTGCAAATGATCCTGAACGGTATCAACAACCTACAAGAACCCAAAGCTGACCAGA ATGCTGACCGCTAAGTTCGCTATGCCAAAGAAGGCTACCGAGCTGAAGCACTTGCAA TGTTTGGAGGAGGAGTTGAAGCCATTGGAGGAGGTCCTGAACTTGGCTCAATCCAA GAACTTCCACCTGAGACCAAGAGACTTGATCTCCAACATCAACGTCATCGTCTTGGA GCTGAAGGGTCCGAGACCACCTTCATGTGTGAGTACGCTGACGAGACCGCTACCAT CGTCGAGTTCTTGAACAGATGGATCACCTTCTCCAATCCATCATCTCCACCCTGACC GGTGGTGGAGTTCTGGTGGTGGTGGATCTATTACATGCCCTCCCCCTATGTCCGTA GAACATGCAGACATCTGGGTAAAGTCATACTCCTTGATTCCAGAGAAAGGTACATC TGTAACCTCCGGCTTCAAAAGGAAGGCTGGTACGTCATCTCTAACCGAGTGTGTTTTG AACAAGGCAACGAATGTCGCACATTGGACCACACCATCTCTTAAGTGTATCAGGGAC TAA	A tag-encoding sequence may be added to the N- or C-terminus (e.g., 6His tag (SEQ ID NO: 120)-encoding sequence at the N-terminus)

SEQ ID NO:	Name	Sequence	Note
94	HSA-IL15 chain of IL2-IL15 (Format 1)	GACGCTCACAAGTCTGAAGTTGCTCACAGATTCAAGGACTTGGGTGAAGAAAACCTC AAGGCTTTGGTTTTGATTGCTTCGCTCAATACTTGCAACAATCTCCATTGGAAGACC ACGTTAAGTTGGTTAACGAAGTTACTGAATTTGCTAAGACTTGTGTTGCTGACGAAT CTGCTGAAAACGTGACAAGTCTTTCACACTTTGTTCCGGTGACAAGTTGTGTACTGT TGCTACTTTGAGAGAAAACCTACGGTGAAATGGCTGACTGTTGTGCTAAGCAAGAACC AGAAAGAAAACGAATGTTTCTTGCAACACAAGGACGACAACCCAAACTTGCCAAGATT GGTTAGACCAGAAGTCGACGTTATGTGTACTGCTTCCACGACAACGAAGAACTTT CTTGAAGAAGTACTTGTACGAAATTGCTAGAAGACACCCATACTTCTACGCTCCAGA ATTGTTGTTCTTCGCTAAGAGATACAAGGCTGCTTCACTGAATGTTGTCAAGCTGCT GACAAGGCTGCTTGTGTTGTTGCCAAAGTTGGACGAATTGAGAGACGAAGGTAAGGC TTCTTCTGCTAAGCAAAGATTGAAGTGTGCTTCTTTCAAAAGTTCCGGTGAAAGAGC TTTCAAAGCTTGGGCTGTTGCTAGATTGTCTCAAAGATCCCAAAGGCTGAATTTGCT GAAGTTTCTAAGTTGGTTACTGACTTGACTAAGGTTTCACTGAATGTTGTCACGGT GACTTGTTGGAATGTGCTGACGACAGAGCTGACTTGGCTAAGTACATTTGTGAAAAC CAAGACTCTATTTCTTAAGTTGAAGGAATGTTGTGAAAAGCCATTGTTGGAAGAG TCTCACTGTATTGCTGAAGTTGAAAACGACGAAATGCCAGCTGACTTGCCATCTTTG GCTGCTGACTTCGTTGAATCTAAGGACGTTTGTAGAAGTACGCTGAAGCTAAGGAC GTTTTCTTGGGTATGTTCTTGACGAATACGCTAGAAGACACCCAGACTACTCTGTTG TTTTGTTGTTGAGATTGGCTAAGACTTACGAACTACTTTGGAAGAGTTGTGTCGG CCGCTGACCCACACGAATGTTACGCTAAGGTTTTGACGAATTTAAGCCATTGGTTG AAGAACCACAAAACCTTGATTAAGCAAACTGTGAATTGTTGCAACAATTGGGTGAAT ACAAGTTCCAAAACGCTTTGTTGGTTAGATACACTAAGAAGTTCCACAAGTTTCTAC TCCAACTTTGGTTGAAGTTTCTAGAAAACCTGGGTAAAGGTTGGTTCTAAGTGTGTAA GCACCCAGAAGCTAAGAGAATGCCATGTGCTGAAGACTACTTGTCTGTTGTTTTGAA CCAATTGTGTGTTTTGCACGAAAAGACTCCAGTTTCTGACAGAGTTACTAAGTGTGT ACTGAATCTTTGGTTAACAGAAGACCATGTTTCTCTGCTTTGGAAGTTGACGAACTT ACGTTCCAAAGGAATTTAACGCTGAACTTTCACTTTCCACGCTGACATTTGTACTTT GTCTGAAAAGGAAAGACAAATTAAGAAGCAAACCTGCTTTGGTTGAATTGGTTAAGC ACAAGCCAAAGGCTACTAAGGAACAATTGAAGGCTGTTATGGACGACTTCGCTGCTT TCGTTGAAAAGTGTTGAAGGCTGACGACAAGGAACTTGTTCGCTGAAGAAGGT AAGAAGTTGGTTGCTGCTTCTCAAGCTGCTTTGGGTTGGGTGGTGGAGTTCTGGT GGTGGTGGATCTAACTGGGTTAACGTTATCTCCGACTTGAAGAAGATCGAGGACTTG ATCCAATCCATGCACATCGACGCTACCTGTACACCGAGTCCGACGTTCACCATCCT GTAAGGTTACCGCTATGAAGTGTCTTGTGGAGTTGCAAGTTATCTCCTTGGAGTC CGGTGACGCTTCCATCCACGACACCGTTGAGAACTTGATCATCTTGGCTAACAACGC TTTGTCTCCAACGGAACGTTGCTGAGTCCGGTTGTAAGGAGTGTGAGGAGTTGG AGGAGAAGAACATCAAGGAGTTCTTGCAATCCTTCGTTACATCGTTCAAATGTTTCA CAACACCTCTTAA	A tag-encoding sequence may be added to the N- or C-terminus (e.g., 6His tag (SEQ ID NO: 120)-encoding sequence)

CLAIMS

What is claimed is:

1. A cytokine hybrid protein, comprising:

- (a) an (interleukin 2) IL2 domain; and
- (b) an (interleukin 15) IL15 domain.

2. The cytokine hybrid protein of **claim 1**, which:

(I) does not bind to human IL2 receptor subunit alpha (IL2RA) or displays reduced binding to human IL2RA relative to wildtype human IL2, optionally wherein:

(i) the wildtype human IL2 comprises the amino acid sequence of SEQ ID NO: 1; and/or

(ii) the human IL2RA comprises the amino acid sequence of SEQ ID NO: 8, positions 1-219 of SEQ ID NO: 8, or positions 1-165 of SEQ ID NO: 8; and/or

(II) (i) the amino acid sequence of the IL2 domain comprises at least one amino acid substitution, insertion, or deletion (relative to SEQ ID NO: 1) which blocks or reduces binding to human IL2RA;

(ii) the IL2 domain comprises at least one amino acid modification, optionally a chemical modification or conjugation, which blocks or reduces binding to human IL2RA; and/or

(iii) the cytokine hybrid protein further comprises a IL2RA domain which is bound to the IL2 domain, optionally wherein:

(iii-1) the IL2 domain comprises a wildtype human IL2 amino acid sequence, optionally SEQ ID NO: 1, or a variant thereof and/or

(iii-2) the IL2RA domain comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 98%, at least 99%, or at least 99.5% identity to SEQ ID NO: 8, optionally comprising the amino acid sequence of SEQ ID NO: 80 or 81; and/or

(III) the cytokine hybrid protein further comprises a IL15 receptor subunit alpha (IL15RA) domain which is bound to the IL15 domain, optionally wherein the IL15 domain comprises a wildtype human IL15 amino acid sequence, optionally SEQ ID NO: 4, or a variant thereof.

3. The cytokine hybrid protein of **claim 1 or 2**, wherein the IL2 domain comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 98%, at least 99%, or at least 99.5% identity to SEQ ID NO: 1,

optionally wherein the IL2 domain comprises an amino acid substitution, insertion, or deletion at one or more of amino acid positions 3, 35, 38, 41, 42, 43, 45, 61, 62, 65, 68, 69, 72, 80, 81, 85, 86, 92, and/or 125 relative to SEQ ID NO: 1,

further optionally wherein the IL2 domain comprises an amino acid substitution (relative to SEQ ID NO: 1) at:

- (i) positions 42 and 45;
- (ii) position 38;
- (iii) position 62;
- (iv) position 65;
- (v) position 72;
- (vi) positions 42 and 45 and one or more of positions 38, 62, 65, and/or 72;
- (vii) position 45;
- (viii) positions 43 and 45;
- (ix) positions 35, 38, 43, and 45;
- (x) positions 35, 38, and 72;
- (xi) positions 42, 45, and 72;
- (xii) positions 38, 42, 45, and 62;
- (xiii) positions 80, 81, 85, 86, and 92; or
- (xiv) positions 38, 43, and 61,
- (xv) F42A and Y45A;
- (xvi) R38A;
- (xvii) E62A;
- (xviii) P65A;
- (xix) L72A;
- (xx) R38A, F42A, and Y45A;
- (xxi) F42A, Y45A, and E62A;
- (xxii) F42A, Y45A, and P65A;
- (xxiii) F42A, Y45A, and L72A;
- (xxiv) R38A, F42A, Y45A, and E62A;
- (xxv) R38A, F42A, Y45A, and P65A;
- (xxvi) R38A, F42A, Y45A, and L72A;

- (xxvii) F42A, Y45A, E62A, and P65A;
- (xxviii) F42A, Y45A, E62A, and L72A;
- (xxix) F42A, Y45A, P65A, and L72A;
- (xxx) R38A, F42A, Y45A, E62A, and P65A;
- (xxxi) R38A, F42A, Y45A, E62A, and L72A;
- (xxxii) R38A, F42A, Y45A, P65A, and L72A;
- (xxxiv) F42A, Y45A, E62A, P65A, and L72A;
- (xxxv) R38A, F42A, Y45A, E62A, P65A, and L72A;
- (xxxvi) Y45S;
- (xxxvii) K43A and Y45A;
- (xxxviii) K35A, R38A, K43A, and Y45A;
- (xxxix) K35A, R38A, and L72G;
- (xl) F42A, Y45A, and L72G;
- (xli) R38A, F42A, Y45A, and E62A;
- (xlii) L80F, R81D, L85V, I86V, and I92F; or
- (xlili) R38D, K43E, and E61R,

optionally wherein in any of (i) to (xlili) the IL2 domain further comprises a substitution at position 125, optionally C125S or C125A.

4. The cytokine hybrid protein of **any one of claims 1-3**, wherein the IL2 domain comprises the amino acid sequence of SEQ ID NO: 10, optionally wherein:

- (i) X₅ and X₇ are A and A, respectively;
- (ii) X₃ is A;
- (iii) X₉ is A;
- (iv) X₁₀ is A;
- (v) X₁₃ is A;
- (vi) X₃, X₅, and X₇ are A, A, and A, respectively;
- (vii) X₅, X₇, and X₉ are A, A, and A, respectively;
- (viii) X₅, X₇, and X₁₀ are A, A, and A, respectively;
- (ix) X₅, X₇, and X₁₃ are A, A, and A, respectively;

- (x) X₃, X₅, X₇, and X₉ are A, A, A, and A, respectively;
- (xi) X₃, X₅, X₇, and X₁₀ are A, A, A, and A, respectively;
- (xii) X₃, X₅, X₇, and X₁₃ are A, A, A, and A, respectively;
- (xiii) X₅, X₇, X₉, and X₁₀ are A, A, A, and A, respectively;
- (xiv) X₅, X₇, X₉, and X₁₃ are A, A, A, and A, respectively;
- (xv) X₇, X₉, X₁₀, and X₁₃ are A, A, A, and A, respectively;
- (xvi) X₃, X₅, X₇, X₉, and X₁₀ are A, A, A, A, and A, respectively;
- (xvii) X₃, X₅, X₇, X₉, and X₁₃ are A, A, A, A, and A, respectively;
- (xviii) X₃, X₅, X₇, X₁₀, and X₁₃ are A, A, A, A, and A, respectively;
- (xix) X₅, X₇, X₉, X₁₀, and X₁₃ are A, A, A, A, and A, respectively;
- (xx) X₃, X₅, X₇, X₉, X₁₀, and X₁₃ are A, A, A, A, A, and A, respectively;
- (xxi) X₇ is S;
- (viii) X₆ and X₇ are A and A, respectively;
- (ix) X₂, X₃, X₆, and X₇ are A, A, A, and A, respectively;
- (x) X₂, X₃, and X₁₃ are A, A, and G, respectively;
- (xi) X₅, X₇, and X₁₃ are A, A, and G, respectively;
- (xii) X₃, X₅, X₇, and X₉ are A, A, A, and A, respectively;
- (xiii) X₁₄, X₁₅, X₁₆, X₁₇, and X₁₈ are F, D, V, V, and F, respectively; or
- (xiv) X₃, X₆, and X₈ are D, E, and R, respectively,

optionally wherein X₁₃ is not C, further optionally wherein X₁₃ is S or A.

5. The cytokine hybrid protein of **any one of claims 1-4**, wherein the IL2 domain comprises the amino acid sequence of SEQ ID NO: 11 or any one of SEQ ID NOS: 101-119.

6. The cytokine hybrid protein of **any one of claims 1-5**, which comprises:

- (a) the IL2 domain according to any one of **claims 2(III)(i), 4, and 5**;
- (b) the IL15 domain; and
- (c) a/the IL15RA domain; and
- (d) optionally one or more scaffold domains,

optionally wherein:

- (b) the IL15 domain comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 98%, at least 99%, or at least 99.5% identity to SEQ ID NO: 4,

optionally wherein the IL15 domain comprises the amino acid sequence of SEQ ID NO: 40, further optionally wherein X_1 , X_2 , X_4 , and/or X_6 is/are an amino acid which is not N and/or one or more of X_3 , X_5 , and/or X_7 is/are an amino acid which is not S or T,

yet further optionally wherein the IL15 domain comprises the amino acid sequence of SEQ ID NO: 41;

- (c) the IL15RA domain comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 98%, at least 99%, or at least 99.5% identity to SEQ ID NO: 2, positions 1-175 of SEQ ID NO: 2, or positions 1-66 of SEQ ID NO: 2,

optionally wherein the IL15RA domain comprises the amino acid sequence of SEQ ID NO: 21; and/or

- (d) each of the one or more scaffold domains is or comprises:

- (i) an albumin domain, optionally human albumin (HSA) domain,

optionally wherein the HSA domain comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 98%, at least 99%, or at least 99.5% identity to SEQ ID NO: 3,

further optionally wherein the HSA domain comprises the amino acid sequence of SEQ ID NO: 31;

- (ii) one or more immunoglobulin constant domains, optionally comprising a CH1 domain, a CH2 domain, and/or CH3 domain, further optionally wherein:

(ii-1) the CH1 domain is of an IgG1, IgG2, IgG3, or IgG4 isotype and/or comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 98%, at least 99%, at least 99.5%, or 100% identity to SEQ ID NO: 61, 62, or 71;

(ii-2) the CH2 domain is of an IgG1, IgG2, IgG3, or IgG4 isotype and/or comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 98%, at least 99%, at least 99.5%, or 100% identity to SEQ ID NO: 64 or 74;

(ii-3) the CH3 domain is of an IgG1, IgG2, IgG3, or IgG4 isotype and/or comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 98%, at least 99%, at least 99.5%, or 100% identity to SEQ ID NO: 65, 66, 67, 68, or 75;

- (iii) an immunoglobulin hinge,

optionally wherein the immunoglobulin hinge is of an IgG1, IgG2, IgG3, or IgG4 isotype and/or comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 98%, at least 99%, at least 99.5%, or 100% identity to SEQ ID NO: 63 or 73; and/or

(iv) an immunoglobulin fragment crystallizable (Fc) domain,

optionally wherein the Fc domain is of an IgG1, IgG2, IgG3, or IgG4 isotype and/or comprises the amino acid sequence of the immunoglobulin hinge according to (iii) or a partial sequence thereof, the amino acid sequence of the CH2 domain according to (ii-2), and the amino acid sequence of the CH3 domain according to (ii-3),

further optionally the Fc domain comprises one or more amino acid substitutions which increases *in vivo* half-life and/or which reduces effector functions, optionally one or more of: a substitution at one or more of positions 234, 235, 236, 237, 265, 297 and/or 329; N297A; N297Q; L234A and L235A; L235E; L234F, L235E, and P331S; L234F, L235Q, and K322Q; A330S and P331S; L234A, L235A, P329G; L234A and G237A; L234A, L235A, and G237A; L234A, L235A, G237A, P238S, H268A, A330S, and P330S; L234A and L235E; G236R and L328R; L234A, L235A, and K322A; M252Y, S254T, and T256E; D265A and P329A; M428L and N434S; N434A; R435H;

and optionally wherein the IL15 domain is bound to the IL15RA domain,

further optionally wherein the cytokine hybrid protein further comprises one or more linkers between two domains, optionally wherein at least one of the linker(s):

- (1) comprises one or more amino acids, optionally one, two, three, four, five, six, seven, eight, nine, ten, eleven, or twelve amino acids;
- (2) consists of small amino acids consisting of G, S, and/or A;
- (3) comprises an amino acid sequence which comprises or consists of the amino acid sequence selected from the group consisting of SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, G, GG, GGG, GS, SG, GGS, GSG, SGG, GSS, SGS, and SSG;
- (4) comprises an amino acid sequence which comprises or consists of multiple repeats, optionally two, three, four, or five repeats, of the amino acid sequence selected from the group consisting of SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, G, GG, GGG, GS, SG, GGS, GSG, SGG, GSS, SGS, and SSG; and/or
- (5) comprises or consists of the amino acid sequence of SEQ ID NO: 58 or 59.

7. The cytokine hybrid protein of claim 6, wherein:

- (a) the IL2 domain comprises the amino acid sequence of SEQ ID NO: 11;
- (b) the IL15 domain comprises the amino acid sequence of SEQ ID NO: 41; and
- (c) the IL15RA domain comprises the amino acid sequence of SEQ ID NO: 21; and

(d) the cytokine hybrid protein at least scaffold domain which comprises a HSA domain comprising the amino acid sequence of SEQ ID NO: 31.

8. The cytokine hybrid protein of claim 6 or 7, which:

(I) comprises:

(A) a first polypeptide comprising the IL15RA domain; and

(B) a second polypeptide comprising the IL15 domain,

optionally wherein:

(i) the first polypeptide further comprises the IL2 domain; and/or

(ii) the second polypeptide further comprises the IL2 domain,

and optionally wherein:

(i) the first polypeptide further comprises the optional scaffold domain; and/or

(ii) the second polypeptide further comprises the optional scaffold domain,

further optionally wherein the cytokine hybrid protein comprises any of the formats depicted in **FIGS. 1B-1C and 1F-1G**, optionally Format 1; or

(II) comprises a polypeptide comprising the IL2 domain, the IL15 domain, and the IL15RA domain and optionally the optional scaffold domain,

optionally wherein the cytokine hybrid protein comprises any of the formats depicted in **FIG. 1D-1E**.

9. The cytokine hybrid protein of claim 8 (I), wherein:

(A) the first polypeptide comprising an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 98%, at least 99%, or at least 99.5% identity to SEQ ID NO: 91; and

(B) the second polypeptide comprising an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 98%, at least 99%, or at least 99.5% identity to SEQ ID NO: 92; or

wherein:

(A) the first polypeptide comprising the amino acid sequence of SEQ ID NO: 91; and

(B) the second polypeptide comprising the amino acid sequence of SEQ ID NO: 92.

10. An isolated nucleic acid, which encodes the cytokine hybrid protein of any one of claims 1-9.

11. The isolated nucleic acid **of claim 10**, which encodes the cytokine hybrid protein is according to claim 10(I), or 11 and optionally comprises:

- (A) a first nucleic acid encoding the first polypeptide; and
- (B) a second nucleic acid encoding the second polypeptide,

further optionally wherein:

- (A) the first nucleic acid comprises a nucleic acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 98%, at least 99%, at least 99.5%, or 100% identity to SEQ ID NO: 93; and/or
- (B) the second nucleic acid comprises a nucleic acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 98%, at least 99%, at least 99.5%, or 100% identity to SEQ ID NO: 94.

12. A vector comprising the isolated nucleic acid **of claim 10 or 11**, optionally wherein:

- (i) the vector is an expression vector; and/or
- (ii) the vector comprises a plasmid, a viral vector (optionally adenoviral, lentiviral, or retroviral), a lipid-based vector, a self-replicating RNA vector, a virus-like particle, a polymer-based vector, and/or a nanoparticle, optionally a lipid-based nanoparticle.

13. An isolated, and/or recombinant host cell or cells comprising the nucleic acid **of claim 10 or 11** or the vector **of claim 12**, optionally wherein the isolated and/or recombinant host cell or cells is/are:

- (i) non-mammalian, optionally bacterial, yeast, fungal, protozoa, plant, or insect, bacterial; or
- (ii) mammalian, optionally human, non-human primate, monkey, rabbit, rodent, hamster, rat, or mouse; or
- (iii) a population of cells comprising the isolated, recombinant, and/or host cell of (i) or (ii).

14. A composition comprising:

- (I) at least one of:
 - (a) the cytokine hybrid protein of **any one of claims 1-9**;
 - (b) the nucleic acid **of claim 10 or 11**;
 - (c) the vector **of claim 12**; and/or
 - (d) the isolated and/or recombinant host cell or a population of cells comprising **of claim 13**; and
- (II) a pharmaceutically acceptable carrier.

15. A method of treating a disease, disorder, or condition in a subject, the method comprising administering to the subject an effective amount of at least one of:

- (a) the cytokine hybrid protein of **any one of claims 1-9**;
- (b) the nucleic acid **of claim 10 or 11**;
- (c) the vector **of claim 12**; and/or
- (d) the isolated and/or recombinant host cell or a population of cells **of claim 13**; and/or
- (e) the composition **of claim 14**,

optionally wherein:

(i) the subject is

- (i-1) a mammal, optionally a human, a non-human primate, a monkey, a horse, a cow, sheep, a goat, a pig, a dog, a cat, a rabbit, a rodent, a hamster, a rat, or a mouse; or
- (i-2) a non-mammalian vertebrate, optionally a bird, fish, an amphibian, or a reptile; and/or

(ii) the method further comprises administering to the subject an additional agent, optionally an adjuvant or a therapeutic agent.

16. The method **of claim 15**, optionally wherein the disease, disorder, or condition comprises cancer, an infectious disease, or another disease, optionally wherein:

(i) the cancer is:

- (i-1) a solid cancer, optionally chosen from: one or more of mesothelioma, malignant pleural mesothelioma, non-small cell lung cancer, small cell lung cancer, squamous cell lung cancer, large cell lung cancer, pancreatic cancer, pancreatic ductal adenocarcinoma, esophageal adenocarcinoma, breast cancer, glioblastoma, ovarian cancer, colorectal cancer, prostate cancer, cervical cancer, skin cancer, melanoma, renal cancer, liver cancer, brain cancer, thymoma, sarcoma, carcinoma, uterine cancer, kidney cancer, gastrointestinal cancer, urothelial cancer, pharynx cancer, head and neck cancer, rectal cancer, esophagus cancer, or bladder cancer, or a metastasis thereof; and/or
- (i-2) a liquid cancer, optionally chosen from: chronic lymphocytic leukemia (CLL), mantle cell lymphoma (MCL), multiple myeloma, acute lymphoid leukemia (ALL), Hodgkin lymphoma, B-cell acute lymphoid leukemia (BALL), T-cell acute lymphoid leukemia (TALL), small lymphocytic leukemia (SLL), B cell prolymphocytic leukemia, blastic plasmacytoid dendritic cell neoplasm, Burkitt's lymphoma, diffuse large B cell lymphoma (DLBCL), DLBCL associated with chronic inflammation, chronic myeloid leukemia, myeloproliferative neoplasms, follicular lymphoma, pediatric follicular lymphoma, hairy cell leukemia, small cell- or a large cell-follicular lymphoma, malignant lymphoproliferative conditions, MALT lymphoma (extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue), Marginal zone lymphoma, myelodysplasia, myelodysplastic syndrome, non-Hodgkin lymphoma, plasmablastic lymphoma, plasmacytoid dendritic cell neoplasm, Waldenstrom macroglobulinemia, splenic marginal zone lymphoma, splenic lymphoma/leukemia, splenic

- diffuse red pulp small B-cell lymphoma, hairy cell leukemia-variant, lymphoplasmacytic lymphoma, a heavy chain disease, plasma cell myeloma, solitary plasmacytoma of bone, extraosseous plasmacytoma, nodal marginal zone lymphoma, pediatric nodal marginal zone lymphoma, primary cutaneous follicle center lymphoma, lymphomatoid granulomatosis, primary mediastinal (thymic) large B-cell lymphoma, intravascular large B-cell lymphoma, ALK+large B-cell lymphoma, large B-cell lymphoma arising in HHV8-associated multicentric Castleman disease, primary effusion lymphoma, B-cell lymphoma, acute myeloid leukemia (AML), or unclassifiable lymphoma; and/or
- (iv) the infectious disease is a viral, bacterial, fungal, yeast, protozoan, prion or parasitic disease, optionally wherein (1) the viral disease is human immunodeficiency virus (HIV), hepatitis virus (optionally hepatitis A, B, or C virus), human papillomavirus (HPV), herpes simplex virus (HSV) (optionally HSV-1 or HSV-2), enterovirus, human cytomegalovirus, adenovirus, rhinovirus, Pox virus, Influenza virus, coronavirus (optionally MERS-CoV, SARS-CoV, or SARS-CoV-2, or common human coronavirus), norovirus, West Nile Virus, Zika virus, poliovirus, Ebola virus, or dengue virus (DENV) infection, (2) the bacterial disease is *Salmonella*, *Escherichia coli*, *Mycobacterium tuberculosis*, *methicillin-resistant staphylococcus aureus* (MRSA), *Clostridium difficile*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Helicobacter pylori*, *Neisseria gonorrhoeae*, *Vibrio vulnificus*, and/or (3) the fungal disease is *Aspergilliosis*, *Candida*, *Candida auris*, *Cryptococcus neoformans*, *Pneumocystis jirovecii*, *Mucoromycetes*, *Talaromyces*, ringworm, *Blastomyces*, *Coccidioides*, *Cryptococcus gattii*, *Histoplasma*, *Paracoccidioides*, or *Sporothrix* infection.

17. A method of manufacturing the cytokine hybrid protein of **any one of claims 1-9**, comprising:

- (a) culturing cells comprising the nucleic acid **of claim 10 or 11** in a condition that allows for expression of said cytokine hybrid protein, and
- (b) harvesting and purifying the cytokine hybrid protein from the cell culture from (a).

18. A method of manufacturing the isolated, recombinant, and/or host cell or the population of cells **of claim 13**, comprising introducing the nucleic acid **of claim 10 or 11** and/or the vector **of claim 12** into one or more cells, optionally wherein the introducing occurs *in vitro*, *ex vivo*, or *in vivo*.

19. The cytokine hybrid protein of **any one of claims 1-9**, the nucleic acid **of claim 10 or 11**, the vector **of claim 12**, the isolated and/or recombinant host cell or the population of cells **of claim 13**, and/or the composition **of claim 14**,

- (i) for use in medicine; and/or
- (ii) for use in treating a disease, disorder, or condition, optionally wherein the disease, disorder, or condition comprises any of those **according to claim 16**.

20. Use of the cytokine hybrid protein of **any one of claims 1-9**, the nucleic acid **of claim 10 or 11**, the vector **of claim 12**, the isolated and/or recombinant host cell or the population of cells **of claim 13**, and/or the composition **of claim 14**, for the manufacture of a medicament for treatment of a disease, disorder, or condition, optionally wherein the disease, disorder, or condition comprises any of those **according to claim 16**.

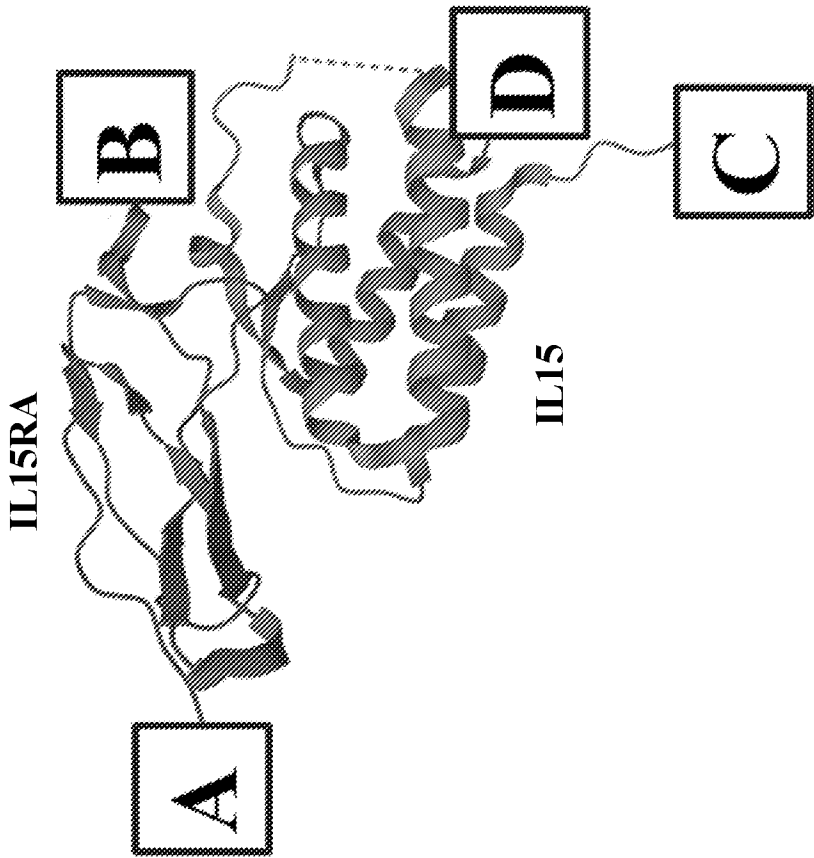


FIG. 1A

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FIG. 1B

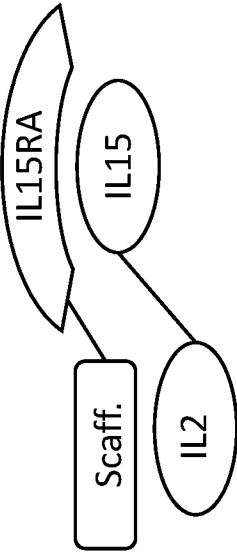
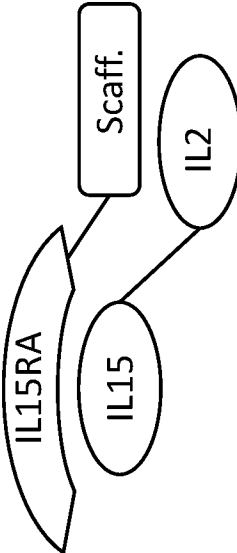
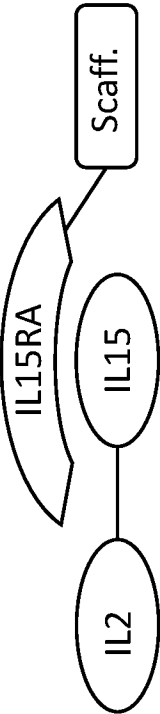
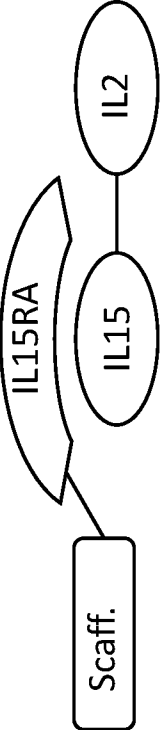
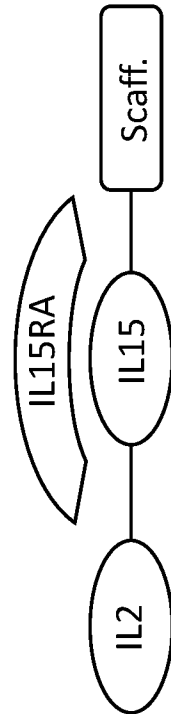
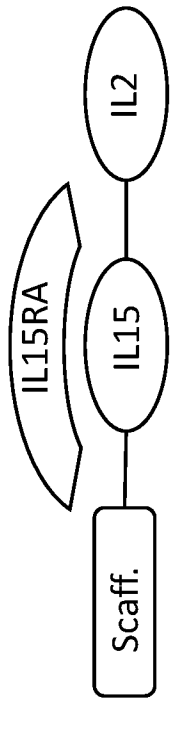
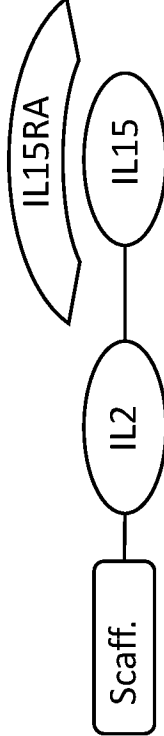
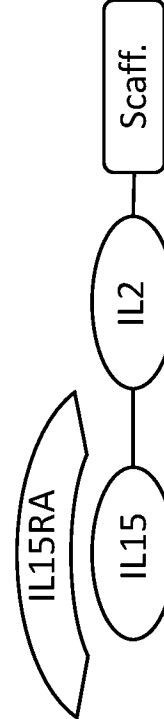
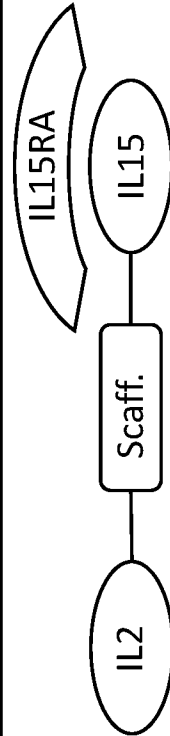
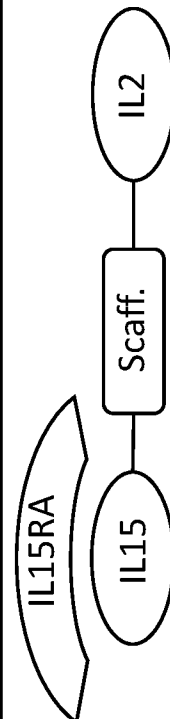
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FIG. 1C

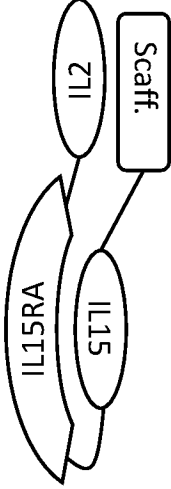
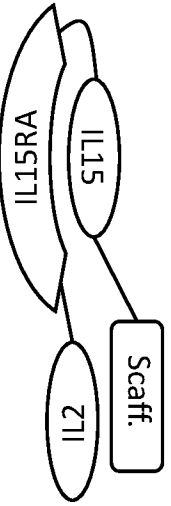
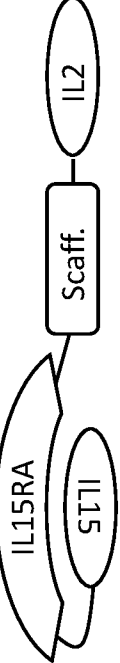
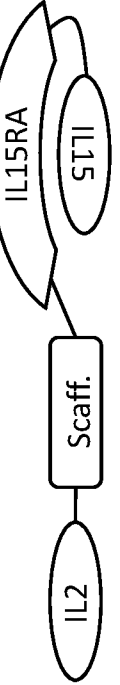
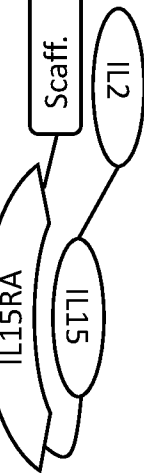
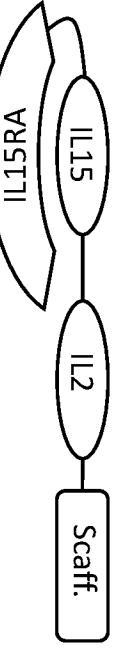
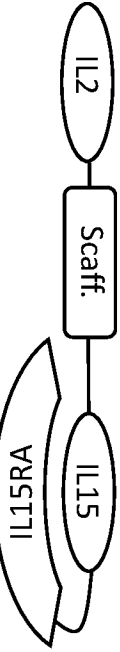
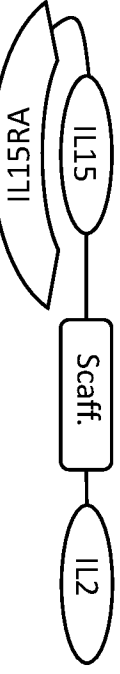
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FIG. 1D

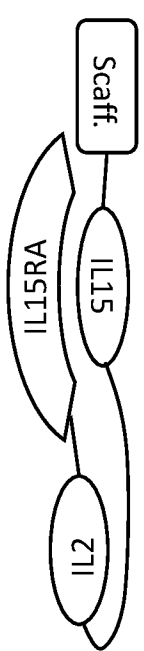
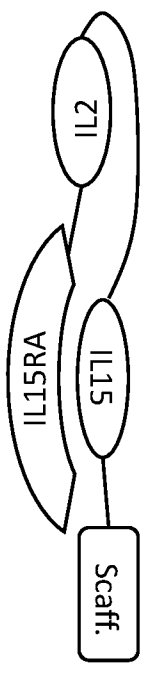
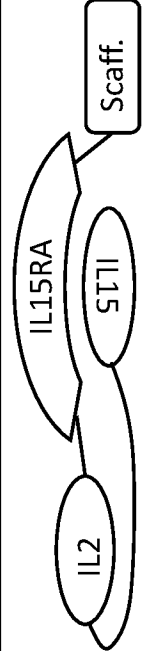
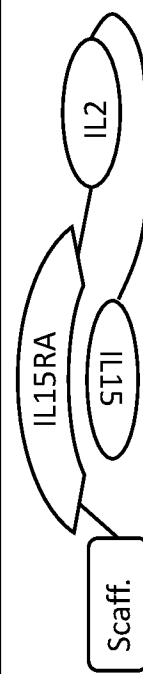
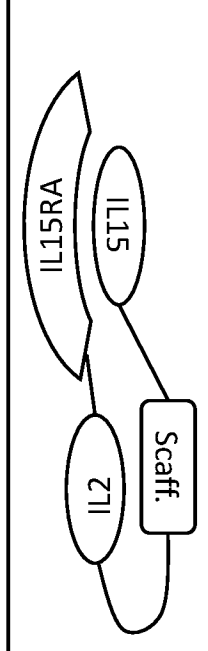
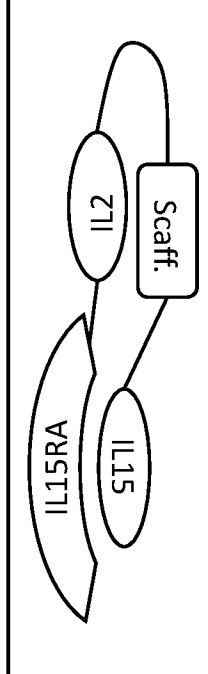
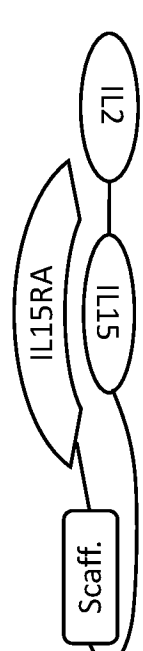
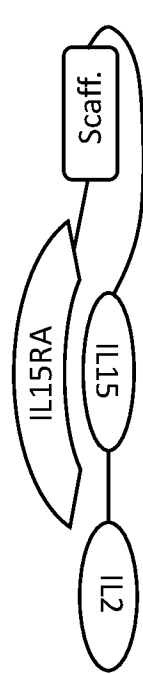
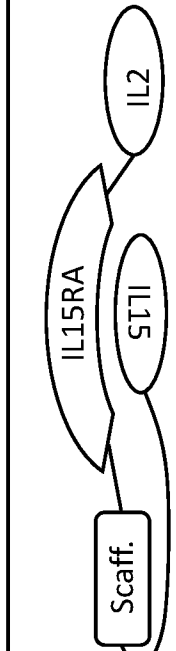
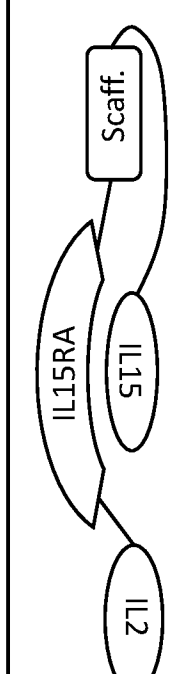
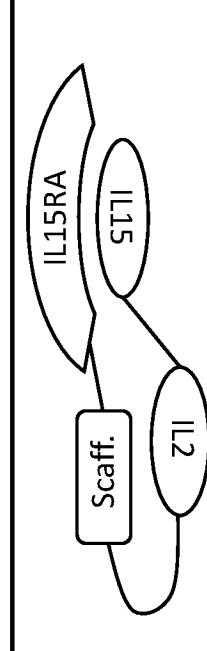
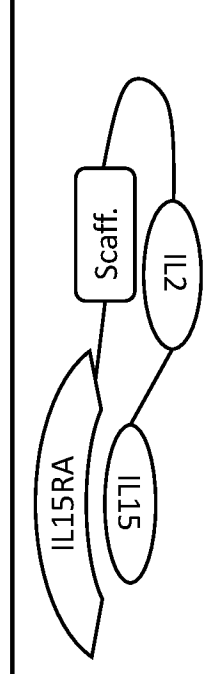
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FIG. 1E

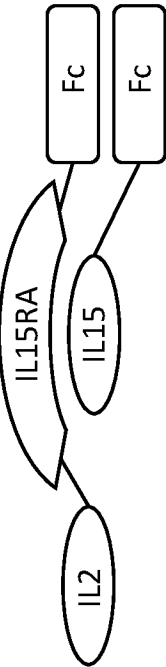
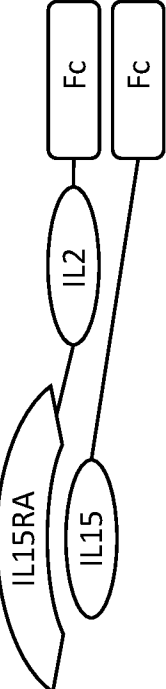
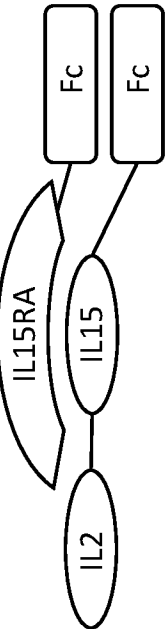
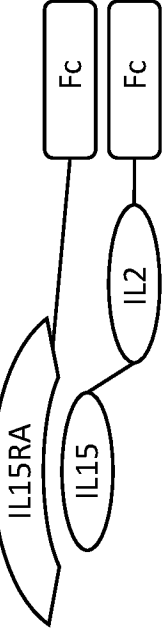
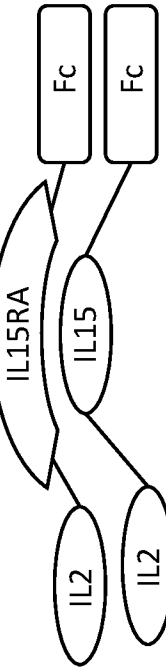
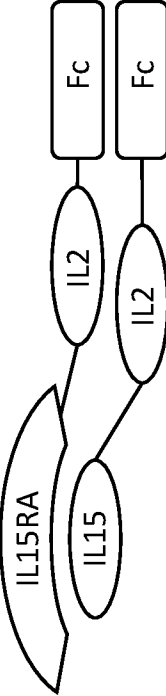
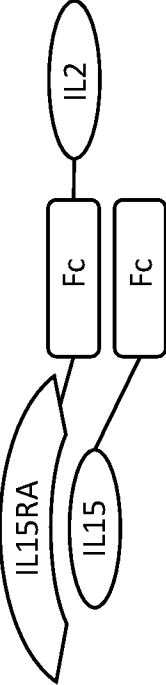
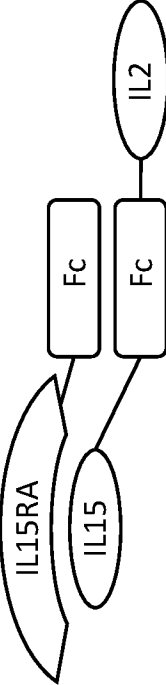
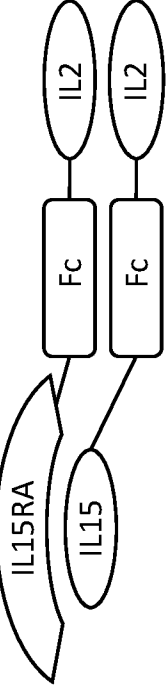
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FIG. 1F

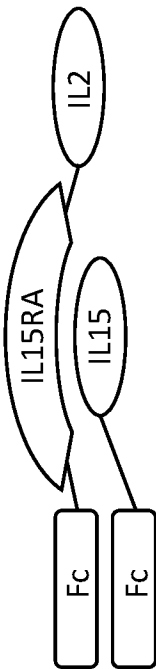
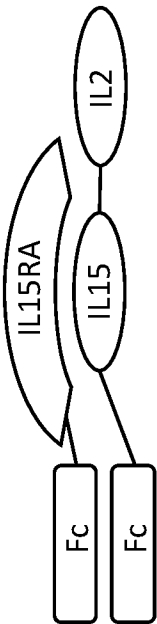
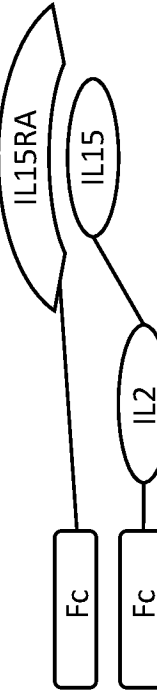
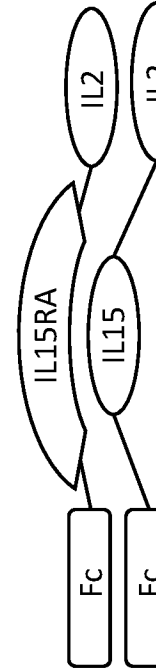
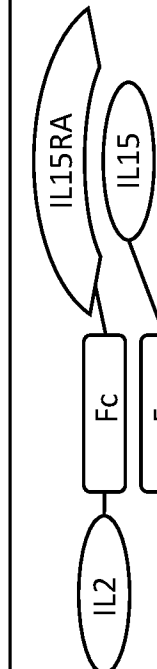
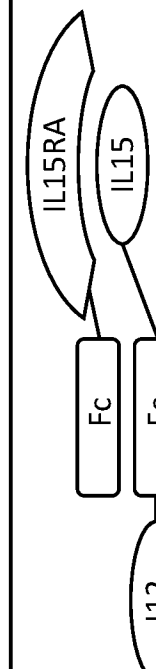
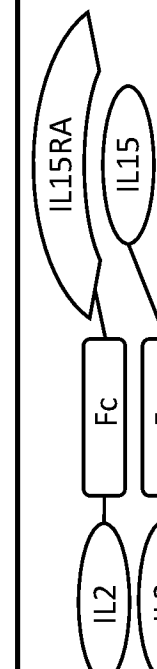
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FIG. 1G

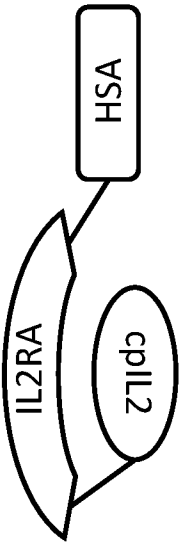


FIG. 2

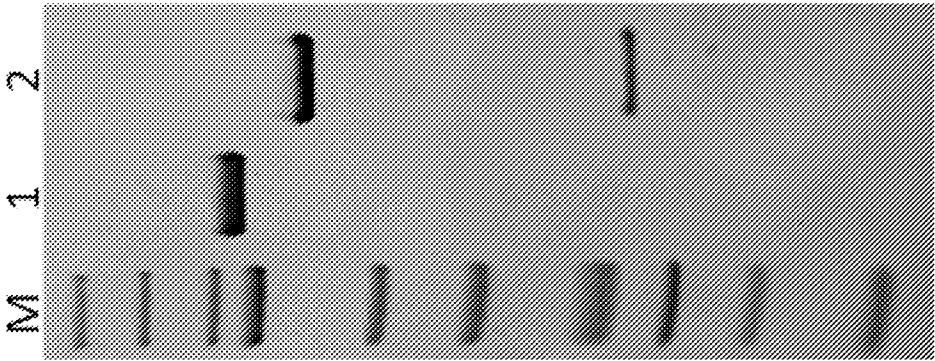


FIG. 3

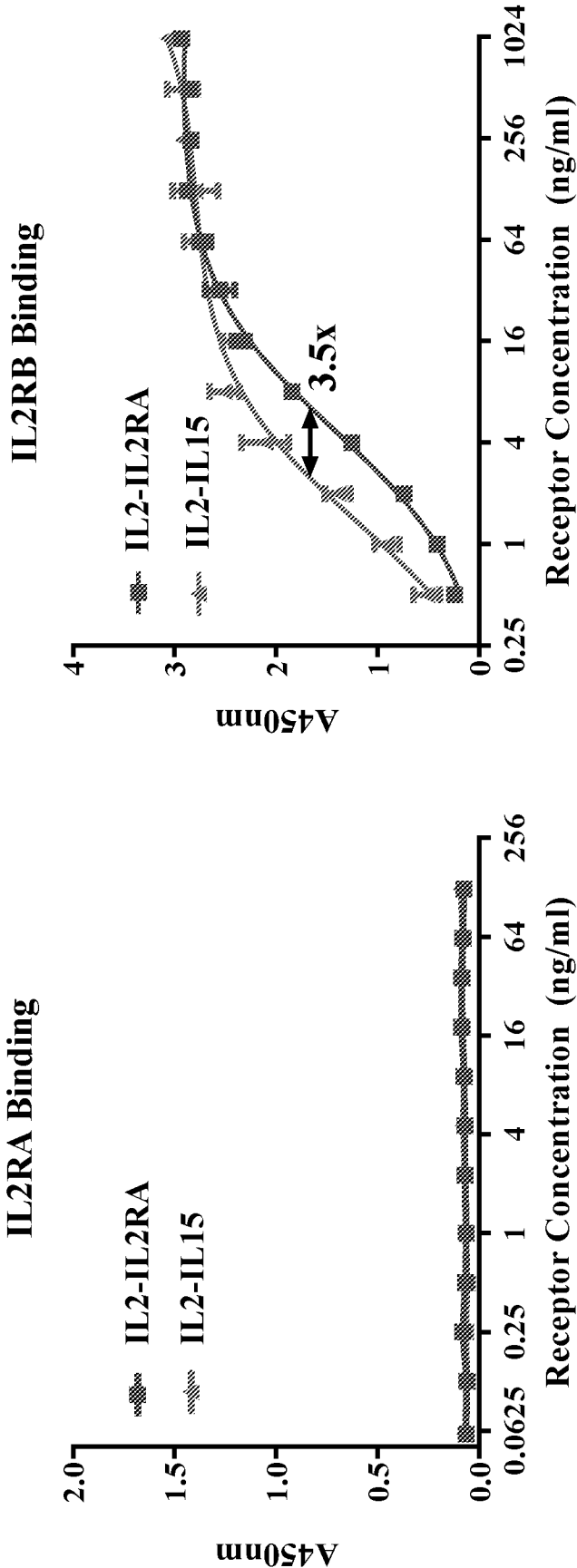


FIG. 4

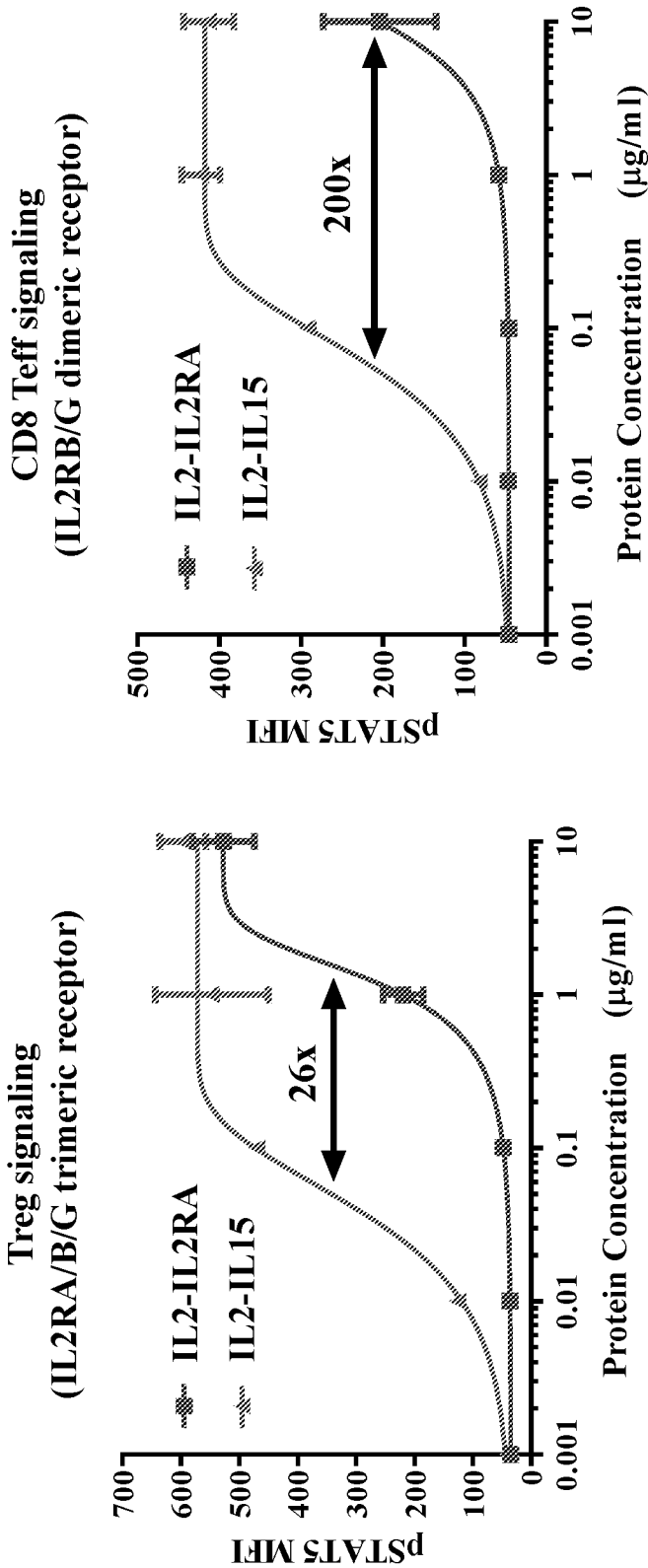


FIG. 5

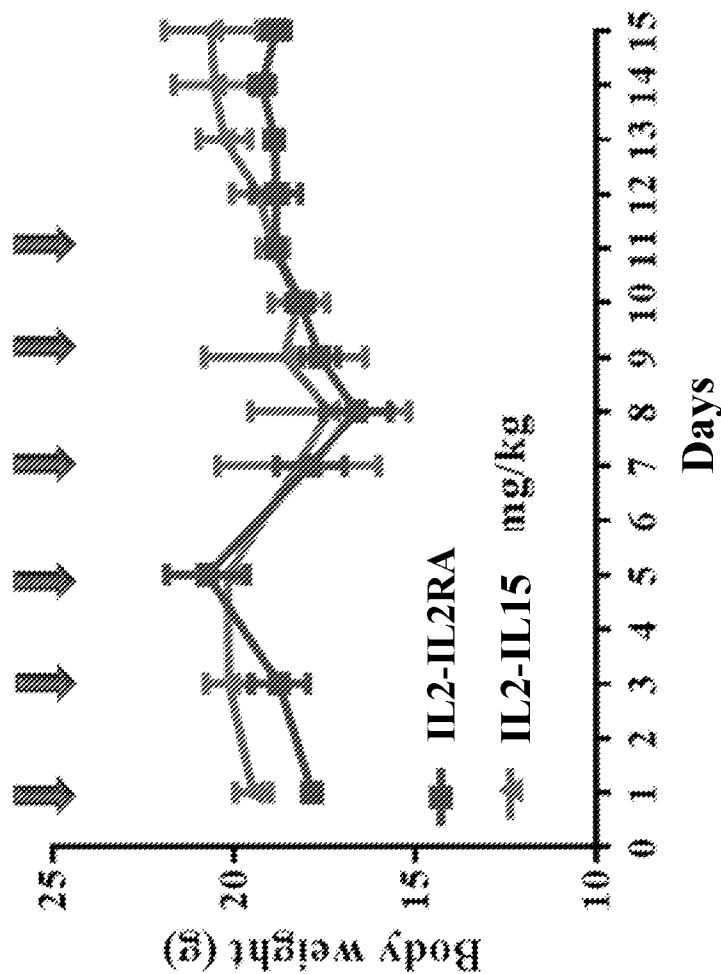


FIG. 6

FIG. 7A

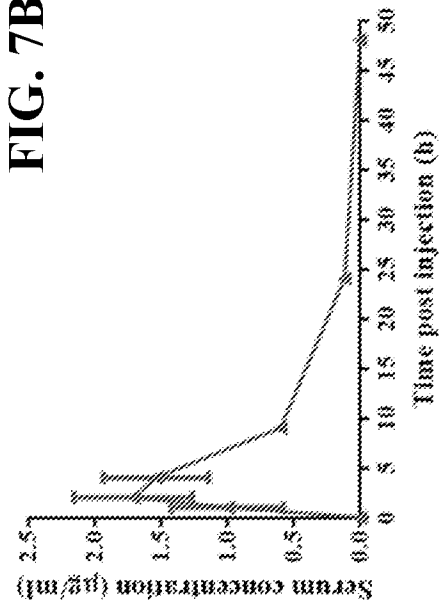


FIG. 7B

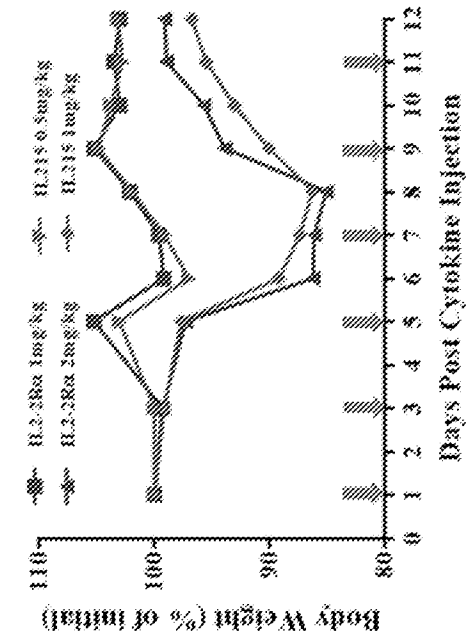


FIG. 7C

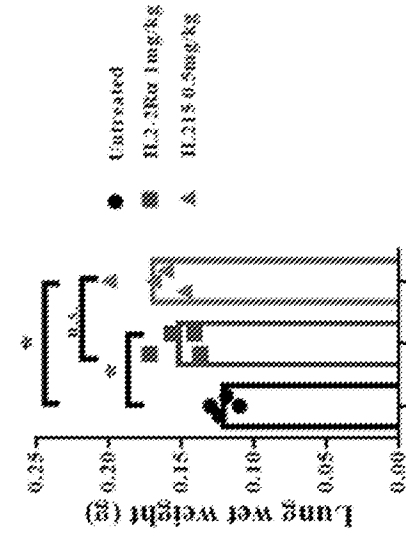


FIG. 7D

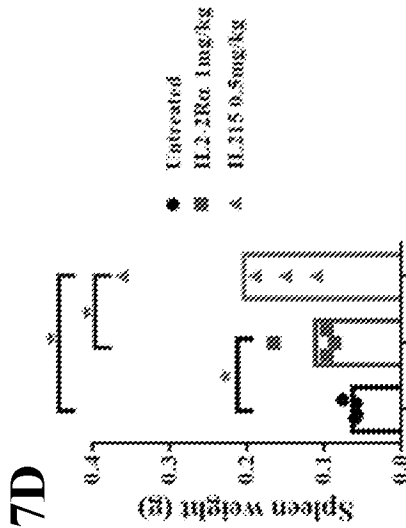


Fig. 7A-D In vivo comparison of IL215 and IL2-2R α in normal mice. A) The in vivo pharmacokinetics of 0.5 mg/kg IL215 after intraperitoneal (i.p.) injection. B) The toxicity after repeated dosing was assessed by the change in body weight. C) Comparison of the lung wet weight, an indicator of pulmonary edema after repeated dosing. Arrows indicate cytokine administration. Statistical analysis was performed using Welch's ANOVA analysis. *: $p < 0.05$; n.s.: not significant.

FIG. 8A

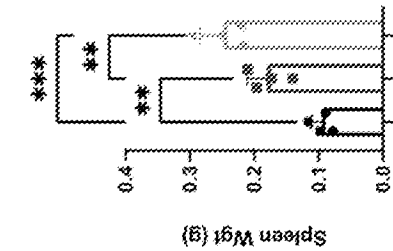


FIG. 8B

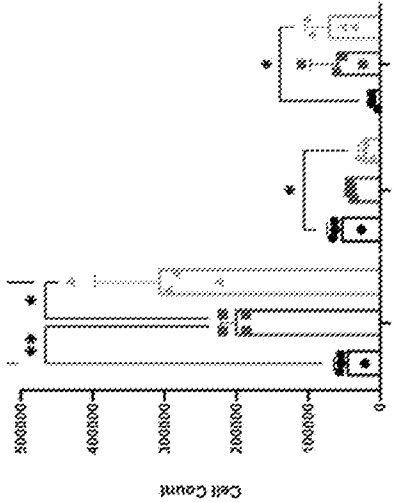


FIG. 8C

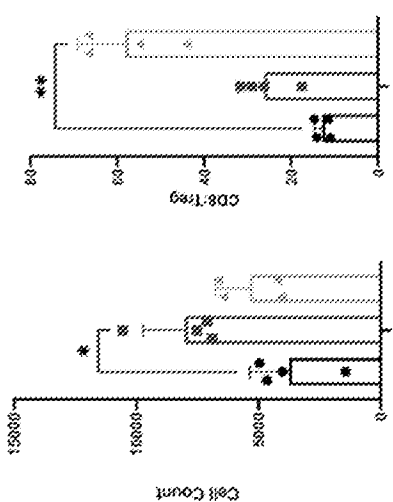


FIG. 8D

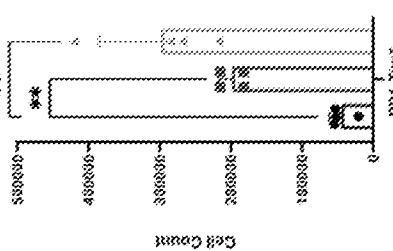


FIG. 8E

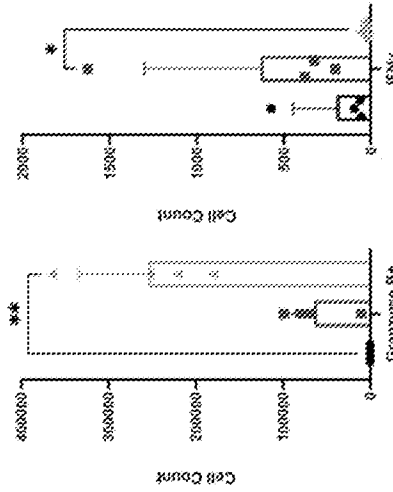


Fig.8A-E Pharmacodynamics in naive C57BL/6 mice. A) Spleen weight, B) T and NK cell numbers, C) CD8.Treg ratio, D) PD1 and TIM3 expression on CD8 T-cells, E) Granzyme B and IFN-γ expression on CD8 T-cells in mice after cytokine administration. Differences in cell number were analyzed using one-way ANOVA followed by either Tukey's or Kruskal Wallis multiple comparison tests. *: p<0.05; **: p<0.01; *: p<0.001.**

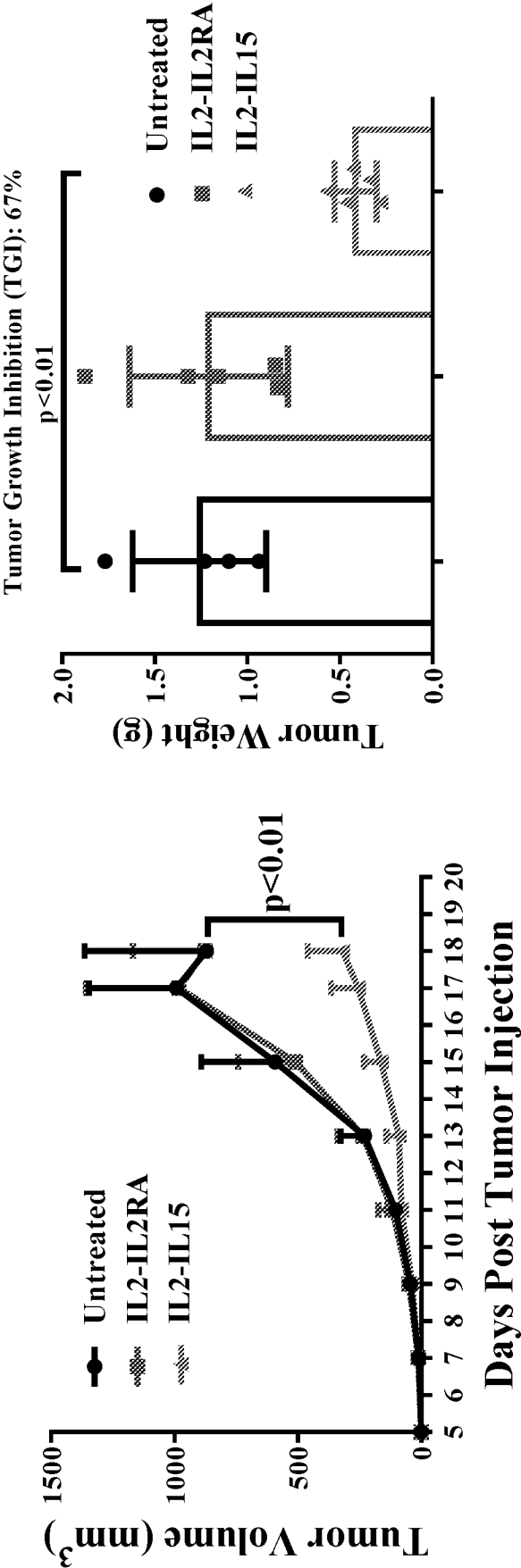


FIG. 9A

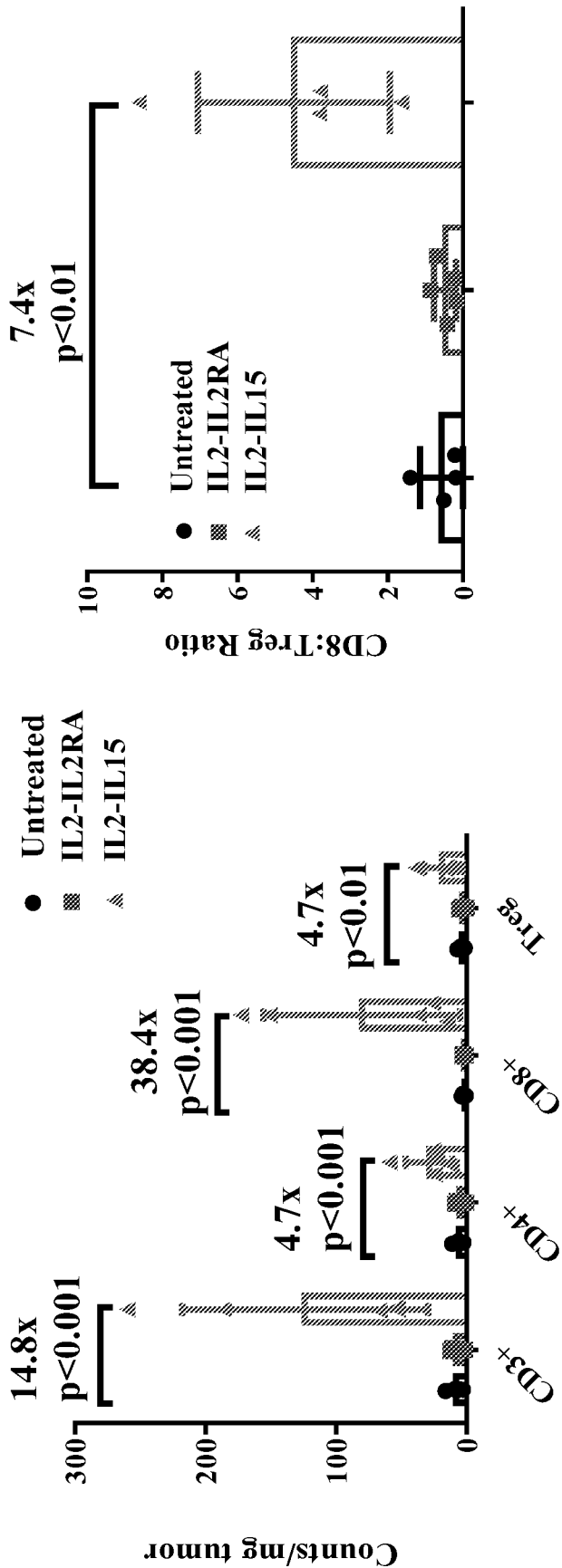


FIG. 9B

FIG. 10C

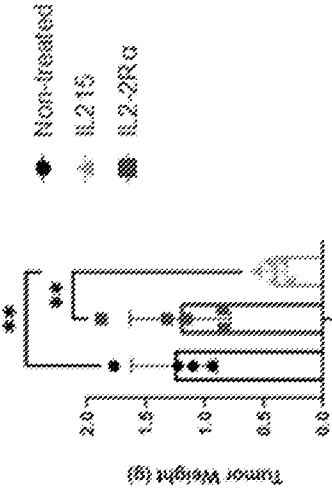


FIG. 10B

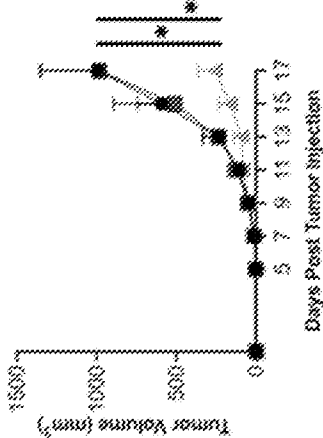


FIG. 10A

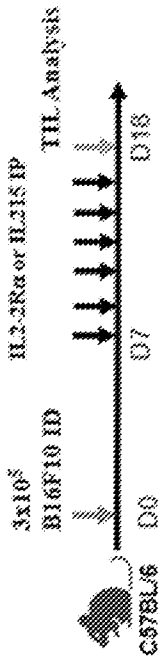


FIG. 10F



FIG. 10E

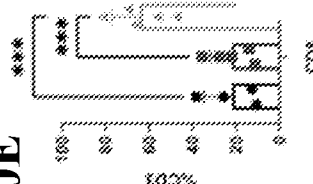


FIG. 10D



FIG. 10G

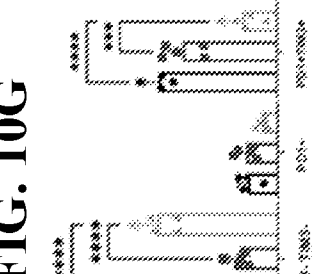


Fig.10A-G Anticancer effect in syngeneic B16F10 melanoma model. A) Schematic depicting protein treatment in tumor-bearing

mice. Both tumor volume (B) and tumor weight (C) were compared. D-G) Tumor-infiltrating lymphocytes (TIL) analysis. The absolute

T-cell counts per mg of tumor (D) and percentage of different populations of T-cells (E) the CD8 and Treg ratios (F) and the state of CD8

T-cells (G). Differences between curves and bars were analyzed using one-way ANOVA followed by Tukey's multiple comparison test.