

[0007] IL-2 orthologs with diminished affinity for the non-engineered intermediate affinity (CD122/CD132) IL-2 receptor complex or high-affinity (CD25/CD122/CD132) IL-2 receptor complex are also useful to selectively target the activity of ortholog IL-2 towards cells which exhibit high expression of CD25, *e.g.* in the treatment of autoimmune disease. IL-2 orthologs with significantly diminished affinity for the native wild-type hCD122 extracellular domain (ECD) but retain binding to the ECD of CD25 may also be used as competitive antagonists of wild-type IL-2 by interfering with the high-affinity IL-2 receptor complex formation and consequently may be employed in the treatment of autoimmune diseases or graft-versus-host (GVH) disease.

[0008] The present disclosure is directed to ligands that interact with an orthogonal hCD122 receptor. Specifically provided are hIL-2 orthogonal ligands (hIL2 orthologs) that provide selective binding and signaling via receptors comprising the extracellular domain of a hCD122 orthogonal receptor, in particular the extracellular domain of human CD122 comprising the amino acid substitutions H133D and Y134F. The IL2 activity on cells expressing the wild-type hCD122 of the present hIL2 orthologs is significantly diminished compared to the activity present hIL2 orthologs on cells expressing the orthogonal hCD122. Thus, selective activation and/or expansion of engineered cells expressing a receptor comprising the extracellular domain of orthogonal hIL2 using hIL-2 orthologs on the engineered cell populations is provided.

SUMMARY OF THE INVENTION

[0008a] In one aspect, provided herein is a human IL-2 (hIL2) ortholog polypeptide consisting of the amino acid sequence set forth in SEQ ID NO:20, wherein polyethylene glycol (PEG) is linked to the amino terminus of the polypeptide and the PEG is a 40kD branched PEG comprising two 20 kD arms.

[0008b] In one aspect, provided herein is a pharmaceutical formulation comprising the hIL2 ortholog polypeptide as described herein.

[0009] The present disclosure relates to human IL2 orthogonal ligands (“hIL2 orthologs”) that specifically and selectively bind to the extracellular domain (ECD) a transmembrane polypeptide comprising of a modified hCD122 polypeptide comprising modifications at positions 133 and/or 134 of the ECD of the orthogonal hCD122 polypeptide. In some

embodiments, the orthogonal hCD122 polypeptide comprising the amino acid substitutions H133D and Y134F. The binding of the hIL2 ortholog to the modified hCD122 polypeptide participates in the transduction pathway of intracellular signaling resulting in a biological activity of the native intracellular signaling patterns associated with IL2 binding to either the intermediate or high affinity IL2 receptor but which exhibits selectivity to an engineered cell

CLAIMS

WE CLAIM:

1. ~~An~~ A human IL-2 (hIL2) ortholog; polypeptide consisting of the amino acid sequence ~~of which has at least 90% identity to polypeptide of the Formula #1:~~

~~(AA1) (AA2) (AA3) (AA4) (AA5) (AA6) (AA7) (AA8) (AA9); T10 Q11 L12-
(AA13) (AA14) (AA15) (AA16) L17 (AA18) (AA19) (AA20) L21 (AA22)-
(AA23) I24 L25 N26 (AA27) I28 N29 N30 Y31 K32 N33 P34 K35 L36 T37-
(AA38) (AA39) L40 T41 (AA42) K43 F44 Y45 M46 P47 K48 K49 A50 (AA51)-
E52 L53 K54 (AA55) L56 Q57 C58 L59 E60 E61 E62 L63 K64 P65 L66 E67-
E68 V69 L70 N71 L72 A73 (AA74) S75 K76 N77 F78 H79 (AA80) (AA81) P82-
R83 D84 (AA85) (AA86) S87 N88 (AA89) N90 (AA91) (AA92) V93 L94 E95-
L96 (AA97) G98 S99 E100 T101 T102 F103 (AA104) C105 E106 Y107 A108-
(AA109) E110 T111 A112 (AA113) H114 V115 E116 F117 L118 N119 R120-
W121 H122 T123 F124 (AA125) (AA126) S127 H128 H129 (AA130) T131 L132-
T133~~

{1}

set forth in SEQ ID NO:20, wherein:

~~AA1 is A (wild type) or deleted;~~

~~AA2 is P (wild type) or deleted;~~

~~AA3 is T (wild type), C, A, G, Q, E, N, D, R, K, P, or deleted;~~

~~AA4 is S (wild type) or deleted;~~

~~AA5 is S (wild type) or deleted;~~

~~AA6 is S (wild type) or deleted;~~

~~AA7 is T (wild type) or deleted;~~

~~AA8 is K (wild type) or deleted;~~

~~AA9 is K (wild type) or deleted;~~

~~AA13 is Q (wild type), W or deleted;~~

~~AA14 is L (wild type), M, W or deleted;~~

~~AA15 is E (wildtype), K, D, T, A, S, Q, H or deleted;~~

~~AA16 is H (wildtype), N or Q or deleted;~~

AA18 is ~~L (wild type) or R, L, G, M, F, E, H, W, K, Q, S, V, I, Y, H, D or T;~~
AA19 is ~~L (wildtype), A, V, I or deleted;;~~
AA20 is ~~D (wildtype), T, S M L, or deleted;;~~
AA22 is ~~Q (wild type) or F, E, G, A, L, M, F, W, K, S, V, I, Y, H, R, N, D, T, F or deleted;~~
AA23 is ~~M (wild type), A, W, H, Y, F, Q, S, V, L, T, or deleted;~~
AA27 IS ~~G (wildtype), K, S or deleted;~~
AA38 is ~~R (wild type), W or G;~~
AA39 is ~~M (wildtype), L or V;~~
AA42 is ~~F (wildtype) or K;~~
AA51 is ~~T (wildtype), I or deleted~~
AA55 is ~~H (wildtype) or Y;~~
AA74 is ~~Q (wild type), N, H, S;~~
AA80 is ~~L (wild type), F or V;~~
AA81 is ~~R (wild type), I, D, Y, T or deleted~~
AA85 is ~~L (wild type) or V;~~
AA86 is ~~I (wild type) or V;~~
AA88 is ~~N (wildtype), E or Q or deleted;~~
AA89 is ~~I (wild type) or V;~~
AA91 is ~~V (wild type), R or K;~~
AA92 is ~~I (wild type) or F;~~
AA97 is ~~K (wild type) or Q;~~
AA104 is ~~M (wild type) or A;~~
AA109 is ~~D (wildtype), C or a non-natural amino acid with an activated side chain;~~
AA113 is ~~T (wild type) or N;~~
AA125 is ~~C (wild type), A or S;~~
AA126 is ~~Q (wild type) or H, M, K, C, D, E, G, I, R, S, or T; and/or~~
AA130 is ~~S (wild type), T or R.~~

2. ~~The hIL2 ortholog of Claim 1, wherein said hIL2 ortholog comprises modifications at positions: AA15, AA16, AA19, AA20, and AA24.~~

3. ~~The hIL2 ortholog of Claim 2, wherein said hIL2 ortholog further comprises an amino acid substitution at one or more positions selected from the group consisting of L12, Q13, H16, L19, D20, M23, R81, D84, S87, N88, V91, I92 and E95.~~

4. ~~The IL2 ortholog of Claim 3 where said one or more substitutions are selected from the group consisting of Q74N, Q74H, Q74S, L80F, L80V, R81D, R81T, L85V, I86V, I89V, and/or I92F.~~

5. ~~The IL2 ortholog of claim 3 wherein said one or more amino acid substitutions are selected from the following sets of amino acid substitutions:~~

~~[E15S-H16Q-L19V-D20L-M23A-L80F-R81D-L85V-I86V-I92F];~~

~~[E15S-H16Q-L19V-D20L-Q22K-L80F-R81D-L85V-I86V-I92F];~~

~~[E15S-H16Q-L19V-D20L-Q22K-M23A-L80F-R81D-L85V-I86V-I92F];~~

~~[E15S-H16Q-L19V-D20L-M23A-L80F-R81D-L85V-I86V-I92F-Q126H];~~

~~[E15S-H16Q-L19V-D20L-Q22K-L80F-R81D-L85V-I86V-I92F-Q126H];~~

~~[E15S-H16Q-L19V-D20L-Q22K-M23A-L80F-R81D-L85V-I86V-I92F-Q126H];~~

~~[E15S-H16Q-L19V-D20L-M23A-L80F-R81D-L85V-I86V-I92F-Q126M];~~

~~[E15S-H16Q-L19V-D20L-Q22K-L80F-R81D-L85V-I86V-I92F-Q126M]; or~~

~~[E15S-H16Q-L19V-D20L-Q22K-M23A-L80F-R81D-L85V-I86V-I92F-Q126M].~~

6. ~~The IL2 ortholog hIL2 ortholog of Claim 2, wherein said hIL2 ortholog further comprises an amino acid substitution at one or more positions selected from the group consisting of S4, K8, K9, T10, Q11, Q13, N26, N29, N30, N30, Y31, K35, T37, R38, T41, F42, K43, F44, Y45, M46, K48, K49, K54, E61, E62, K64, P65, E67G, E68, V69, N71, L72, Q74 S75, K76, H79, I89, N90, I92, S99, T101, F103, Y107, H114, H128 and T133.~~

7. ~~The IL2 ortholog of Claim 6 where said one or more substitutions are selected from the group consisting of S4P, K8R, K9T, T10A, Q11R, Q13R, N26D, N29S, N30S, N30D, N30T, Y31H, Y31C, K35R, T37A, T37R, M46L, K48E, K49R, K49E, K54R, E61D, K64R, E67G, E68D, V69A, N71T, N71A, N71R, A73V, Q74P S75P, K76E, K76R, H79R, I89V, N90H, I92T, S99P, T101A, F103S, H114V, H128T, T133A, and T133N.~~

8. ~~———— The IL2 ortholog of claim 6 wherein said one or more amino acid substitutions are selected from the following sets of amino acid substitutions:~~

~~[R38A-F42A-Y45A-E62A]; [F42A-Y45A-L72G]; [V69A, Q74P]; [V69A, Q74, T101A]; [V69A, Q74P, H128T]; [N30D, V69A, Q74P, F103S]; [K49E, V69A, A73V, K76E]; [V69A, Q74P, T101A, T133N]; [N30S, V69A, Q74P, H128A]; [N30S, V69A, Q74P, H128T]; [K9T, Q11R, K35R, V69A, Q74P]; [A1T, M46L, K49R, E61D, V69A, H79R]; [K48E, E68D, N71T, N90H, F103S, H114V]; [S4P-T10A, Q11R, V69A, Q74P, T133A]; [N30S, Y31H, K35R, K48E, V69A, Q74P, I92T]; [N30S, E68D, V69A, N71A, Q74P, S75P, K76R, N90H]; [N30S, Y31C, T37A, V69A, A73V, Q74P, H79R, H128T]; [N26D, N29S, N30S, K54R, E67G, V69A, Q74P, I92T]; [K8R, Q13R, N26D, N30T, K35R, T37R, V69A, Q74P, I92T]; [N29S, Y31H, K35R, T37A, K48E, V69A, N71R, Q74P, I39V] and [T41P-T51P].~~

9. ~~———— The IL2 ortholog of Claim 2, wherein said hIL2 ortholog further comprises an amino acid substitution at one or more positions selected from the group consisting of Q11, L18, Q22, E110, N119, T123, Q126, S127, Q126, S127, H129, S130, and T133.~~

10. ~~———— The hIL2 ortholog of Claim 9, wherein said hIL2 ortholog further comprises an amino acid substitution at one or more positions selected from the group consisting of L18R, L18G, L18M, L18F, L18E, L18H, L18W, L18K, L18Q, L18S, L18V, L18I, L18Y, L18H, L18D, L18T, Q22E, Q22E, Q22E, Q22E, , Q22G, Q22E, , Q22A, Q22L, Q22M, Q22F, Q22W, Q22K, Q22S, Q22V, Q22I, Q22Y, Q22H, Q22R, Q22N, Q22D, Q22T, Q22F, Q126H, Q126M, Q126K, Q126C, Q126D, Q126E, Q126G, Q126I, Q126R, Q126S, or Q126T.~~

11. ~~———— The hIL2 ortholog of Claim 1 wherein said hIL2 ortholog comprises one of the following sets of amino acid modifications:~~

~~[E15S-H16Q-L18R-L19V-D20L-Q22E-M23A];
[E15S-H16Q-L18R-L19V-D20L-Q22K-M23A];
[E15S-H16Q-L18R-L19V-D20L-M23A];
[E15S-H16Q-L18R-L19V-D20L-Q22K-M23A];
[E15S-H16Q-L18R-L19V-D20L-M23A-Q126H];
[E15S-H16Q-L18R-L19V-D20L-Q22K-M23A-Q126H];~~

~~[E15S-H16Q-L18R-L19V-D20L-M23A-Q126K];~~
~~[E15S-H16Q-L18R-L19V-D20L-Q22K-M23A-Q126K];~~
~~[E15S-H16Q-L19V-D20L-M23A-Q126H];~~
~~[E15S-H16Q-L19V-D20L-Q22K-M23A-Q126H];~~
~~[E15S-H16Q-L19V-D20L-M23A-Q126K];~~
~~[E15S-H16Q-L19V-D20L-Q22K-M23A-Q126K];~~
~~[E15S-H16Q-L18R-L19V-D20L-M23A-Q126H];~~
~~[E15S-H16Q-L18R-L19V-D20L-Q22K-M23A-Q126H];~~
~~[E15S-H16Q-L18R-L19V-D20L-M23A-Q126K];~~
~~[E15S-H16Q-L18R-L19V-D20L-Q22K-M23A-Q126K];~~
~~[E15S-H16Q-L19V-D20L-Q22K-Q126H];~~
~~[E15S-H16Q-L19V-D20L-M23A-Q126M];~~
~~[E15S-H16Q-L19V-D20L-Q22K-M23A-Q126M];~~
~~[E15S-H16Q-L19V-D20L-Q22K-Q126M];~~
~~[desAla1-E15S-H16Q-L19V-D20L-Q126M];~~
~~[desAla1-E15S-H16Q-L19V-D20L-Q22K-Q126M];~~
~~[desAla1-E15S-H16Q-L19V-D20L-M23A-Q126M];~~
~~[desAla1-E15S-H16Q-L19V-D20L-Q22K-M23A-Q126M];~~
~~[desAla1-E15S-H16Q-L19V-D20L-Q22K-M23A-Q126M];~~
~~[E15S-H16Q-L19V-D20L-M23A-L80F-R81D-I86V-I92F-Q126H];~~
~~[E15S-H16Q-L19V-D20L-Q22K-L80F-R81D-I86V-I92F-Q126H];~~
~~[E15S-H16Q-L19V-D20L-Q22K-M23A-L80F-R81D-I86V-I92F-Q126H];~~
~~[E15S-H16Q-L19V-D20L-M23A-L80F-R81D-I86V-I92F-Q126M];~~
~~[E15S-H16Q-L19V-D20L-Q22K-M23A-L80F-R81D-I86V-I92F-Q126M];~~
~~[E15S-H16Q-L19V-D20L-M23A-L85V-Q126H];~~
~~[E15S-H16Q-L19V-D20L-Q22K-L85V-Q126H];~~
~~[E15S-H16Q-L19V-D20L-Q22K-M23A-L85V-Q126H];~~
~~[E15S-H16Q-L19V-D20L-M23A-L85V-Q126M];~~
~~[E15S-H16Q-L19V-D20L-Q22K-L85V-Q126H]; or~~
~~[E15S-H16Q-L19V-D20L-Q22K-M23A-L85V-Q126M].~~

~~12. The hIL2 ortholog of Claim 1 wherein the hIL2 ortholog is selected from the group consisting of SEQ ID NOs: 5-138.~~

~~13. The hIL2 ortholog of Claim 1 wherein the hIL2 ortholog is operably linked to at least one carrier molecule.~~

~~14. The hIL2 ortholog of Claim 13, wherein carrier molecule is independently selected from the group consisting of water-soluble polymers, Fc domain of an IgG, sugars, and/or albumin.~~

~~15. The hIL2 ortholog of Claim 14, wherein the least one carrier molecule is polyethylene glycol (PEG).~~

~~16. The hIL2 ortholog of Claim 14, wherein the least one carrier molecule is polyethylene glycol (PEG).~~

~~17. The hIL2 ortholog of Claim 14 wherein the hIL2 ortholog comprises the structure:~~

~~— [PEG]-[linker]_n-[hoIL2]~~

~~wherein where n = 0 or 1 and hoIL2 is a human orthogonal IL2-) is linked to the amino terminus of the polypeptide variant. and the PEG is a 40kD branched PEG comprising two 20 kD arms.~~

~~18. The hIL2 ortholog of Claim 17 wherein the PEG has a molecular weight of from between 5kDa and 80kDa.~~

~~19. The hIL2 ortholog of Claim 17 wherein the PEG has a molecular weight of approximately 40kDa.~~

~~20. The~~

2. A pharmaceutical formulation comprising the hIL2 ortholog polypeptide of Claim 19 wherein the hoIL2 is an IL2 polypeptide variant comprising the set of amino acid substitution—[desAla1-E15S-H16Q-L19V-D20L-Q22K-M23A]claim 1.

~~21. The hIL2 ortholog polypeptide of Claim 19 wherein the hoIL2 is an IL2 polypeptide variant of the amino acid sequence:~~

~~—PTSSSTKKTQLQLSQLLVLLKAILNGINNYKNPKLTRM~~

~~-LTFKFYMPKKATELKHLQCLEEEELKPLEEVLNLAQSK
-NFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIV
-EFLNRWITFCQSIHSTLT (SEQ ID NO:5).~~

~~22. — A nucleic acid sequence encoding an hIL2 ortholog polypeptide of any one of claims 1.~~

~~23. — A recombinant vector comprising the nucleic acid sequence of Claim 22.~~

~~24. — A method of treating a disease, disorder or condition in a subject suffering therefrom by administering to the subject:~~

~~a. — an engineered mammalian cell comprising a nucleic acid sequence encoding a transmembrane receptor molecule comprising an extracellular domain (ECD) of an orthogonal hCD122 operably linked to one or more expression control elements capable of effecting the expression and surface presentation of the ECD of the transmembrane receptor molecule; and~~

~~b. — administering to said subject a therapeutically effective dose of hIL2 ortholog of Claim 1.~~

~~25. — The method of Claim 24 wherein engineered mammalian cells is engineered T cell.~~

~~26. — The method of Claim 25 wherein the engineered T cells is a CAR T cell.~~

~~27. — The method of Claim 26 wherein the hIL2 ortholog is an hIL2 ortholog of Claim 12.~~

~~28. — The method of Claim 24, wherein the hIL2 ortholog is of Claim 17.~~

~~29. — A method of preparing an engineered T cell product said T cell product comprising at least 20% of an hCD122 T cell, the method comprising the steps of:~~

~~a. — isolated a population a population of T cells from a mammalian subject;~~

~~b. — contacting the isolated a population of T cells ex vivo with recombinant vector comprising a nucleic acid sequence encoding a hCD122 operably linked to one or more expression control sequences so as to facilitate expression in a mammalian T cell under conditions that permit uptake of the recombinant vector by a T cell;~~

~~c. contacting the isolated a population of T cells an effective amount of an hIL2 ortholog of Claim 1.~~

~~30. A cell population product of the process of claim 29 wherein the com cell population at least 20% engineered hoCD122 T cells.~~