

INTERNATIONAL APPLICATION FOR PATENT FILED UNDER
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Title

IL-2 ORTHOLOGS AND METHODS OF USE

Cross-Reference to Other Applications

[0001] This application claims the priority of United States Provisional Patent Application Serial No. 62/948066 filed December 13, 2019.

Statement Regarding Government Funding

[0002] No United States government funding was used in the conception or reduction to practice of the subject matter of the present invention.

Background of the Invention

[0003] The controlled manipulation of the differentiation, development and proliferation of cells, particularly engineered immune cells, is of significant clinical interest. T cells have been engineered for use in therapeutic applications such as the recognition and killing of cancer cells, intracellular pathogens and cells involved in autoimmunity. The use of engineered cell therapies in the treatment of cancer is facilitated by the selective activation and expansion of engineered T cells that provide specific functions and are directed to selectively attack cancer cells. In some examples of adoptive immunotherapy, T cells are isolated from the blood of a subject, processed *ex vivo*, and re-infused into the subject. Compositions and methods that enable selective activation of a targeted engineered cell population are therefore desirable.

[0004] A challenge with the manufacture of cell therapy products is that such ‘living drugs’ require close control of their environment to preserve viability and functionality. In practice, isolated cells, whether derived from a patient (autologous) or from a single donor source

(allogeneic), begin to lose function rapidly following removal from the subject or the controlled culture conditions. Successful maintenance of the health and function of isolated cells while outside the subject or controlled culture conditions enables the isolated cells to return to functionality for reinsertion into the cell product manufacturing workflow or into patients.

[0005] Additionally, a challenge with the clinical application of engineered T cell therapies is to selectively stimulate these engineered cells to maximize their therapeutic effectiveness. Typical means to provide for the continued maintenance of activated engineered T cell products is the systemic administration of cytokines such as IL2. However, the systemic administration of IL2 is associated with non-specific stimulatory effects beyond the population of engineered cells and is associated, particularly in high doses, and is associated with significant toxicity in human subjects. Furthermore, IL2 has a short lifespan *in vivo* which requires that the IL-2 be dosed frequently to maintain the engineered T cells in an activated state. Although engineered cells from an initial administration of an initial population may be detectable for months or even years following the administration of the engineered cell product, a significant fraction of these engineered cells lapse into a quiescent state that requires reactivation for them to exhibit significant therapeutic effect. Consequently, a challenge in cell-based therapies is to confer a desired regulatable behavior into the transferred cells that is protected from endogenous signaling pathways, that does not affect non-targeted endogenous cells, and that can be controlled selectively following administration of the engineered cell population to a subject.

[0006] CD122 is a component of the intermediate and high affinity IL2 receptor complexes. Sockolosky, *et al.* (Science (2018) 359: 1037–1042) and Garcia, et al. (United States Patent Application Publication US2018/0228841A1 published August 16, 2018) describe an orthogonal IL2/CD122 ligand/receptor system to facilitate selective stimulation of cells engineered to express the orthogonal CD122 receptor. IL2 muteins that are cognate ligands for the orthogonal receptor are also described. The contact of engineered T cells that express the orthogonal CD122 with a corresponding orthogonal ligand for such orthogonal CD122 (“IL2 orthologs”) enables specific activation of such engineered T cells. In particular this orthogonal IL2 receptor ligand complex provides for selective expansion of cells engineered to express the orthogonal receptor in a mixed population of cells, in particular a mixed population of T cells.

[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

[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

expressing an hCD122 orthogonal receptor. In some embodiments, the hIL2 ortholog is a hIL2 variant of Formula 1 described hereinbelow. The hIL2 orthologs of the present d exhibit significantly reduced binding relative to the extracellular domain of wild-type hCD122, either alone or when hCD122 is present in the form of the endogenous high or intermediate affinity hIL2 receptor relative to the hIL2 ortholog's binding to the hCD122 orthogonal receptor. In some embodiments, the affinity of the hIL2 ortholog for the extracellular domain of the orthogonal hCD122 is comparable to the affinity of wild-type hIL2 for wild-type hCD122. In some embodiments, the affinity of the hIL2 ortholog for the extracellular domain ("ECD") of the orthogonal hCD122 is greater than to the affinity of wild-type hIL2 for extracellular domain of wild-type hCD122. In some embodiments, the affinity of the hIL2 ortholog for the extracellular domain of the orthogonal hCD122 is less than to the affinity of wild-type hIL2 for the extracellular domain of the wild-type hCD122. In one embodiment, the ECD of the orthogonal hCD122 receptor comprises a modified human hCD122 ECD polypeptide comprising the substitutions H133D and Y134F (numbered in accordance with wild-type hCD122) wherein the amino acid sequence of the ECD of the orthogonal hCD122 receptor comprises a 214 amino acid polypeptide having the sequence:

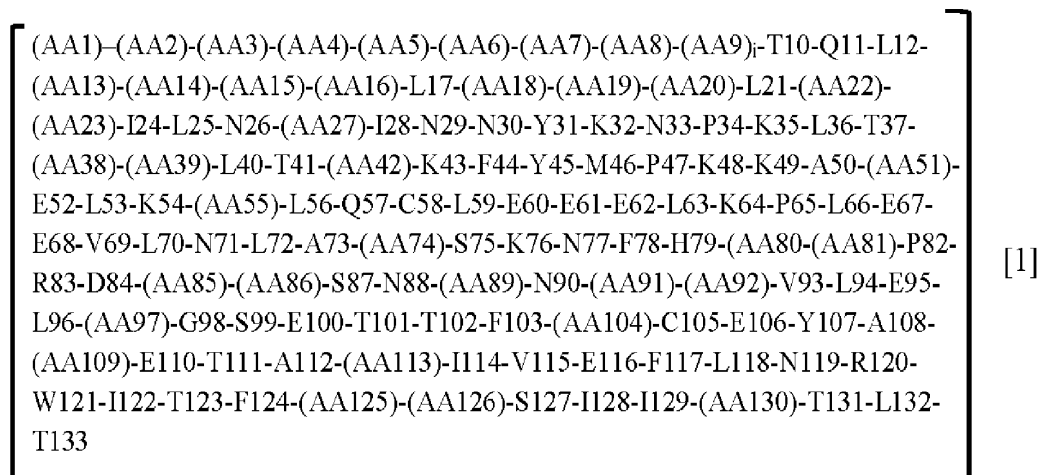
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AVNGTSQFTC FYNSRANISC VWSQDGALQD TSCQVHAWPD RRRWNQTCEL
LPVSQASWAC NLILGAPDSQ KLTTVDIVTL RVLCREGVRW RVMAIQDFKP
FENLRMLAPI SLQVVHVETH RCNISWEISQ ASDFFERHLE FEARTLSPGH
TWEEAPLLTL KQKQEWICLE TLTPDTQYEF QVRVKPLQGE FTTWSPWSQP
LAFRTKPAAL GKDT (SEQ ID NO:1)
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[0010] In one embodiment, the orthogonal hCD122 receptor is a modified human CD122 having the comprising amino acid sequence (less the signal peptide) of the ECD of hCD122 having substitutions H133D and Y134F (SEQ ID NO:1) and the transmembrane (TM) and intracellular domain (ICD) of the wild-type hCD122 molecule, the orthogonal hCD122 receptor (hoRb) comprising the amino acid sequence:

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AVNGTSQFTC FYNSRANISC VWSQDGALQD TSCQVHAWPD RRRWNQTCEL
LPVSQASWAC NLILGAPDSQ KLTTVDIVTL RVLCREGVRW RVMAIQDFKP
FENLRMLAPI SLQVVHVETH RCNISWEISQ ASDFFERHLE FEARTLSPGH
TWEEAPLLTL KQKQEWICLE TLTPDTQYEF QVRVKPLQGE FTTWSPWSQP
LAFRTKPAAL GKDTIPWLGH LLVGLSGAFG FIILVYLLIN CRNTGPWLKK
VLKCNTPDPS KFFSQLSSEH GGDVQKWLSS PFPSSSFSPG GLAPEISPLE
VLERDKVTQL LLQQDKVPEP ASLSSNHSLT SCFTNQGYFF FHLPDALIEI
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ACQVYFTYDP YSEEDPDEGV AGAPTGSSPQ PLQPLSGEDD AYCTFPSRDD
 LLLFSPSLLG GPSPPSTAPG GSGAGEERMP PSLQERVPRD WDPQPLGPPT
 PGVPDLVDFQ PPPELVLEA GEEVPDAGPR EGVSPFWSRP PGQGEFRLN
 ARLPLNTDAY LSLQELQGQD PTHL (SEQ ID NO:2)

[0011] In one embodiment, the present disclosure provides an hIL2 ortholog, the amino acid sequence of which has at least 80% identity to polypeptide of the Formula #1:



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.

[0012] In some embodiments the the present disclosure provides an hIL2 ortholog comprising a IL2 variant polypeptide comprising amino acid modifications at positions: E15, L16, L19, D20, and M23 and optionally Q22. In some embodiments the hIL2 ortholog comprises

a IL2 variant polypeptide comprising the amino acid substitutions: L12, Q13, H16, L19, D20, M23, R81, D84, S87, N88, V91, I92 and E95. In some embodiments the the present disclosure provides an hIL2 ortholog comprising a IL2 variant polypeptide comprising amino acid modifications at positions hIL2 ortholog comprises a IL2 variant polypeptide comprising the amino acid substitutions: Q74N, Q74H, Q74S, L80F, L80V, R81D, R81T, L85V, I86V, I89V, and/or I92F. In some embodiments the present disclosure provides an hIL2 ortholog comprises a IL2 variant polypeptide comprising amino acid modifications 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].

[0013] In some embodiments the present disclosure provides an hIL2 ortholog comprises a IL2 variant polypeptide comprising amino acid modifications at positions: 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, I114, I128 and T133. In some embodiments the present disclosure provides an hIL2 ortholog comprises a IL2 variant polypeptide comprising one or more of the amino acid substitutions: 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, I114V, I128T, T133A, and T133N. In some embodiments the present disclosure provides an a IL2 variant polypeptide comprising amino acid modifications selected from the following sets of amino acid substitutions:

[R38A-F42A-Y45A-E62A]; [F42A-Y45A-L72G]; [V69A, Q74P]; [V69A, Q74, T101A]; [V69A, Q74P, I128T]; [N30D, V69A, Q74P, F103S]; [K49E, V69A, A73V, K76E]; [V69A, Q74P, T101A, T133N]; [N30S, V69A, Q74P, I128A]; [N30S, V69A, Q74P, I128T]; [K9T, Q11R, K35R, V69A, Q74P]; [A1T, M46L, K49R, E61D, V69A, H79R]; [K48E, E68D, N71T, N90H, F103S, I114V]; [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, I128T]; [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].

[0014] In some embodiments the present disclosure provides an hIL2 ortholog comprising a IL2 variant polypeptide comprising amino acid modifications at positions: Q11, L18, Q22, E110, N119, T123, Q126, S127, Q126, S127, I129, S130, and T133. In some embodiments the present disclosure provides an hIL2 ortholog comprising a IL2 variant polypeptide having one or more amino acid substitutions selected from: 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. In some embodiments the present disclosure provides an hIL2 ortholog comprising an IL2 variant polypeptide having a set of amino acid substitutions selected from the following sets of amino acid substitutions:

[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].

[0015] In some embodiments the present disclosure provides an hIL2 ortholog comprises a IL2 variant polypeptide selected from the group consisting of SEQ ID NOs: 5-138.

[0016] In some embodiments the present disclosure provides an hIL2 ortholog is operably linked to at least one carrier molecule. In some embodiments the present disclosure provides an hIL2 ortholog comprising at least one polyethylene glycol (PEG) molecule.

[0017] In some embodiments the present disclosure provides an hIL2 ortholog comprises the structure:



wherein where $n = 0$ or 1 and hoIL2 is a human orthogonal IL2 polypeptide variant of the Formula 1. In some embodiments PEG has a molecular weight of from between 5kDa and 80kDa. In some embodiments the PEG has a molecular weight of approximately 40kDa. In some embodiments the present disclosure provides an hIL2 ortholog of the above structure wherein the hoIL2 is an IL2 polypeptide variant comprising the set of amino acid substitution -[desAla1-E15S-H16Q-L19V-D20L-Q22K-M23A]. In some embodiments the present disclosure provides an hIL2 ortholog of the above structure wherein the hoIL2 is an IL2 polypeptide variant comprising amino acid sequence:

PTSSSTKKTQLQLSQLLVLLKAILNGINNYKNPKLTRM
LTFKFYMPKKATELKHLQCLEELKPLEEVNLNAQSK
NFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIV
EFLNRWITFCQSIISTLT (SEQ ID NO:5).

[0018] In some embodiments the present disclosure provides a nucleic acid sequence encoding an hIL2 ortholog polypeptide of Formula #1.

[0019] In some embodiments the present disclosure provides a recombinant vector encoding a nucleic acid sequence encoding an hIL2 ortholog polypeptide of Formula #1A recombinant vector comprising the nucleic acid sequence of Claim 22.

[0020] In some embodiments the present disclosure provides 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 Formula #1

[0021] In some embodiments the present disclosure provides a preparing an engineered T cell product said T cell product comprising at least 20% of an hoCD122 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 hoCD122 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.

[0022] In some embodiments the present disclosure provides a cell population at least 20% engineered hoCD122 T cells.

[0023] The present disclosure further provides methods of making the hIL2 orthologs of the present invention. In particular, the present disclosure provides recombinant expression vectors comprising a nucleic acid sequence encoding the hIL2 orthologs operably linked to control elements to provide for expression of the nucleic acid sequence encoding the hIL2 ortholog in a host cell.

[0024] The present disclosure further provides a composition comprising a mixed cell population comprising at least 10%, alternatively at least 20%, alternatively at least 30%, alternatively at least 40%, alternatively at least 50%, alternatively at least 60%, alternatively at least 70%, of a T cell (e.g., T cell, CD8+ T cell, Treg, TIL, NK cell, TCR modified cell, CAR-T cell, etc), wherein the T cell has been recombinantly modified to express an orthogonal hCD122 receptor polypeptide. The present disclosure further provides a method of generating a pharmaceutically acceptable dosage form of an engineered cell therapy product the dosage form comprising a population of T cells wherein the population of T cells is substantially enriched for one or more species of engineered T cells, the engineered T cells expressing a receptor

comprising the extracellular domain of an hCD122 orthogonal polypeptide, the method comprising the steps culturing the population of T cells comprising engineered T cells expressing a receptor comprising the extracellular domain of an hCD122 orthogonal polypeptide *ex vivo* in the presence of an hIL2 ortholog of the present invention for a period of time sufficient to enrich the cell population in of one or more such engineered T cells.

[0025] In some embodiments, the present disclosure provides a recombinant vector comprising a nucleic acid sequence encoding the hIL2 ortholog described herein operably linked to control elements to facilitate expression and secretion of the hIL2 ortholog from a mammalian cell is administered to the subject to provide for *in situ* expression of the hIL2 ortholog. In some embodiments, the recombinant vector is administered intratumorally to a subject suffering from cancer. In some embodiments, the recombinant vector is a recombinant viral vector. In some embodiments the recombinant viral vector is a recombinant adeno-associated virus (rAAV) or recombinant adenovirus (rAd), for example in some embodiments, a replication deficient adenovirus derived from human adenovirus serotypes 3 and/or 5. In some embodiments, the replication deficient adenovirus has one or more modifications to the E1 region which interfere with the ability of the virus to initiate the cell cycle and/or apoptotic pathways. The replication deficient adenoviral vector may optionally comprise deletions in the E3 domain. In some embodiments the adenovirus is a replication competent adenovirus. In some embodiments the adenovirus is a replication competent recombinant virus engineered to selectively replicate in neoplastic cells.

[0026] The present disclosure further provides methods of preparing a pharmaceutically acceptable dosage form of a cell therapy product comprising at least one (alternatively 2, 3, 4 or more) species of engineered T cells that express a transmembrane receptor protein wherein the extracellular domain of such transmembrane receptor protein comprises the extracellular domain of an hCD122 orthogonal polypeptide wherein the fraction of engineered cells in the cell therapy product comprises at least 20%, alternatively at least 30%, alternatively at least 40%, alternatively at least 50%, alternatively at least 60%, alternatively at least 70%, alternatively at least 80%, or alternatively at least 90% of the total number of cells in the cell therapy product.

[0027] In some embodiments a therapeutic method is provided, the method comprising introducing into a subject suffering from disease, disorder or condition a population of engineered cells, said engineered cell population comprising nucleic acid sequence encoding a cell membrane spanning orthogonal receptor polypeptide comprising an ECD of SEQ ID NO:1, a transmembrane domain and an intracellular signaling domain that results in an intracellular signal in response to the binding of a hIL2 ortholog ligand to the ECD of said cell membrane spanning orthogonal receptor polypeptide, said nucleic acid sequence operably linked to expression control elements to facilitate transcription and translation and cell surface presentation of the ECD of said membrane spanning polypeptide in combination with the administration of hCD122 ortholog of the present disclosure. Such cell population may comprise cells which have been modified *ex vivo* and are autologous or allogeneic with respect to the subject. In some embodiments, the therapeutic method comprises: (1) contacting engineered cell population, the engineered cells expressing a receptor comprising hIL2 orthogonal CD122 ECD *ex vivo* with an amount of a cognate hIL2 ortholog at a concentration and for a duration of time sufficient to activate said engineered cells; and (2) administering the cell population to the subject; and (3) administering to the subject a cognate hIL2 ortholog in combination with the administration of the engineered cells to the subject. In some embodiments, the subject to which the engineered hIL2 orthogonal CD122 ECD receptor cell population and hIL2 ortholog is suffering from a neoplastic disease. In some embodiments, the orthogonal receptor and ligand are administered in combination with at least one additional/supplemental therapeutic or prophylactic agent.

BRIEF DESCRIPTION OF THE DRAWINGS.

[0028] The invention is best understood from the following detailed description when read in conjunction with the accompanying drawings. It is emphasized that, according to common practice, the various features of the drawings are not to-scale. On the contrary, the dimensions of the various features are arbitrarily expanded or reduced for clarity. Included in the drawings are the following figures.

[0029] Figure 1 of the attached drawing provides Celltiterglo® values for NKL cells treated with 293 transfection supernatant from experiments as more fully described in Example 7 herein. For NKL cells receiving the indicated dilution of each supernatant, indicated in bold, duplicate Celltiterglo® values are shown in side-by-side columns.

[0030] Figure 2 of the attached drawing provides Celltiterglo® values for NKL cells which have been recombinantly modified to express a hCD122 orthogonal receptor of SEQ ID NO2 (“NKL hoRB cells”) treated with 293 transfection supernatants from experiments as described in Example 7. For NKL hoRB cells receiving the indicated dilution of each supernatant, indicated in bold, duplicate Celltiterglo® values are shown in side-by-side columns.

DETAILED DESCRIPTION

[0031] In order for the present disclosure to be more readily understood, certain terms and phrases are defined below as well as throughout the specification. The definitions provided herein are non-limiting and should be read in view of the knowledge of one of skill in the art would know.

[0032] Before the present methods and compositions are described, it is to be understood that this invention is not limited to particular method or composition described, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting.

[0033] Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limits of that range is also specifically disclosed. Each smaller range between any stated value or intervening value in a stated range and any other stated or intervening value in that stated range is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included or excluded in the range, and each range where either, neither or both limits are included in the smaller ranges is also encompassed within the invention, 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 invention.

[0034] 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 invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, some potential and preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited.

[0035] It should be noted that as used herein and in the appended claims, the singular forms "a", "an", and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a cell" includes a plurality of such cells and reference to "the peptide" includes reference to one or more peptides and equivalents thereof, e.g. polypeptides, known to those skilled in the art, and so forth.

[0036] The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed.

[0037] Unless indicated otherwise, parts are parts by weight, molecular weight is weight average molecular weight, temperature is in degrees Celsius (°C), and pressure is at or near atmospheric. Standard abbreviations are used, including the following: bp = base pair(s); kb = kilobase(s); pl = picoliter(s); s or sec = second(s); min = minute(s); h or hr = hour(s); aa = amino acid(s); kb = kilobase(s); nt = nucleotide(s); pg = picogram; ng = nanogram; µg = microgram; mg = milligram; g = gram; kg = kilogram; dl or dL = deciliter; µl or µL = microliter; ml or mL = milliliter; l or L = liter; µM = micromolar; mM = millimolar; M = molar; kDa = kilodalton; i.m. = intramuscular(ly); i.p. = intraperitoneal(ly); SC or SQ = subcutaneous(ly); QD = daily; BID = twice daily; QW = weekly; QM = monthly; HPLC = high performance liquid chromatography; BW = body weight; U = unit; ns = not statistically significant; PBS = phosphate-buffered saline; PCR = polymerase chain reaction; NHS = N-hydroxysuccinimide; HSA = human serum

albumin; MSA = mouse serum albumin; DMEM = Dulbecco's Modification of Eagle's Medium; GC = genome copy; EDTA = ethylenediaminetetraacetic acid.

[0038] It will be appreciated that throughout this disclosure reference is made to amino acids according to the single letter or three letter codes. For the reader's convenience, the single and three letter amino acid codes are provided in Table 1 below:

Table 1. Amino Acid Abbreviations		
G	Glycine	Gly
P	Proline	Pro
A	Alanine	Ala
V	Valine	Val
L	Leucine	Leu
I	Isoleucine	Ile
M	Methionine	Met
C	Cysteine	Cys
F	Phenylalanine	Phe
Y	Tyrosine	Tyr
W	Tryptophan	Trp
H	Histidine	His
K	Lysine	Lys
R	Arginine	Arg
Q	Glutamine	Gln
N	Asparagine	Asn
E	Glutamic Acid	Glu
D	Aspartic Acid	Asp
S	Serine	Ser
T	Threonine	Thr

[0039] Standard methods in molecular biology are described in the scientific literature (see, e.g., Sambrook and Russell (2001) *Molecular Cloning*, 3rd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.; and Ausubel, et al. (2001) *Current Protocols in Molecular Biology*, Vols. 1-4, John Wiley and Sons, Inc. New York, N.Y., which describes cloning in bacterial cells and DNA mutagenesis (Vol. 1), cloning in mammalian cells and yeast (Vol. 2), glycoconjugates and protein expression (Vol. 3), and bioinformatics (Vol. 4)). The scientific literature describes methods for protein purification, including immunoprecipitation, chromatography, electrophoresis, centrifugation, and crystallization, as well as chemical

analysis, chemical modification, post-translational modification, production of fusion proteins, and glycosylation of proteins (see, e.g., Coligan, et al. (2000) Current Protocols in Protein Science, Vols. 1-2, John Wiley and Sons, Inc., NY).

[0040] Unless otherwise indicated, the following terms are intended to have the meaning set forth below. Other terms are defined elsewhere throughout the specification.

[0041] Activate: As used herein the term “activate” is used in reference to a receptor or receptor complex to reflect the biological effect, directly and/or by participation in a multicomponent signaling cascade, arising from the binding of an agonist ligand to a receptor responsive to the binding of the ligand. For example, it is said that the binding of an IL2 agonist to its cognate IL2 receptor “activates” the signaling of the receptor to produce one or more intracellular biological effects (e.g., phosphorylation of STAT5).

[0042] Activity: As used herein, the term “activity” is used with respect to a molecule to describe a property of the molecule with respect to a test system or biological function such as the degree of binding of the molecule to another molecule. Examples of such biological functions include but are not limited to catalytic activity of a biological agent, the ability to stimulate intracellular signaling, gene expression, cell proliferation, the ability to modulate immunological activity such as inflammatory response. “Activity” is typically expressed as a biological activity per unit of administered agent such as [catalytic activity]/[mg protein], [immunological activity]/[mg protein], international units (IU) of activity, [STAT5 phosphorylation]/[mg protein], [T-cell proliferation]/[mg protein], plaque forming units (pfu), etc. The term “proliferative activity” encompasses an activity that promotes cell division including dysregulated cell division as that observed in neoplastic diseases, inflammatory diseases, fibrosis, dysplasia, cell transformation, metastasis, and angiogenesis.

[0043] Administer/Administration: The terms “administration” and “administer” are used interchangeably herein to refer the act of contacting a subject, including contacting a cell, tissue, organ, or biological fluid *in vitro*, *in vivo* or *ex vivo* of the subject, with an agent (e.g. an IL-2 ortholog, a CAR-T cell, a chemotherapeutic agent, an antibody, or modulator or a pharmaceutical formulation comprising one or more of the foregoing). Administration of an agent may be achieved through any of a variety of art recognized methods including but not

limited to the topical, intravascular injection (including intravenous or intraarterial infusion), intradermal injection, subcutaneous injection, intramuscular injection, intraperitoneal injection, intracranial injection, intratumoral injection, transdermal, transmucosal, iontophoretic delivery, intralymphatic injection, intragastric infusion, intraprostatic injection, intravesical infusion (e.g., bladder), respiratory inhalers, intraocular injection, intraabdominal injection, intralesional injection, intraovarian injection, intracerebral infusion or injection, intracerebroventricular injection (ICVI), and the like. The term “administration” includes contact of an agent to the cell, tissue or organ as well as the contact of an agent to a fluid, where the fluid is in contact with the cell.

[0044] Adverse Event: As used herein, the term “adverse event” refers to any undesirable experience associated with the use of a therapeutic or prophylactic agent in a subject. Adverse events do not have to be caused by the administration of the therapeutic or prophylactic agent (e.g. an hIL2 ortholog) but may arise from unrelated circumstances. Adverse events are typically categorized as mild, moderate, or severe. As used herein, the classification of adverse events as used herein is in accordance with the Common Terminology Criteria for Adverse Events v4.03 (CTCAE) dated June 14, 2010 published by the United States Department of Health and Human services, National Institutes of Health National Cancer Institute.

[0045] Affinity: As used herein the term “affinity” refers to the degree of specific binding of a first molecule (e.g. a ligand) to a second molecule (e.g. a receptor) and is measured by the binding kinetics expressed as K_d , a ratio of the dissociation constant between the molecule and the its target (K_{off}) and the association constant between the molecule and its target (K_{on}).

[0046] Agonist: As used herein, the term “agonist” refers a first agent that specifically binds a second agent (“target”) and interacts with the target to cause or promote an increase in the activation of the target. In some instances, agonists are activators of receptor proteins that modulate cell activation, enhance activation, sensitize cells to activation by a second agent, or up-regulate the expression of one or more genes, proteins, ligands, receptors, biological pathways, that may result in cell proliferation or pathways that result in cell cycle arrest or cell death such as by apoptosis. In some embodiments, an agonist is an agent that binds to a receptor and alters the receptor state resulting in a biological response that mimics the effect of the

endogenous ligand of the receptor. The term “agonist” includes partial agonists, full agonists and superagonists. An agonist may be described as a “full agonist” when such agonist which leads to a substantially full biological response (*i.e.* the response associated with the naturally occurring ligand/receptor binding interaction) induced by receptor under study, or a partial agonist. A “superagonist” is a type of agonist that is capable of producing a maximal response greater than the endogenous agonist for the target receptor, and thus has an activity of more than 100% of the native ligand. A super agonist is typically a synthetic molecule that exhibits greater than 110%, alternatively greater than 120%, alternatively greater than 130%, alternatively greater than 140%, alternatively greater than 150%, alternatively greater than 160%, or alternatively greater than 170% of the response in an evaluable quantitative or qualitative parameter of the naturally occurring form of the molecule when evaluated at similar concentrations in a comparable assay. It should be noted that the biological effects associated with the full agonist may differ in degree and/or in kind from those biological effects of partial or superagonists. In contrast to agonists, antagonists may specifically bind to a receptor but do not result the signal cascade typically initiated by the receptor and may to modify the actions of an agonist at that receptor. Inverse agonists are agents that produce a pharmacological response that is opposite in direction to that of an agonist.

[0047] Antagonist: As used herein, the term “antagonist” or “inhibitor” refers a molecule that opposes the action(s) of an agonist. An antagonist prevents, reduces, inhibits, or neutralizes the activity of an agonist, and an antagonist can also prevent, inhibit, or reduce constitutive activity of a target, e.g., a target receptor, even where there is no identified agonist. Inhibitors are molecules that decrease, block, prevent, delay activation, inactivate, desensitize, or down-regulate, e.g., a gene, protein, ligand, receptor, biological pathway including an immune checkpoint pathway, or cell.

[0048] Antibody: As used herein, the term “antibody” refers collectively to: (a) glycosylated and non-glycosylated the immunoglobulins (including but not limited to mammalian immunoglobulin classes IgG1, IgG2, IgG3 and IgG4) that specifically binds to target molecule and (b) immunoglobulin derivatives including but not limited to IgG(1-4) δ C_H2, F(ab')₂, Fab, ScFv, V_H, V_L, tetrabodies, triabodies, diabodies, dsFv, F(ab')₃, scFv-Fc and (scFv)₂ that competes with the immunoglobulin from which it was derived for binding to the target molecule.

The term antibody is not restricted to immunoglobulins derived from any particular mammalian species and includes murine, human, equine, camelids, antibodies, human antibodies. The term antibody includes so called “heavy chain antibodies” or “VHHs” or “Nanobodies®” as typically obtained from immunization of camelids (including camels, llamas and alpacas (see, e.g. Hamers-Casterman, *et al.* (1993) Nature 363:446-448). Antibodies having a given specificity may also be derived from non-mammalian sources such as VHHs obtained from immunization of cartilaginous fishes including, but not limited to, sharks. The term “antibody” encompasses antibodies isolatable from natural sources or from animals following immunization with an antigen and as well as engineered antibodies including monoclonal antibodies, bispecific antibodies, tri-specific, chimeric antibodies, humanized antibodies, human antibodies, CDR-grafted, veneered, or deimmunized (e.g., to remove T-cell epitopes) antibodies, camelized (in the case of VHHs), or molecules comprising binding domains of antibodies (e.g. CDRs) in non-immunoglobulin scaffolds. The term “antibody” should not be construed as limited to any particular means of synthesis and includes naturally occurring antibodies isolatable from natural sources and as well as engineered antibodies molecules that are prepared by “recombinant” means including antibodies isolated from transgenic animals that are transgenic for human immunoglobulin genes or a hybridoma prepared therefrom, antibodies isolated from a host cell transformed with a nucleic acid construct that results in expression of an antibody, antibodies isolated from a combinatorial antibody library including phage display libraries. In one embodiment, an “antibody” is a mammalian immunoglobulin. In some embodiments, the antibody is a “full length antibody” comprising variable and constant domains providing binding and effector functions. The term “single domain antibody” (sdAb) as used herein refers an antibody fragment consisting of a monomeric variable antibody domain that is able to bind selectively to an antigen and compete for binding with the parent antibody from which it is derived. As used herein, the term “VHH” refers to a single domain antibody derived from camelid antibody typically obtained from immunization of camelids (including camels, llamas and alpacas (see, e.g. Hamers-Casterman, *et al.* (1993) Nature 363:446-448). VHHs are also referred to as heavy chain antibodies or Nanobodies® as Single domain antibodies may also be derived from non-mammalian sources such as VHHs obtained from IgNAR antibodies immunization of cartilaginous fishes including, but not limited to, sharks. The term antibody

includes antibody conjugates comprising modifications to prolong duration of action such as fusion proteins or conjugation to polymers (e.g. PEGylated) as described in more detail below.

[0049] Biological Sample: As used herein, the term “biological sample” or “sample” refers to a sample obtained or derived from a subject. By way of example, a biological sample comprises a material selected from the group consisting of body fluids, blood, whole blood, plasma, serum, mucus secretions, saliva, cerebrospinal fluid (CSF), bronchoalveolar lavage fluid (BALF), fluids of the eye (e.g., vitreous fluid, aqueous humor), lymph fluid, lymph node tissue, spleen tissue, bone marrow, and an immunoglobulin enriched fraction derived from one or more of these tissues. In some embodiments, the sample is obtained from a subject who has been exposed to a therapeutic treatment regimen including a pharmaceutical formulation of an hIL2 ortholog, such as repeatedly exposed to the same drug. In other embodiments, the sample is obtained from a subject who has not recently been exposed to the hIL2 ortholog or obtained from the subject prior to the planned administration of the hIL2 ortholog.

[0050] “CAR” or “Chimeric Antigen Receptor”: As used herein, the terms “chimeric antigen receptor” and “CAR” are used interchangeably to refer to a chimeric polypeptide comprising multiple functional domains arranged from amino to carboxy terminus in the sequence: (a) an antigen binding domain (ABD), (b) a transmembrane domain (TD); and (c) one or more cytoplasmic signaling domains (CSDs) wherein the foregoing domains may optionally be linked by one or more spacer domains. The CAR may also further comprise a signal peptide sequence which is conventionally removed during post-translational processing and presentation of the CAR on the cell surface of a cell transformed with an expression vector comprising a nucleic acid sequence encoding the CAR. CARs useful in the practice of the present invention are prepared in accordance with principles well known in the art. See e.g., Eshhaar et al. United States Patent No. 7,741,465 B1 issued June 22, 2010; Sadelain, et al (2013) Cancer Discovery 3(4):388-398; Jensen and Riddell (2015) Current Opinions in Immunology 33:9-15; Gross, et al. (1989) PNAS(USA) 86(24):10024-10028; Curran, et al. (2012) J Gene Med 14(6):405-15. Examples of commercially available CAR-T cell products that may be modified to incorporate an orthogonal receptor of the present invention include axicabtagene ciloleucel (marketed as Yescarta® commercially available from Gilead Pharmaceuticals) and tisagenlecleucel (marketed as Kymriah® commercially available from Novartis).

[0051] CAR-T Cell: As used herein, the terms “chimeric antigen receptor T-cell” and “CAR-T cell” are used interchangeably to refer to a T-cell that has been recombinantly modified to express a chimeric antigen receptor. As used herein, a CAR-T cell may be engineered to express an hCD122 ortho polypeptide.

[0052] CD-122 Ortho: As used herein, the terms “CD122 ortho” or “hoCD122” or “hoIL2Rb” are used interchangeably to refer to a variant of hCD122 polypeptide comprising amino acid substitutions at positions histidine 133 (H133) and tyrosine 134 (Y134) in the ECD of the hCD122 polypeptide. In some embodiments CD-122 ortho comprises the amino acid substitutions at position 133 from histidine to aspartic acid (H133D), glutamic acid (H133E) or lysine (H133K) and/or amino acid substitutions at position 134 to from tyrosine to phenylalanine (Y134F), glutamic acid (Y134E), or arginine (Y134R). In a preferred embodiment, the hCD122 orthogonal receptor is a hCD122 molecule having amino acid substitutions H133D and Y134F. In one embodiment, the hCD122 orthogonal receptor is a polypeptide having the amino acid sequence of Sequence ID NO:2.

[0053] CDRs. As used herein, the term “CDR” or “complementarity determining region” is intended to mean the non-contiguous antigen combining sites found within the variable region of both heavy and light chain immunoglobulin polypeptides. CDRs have been described by Kabat et al., *J. Biol. Chem.* 252:6609-6616 (1977); Kabat et al., U.S. Dept. of Health and Human Services, “Sequences of proteins of immunological interest” (1991) (also referred to herein as Kabat 1991); by Chothia et al., *J. Mol. Biol.* 196:901-917 (1987) (also referred to herein as Chothia 1987); and MacCallum et al., *J. Mol. Biol.* 262:732-745 (1996), where the definitions include overlapping or subsets of amino acid residues when compared against each other. Nevertheless, application of either definition to refer to a CDR of an antibody or grafted antibodies or variants thereof is intended to be within the scope of the term as defined and used herein. In the context of the present disclosure, the numbering of the CDR positions is provided according to Kabat numbering conventions.

[0054] Comparable: As used herein, the term “comparable” is used to describe the degree of difference in two measurements of an evaluable quantitative or qualitative parameter. For example, where a first measurement of an evaluable quantitative parameter (e.g. the level of IL-2

activity as determined by an CTLL-2 proliferation or phospho-STAT5 assay) and a second measurement of the evaluable parameter do not deviate beyond a range that the skilled artisan would recognize as not producing a statistically significant difference in effect between the two results in the circumstances, the two measurements would be considered “comparable.” In some instances, measurements may be considered “comparable” if one measurement deviates from another by less than 35%, alternatively by less than 30%, alternatively by less than 25%, alternatively by less than 20%, alternatively by less than 15%, alternatively by less than 10%, alternatively by less than 7%, alternatively by less than 5%, alternatively by less than 4%, alternatively by less than 3%, alternatively by less than 2%, or by less than 1%. In particular embodiments, one measurement is comparable to a reference standard if it deviates by less than 15%, alternatively by less than 10%, or alternatively by less than 5% from the reference standard.

[0055] Derived From: As used herein in the term “derived from”, in the context of an amino acid sequence or polynucleotide sequence (e.g., an amino acid sequence “derived from” an IL-2 polypeptide), is meant to indicate that the polypeptide or nucleic acid has a sequence that is based on that of a reference polypeptide or nucleic acid (e.g., a naturally occurring IL-2 polypeptide or an IL-2-encoding nucleic acid), and is not meant to be limiting as to the source or method in which the protein or nucleic acid is made. By way of example, the term “derived from” includes homologs or variants of reference amino acid or DNA sequences.

[0043] Effective Concentration (EC): As used herein, the terms “effective concentration” or its abbreviation “EC” are used interchangeably to refer to the concentration of an agent in an amount sufficient to effect a change in a given parameter in a test system. The abbreviation “E” refers to the magnitude of a given biological effect observed in a test system when that test system is exposed to a test agent. When the magnitude of the response is expressed as a factor of the concentration (“C”) of the test agent, the abbreviation “EC” is used. In the context of biological systems, the term E_{max} refers to the maximal magnitude of a given biological effect observed in response to a saturating concentration of an activating test agent. When the abbreviation EC is provided with a subscript (e.g., EC₄₀, EC₅₀, etc.) the subscript refers to the percentage of the E_{max} of the biological response observed at that concentration. For example, the concentration of a test agent sufficient to result in the induction of a measurable biological

parameter in a test system that is 30% of the maximal level of such measurable biological parameter in response to such test agent, this is referred to as the “EC₃₀” of the test agent with respect to such biological parameter. Similarly, the term “EC₁₀₀” is used to denote the effective concentration of an agent that results the maximal (100%) response of a measurable parameter in response to such agent. Similarly, the term EC₅₀ (which is commonly used in the field of pharmacodynamics) refers to the concentration of an agent sufficient to results in the half-maximal (50%) change in the measurable parameter. The term “saturating concentration” refers to the maximum possible quantity of a test agent that can dissolve in a standard volume of a specific solvent (*e.g.*, water) under standard conditions of temperature and pressure. In pharmacodynamics, a saturating concentration of a drug is typically used to denote the concentration sufficient of the drug such that all available receptors are occupied by the drug, and EC₅₀ is the drug concentration to give the half-maximal effect.

[0056] Enriched: As used herein in the term “enriched” refers to a sample is non-naturally manipulated so that a molecule of interest is present in: (a) a greater concentration (*e.g.*, at least 3-fold greater, alternatively at least 5-fold greater, alternatively at least 10-fold greater, alternatively at least 50-fold greater, alternatively at least 100-fold greater, alternatively at least 1000-fold greater) than the concentration of the molecule in the starting sample, such as a biological sample (*e.g.*, a sample in which the molecule naturally occurs or in which it is present after administration); or (b) a concentration greater than the environment in which the molecule was made (*e.g.*, as in a recombinantly modified bacterial or mammalian cell).

[0057] Extracellular Domain: As used herein the term "extracellular domain" or its abbreviation "ECD" refers to the portion of a cell surface protein (*e.g.* a cell surface receptor) which is outside of the plasma membrane of a cell. The ECD may include the entire extra-cytoplasmic portion of a transmembrane protein, a cell surface or membrane associated protein, a secreted protein, a cell surface targeting protein,

[0058] hCD-122: As used herein the term "hCD122" refers to a naturally occurring human CD122 polypeptide including naturally occurring variants thereof. The amino acid sequence of one naturally occurring hCD122 variant is:

AVNGTSQFTC FYNSTRANISC VWSQDGALQD TSCQVHAWPD RRRWNQTCEL

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LPVSQASWAC NLILGAPDSQ KLTTVDIVTL RVLCREGVRW RVMAIQDFKP
FENLRIMAPI SLQVVHVETH RCNISWEISQ ASHYFERHLE FEARTLSPGH
TWEEAPLLTL KQKQEWICLE TLTPDTQYEF QVRVKPLQGE FTTWSPWSQP
LAFRTKPAAL GKDTIPWLGH LLVGLSGAFG FIILVYLLIN CRNTGPWLKK
VLKCNTPDPS KFFSQLSSEH GGDVQKWLS PFPSSSFSPG GLAPEISPLE
VLERDKVTQL LLQQDKVPEP ASLSSNHS LT SCFTNQGYFF FHLPDALEIE
ACQVYFTYDP YSEEDPDEGV AGAPTGSSPQ PLQPLSGEDD AYCTFPSRDD
LLLFSPSLG GPSPPSTAPG GSGAGEERMP PSLQERVPRD WDPQPLGPPT
PGVPDLVDFQ PPELVLR EA GEEVPDAGPR EGVSPFWSRP PGQGEFRALN
ARLPLNTDAY LSLQELQGQD PTHL (SEQ ID NO:3)

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[0059] Identity: The term "identity," as used herein in reference to polypeptide or DNA sequences, refers to the subunit sequence identity between two molecules. When a subunit position in both of the molecules is occupied by the same monomeric subunit (*i.e.*, the same amino acid residue or nucleotide), then the molecules are identical at that position. The similarity between two amino acid or two nucleotide sequences is a direct function of the number of identical positions. In general, the sequences are aligned so that the highest order match is obtained. If necessary, identity can be calculated using published techniques and widely available computer programs, such as the GCS program package (Devereux, *et al.*, (1984) *Nucleic Acids Res.* 12:387), BLASTP, BLASTN, FASTA (Atschul, *et al.* (1990) *J. Molecular Biol.* 215:403-410). Algorithms that are suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul *et al.* (1990) *J. Mol. Biol.* 215: 403-410 and Altschul, *et al.* (1977) *Nucleic Acids Res.* 25: 3389-3402. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (NCBI) web site. The algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W of the query sequence, which either match or satisfy some positive-valued threshold score "T" when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul, *et al.*, *supra*). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are then extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters "M" (the reward score for a pair of matching residues; always >0) and "N" (the penalty score for mismatching residues; always <0). For amino acid sequences, a scoring matrix is used to

calculate the cumulative score. Extension of the word hits in each direction are halted when: (a) the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or (b) the end of either sequence is reached. The BLAST algorithm parameters “W”, “T”, and “X” determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) functions similarly but uses as defaults a word size (“W”) of 28, an expectation (“E”) of 10, M=1, N=-2, and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a word size (W) of 3, an expectation (E) of 10, and the BLOSUM62 scoring matrix (*see* Henikoff & Henikoff, (1989) PNAS(USA) 89:10915-10919).

[0060] IL-2: As used herein, the term “interleukin-2” or “IL-2” refers to a naturally occurring IL-2 polypeptide that possesses IL-2 activity. In some embodiments, IL-2 refers to mature wild-type human IL-2. Mature wild-type human IL-2 (hIL2) occurs as a 133 amino acid polypeptide (less the signal peptide, consisting of an additional 20 N-terminal amino acids), as described in Fujita, et. al., PNAS USA, 80, 7437-7441 (1983). An amino acid sequence of naturally occurring variant of mature wild-type human IL-2 (hIL2) is:

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APTSSSTKKT QLQLEHLLLD LQMILNGINN YKNPKLTRML TFKFYMPKKA
TELKHLQCLE EELKPLEEVL NLAQSKNFHL RPRDLISNIN VIVLELKGSE
TTFMCEYADE TATIVEFLNR WITFCQSIIS TLT (SEQ ID NO:4)
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As used herein, the number of residues is based on the hIL2 sequence UniProt ID P60568 excluding the signal peptide which is the same as that of SEQ ID NO:4.

[0061] IL2 Activity: The term “IL2 activity” refers to one or more the biological effects on a cell in response to contacting the cell with an effective amount of an IL2 polypeptide. IL2 Activity may be measured, for example, in a cell proliferation assay using CTLL-2 mouse cytotoxic T cells, see Gearing, A.J.H. and C.B. Bird (1987) in Lymphokines and Interferons, A Practical Approach. Clemens, M.J. *et al.* (eds): IRL Press. 295. The specific activity of Recombinant Human IL-2 is approximately 2.1×10^4 IU/ μ g, which is calibrated against recombinant human IL-2 WHO International Standard (NIBSC code: 86/500). In some embodiments, for example when the hIL2 orthogonal polypeptide of interest exhibits (or is

engineered to possess) diminished affinity for CD25, IL2 activity may be assessed in human cells such as YT cells which do not require CD25 to provide signaling through the IL2 receptor but rather are capable of signaling through the intermediate affinity CD122/CD132 receptor. An orthogonal human IL-2 of the present disclosure may have less than 20%, alternatively less than about 10%, alternatively less than about 8%, alternatively less than about 6%, alternatively less than about 4%, alternatively less than about 2%, alternatively less than about 1%, alternatively less than about 0.5% of the activity of WHO International Standard (NIBSC code: 86/500) wild-type mature human IL-2 when evaluated at similar concentrations in a comparable assay.

[0062] IL-2 ortholog: As used herein, the term “IL-2 ortholog” refers to a variant of hIL2 derived from an IL-2 parent polypeptide which specifically binds to an orthogonal hCD122 ECD and exhibits significantly reduced binding to the extracellular domain of a wild type hCD122. In some embodiment the hIL2 ortholog exhibits specific binding to a receptor comprising an orthogonal hCD122 ECD and the contacting of a cell expressing a membrane spanning receptor comprising the ECD of an orthogonal hCD122 polypeptide in an amount sufficient to effect a change results in the a signal characteristic of the signal produced by the intracellular domain (ICD) of said membrane spanning receptor. When the membrane spanning receptor comprises an orthogonal hCD122 ECD and hCD122 ICD, the binding of an hIL2 ortholog to such receptor results in an intracellular signal characteristic of the activation of a Cd25/CD122/CD132 high affinity of CD122/CD132 intermediate affinity hIL2 receptor. An IL-2 ortholog exhibits significantly reduced binding to wild-type hCD122. The term hIL2 orthologs includes IL-2 orthogonal variants and modified hIL2 orthologs. In some embodiments, the hIL2 ortholog is derived from a naturally occurring variant of human IL2 and such human IL2 orthologs may be referred to as “hoCD122” or “hoRb.” Certain modified IL-2 polypeptides are provided in Garcia, et al. (United States Patent Application Publication US2018/0228842A1 published August 16, 2018). As used herein, the term hIL2 orthologs does not include the modified hIL2 polypeptides described in Garcia, et al United State Patent Application Publication US2018/0228842A1 published August 16, 2018.

[0063] In An Amount Sufficient Amount to Effect a Change: As used herein the phrase “in an amount sufficient to effect a change” refers to the amount of a test agent sufficient to provide a detectable difference between a level of an indicator measured before (e.g., a baseline level)

and after the application of the test agent to a system such as biological function evaluated in a cell based assay in response to the administration of a quantity of the test agent. “An amount sufficient to effect a change” may be sufficient to be a therapeutically effective amount but “in an amount sufficient to effect a change” may be more or less than a therapeutically effective amount.

[0064] In Need of Treatment: The term “in need of treatment” as used herein refers to a judgment made by a physician or other caregiver with respect to a subject that the subject requires or will potentially benefit from treatment. This judgment is made based on a variety of factors that are in the realm of the physician’s or caregiver's expertise.

[0065] In Need of Prevention: As used herein the term “in need of prevention” refers to a judgment made by a physician or other caregiver with respect to a subject that the subject requires or will potentially benefit from preventative care. This judgment is made based upon a variety of factors that are in the realm of a physician’s or caregiver’s expertise.

[0066] Inhibitor: As used herein the term “inhibitor” refers to a molecule that decreases, blocks, prevents, delays activation of, inactivates, desensitizes, or down-regulates, e.g., a gene, protein, ligand, receptor, or cell. An inhibitor can also be defined as a molecule that reduces, blocks, or inactivates a constitutive activity of a cell or organism.

[0067] Intracellular Domain: As used herein the term “intracellular domain” or its abbreviation “ICD” refers to the portion of a cell surface protein (e.g. a cell surface receptor) which is inside of the plasma membrane of a cell. The ICD may include the entire cytoplasmic portion of a transmembrane protein or membrane associated protein, or intracellular protein.

[0068] Isolated: As used herein the term “isolated” is used in reference to a polypeptide of interest that, if naturally occurring, is in an environment different from that in which it can naturally occur. “Isolated” is meant to include polypeptides that are within samples that are substantially enriched for the polypeptide of interest and/or in which the polypeptide of interest is partially or substantially purified. Where the polypeptide is not naturally occurring, “isolated” indicates that the polypeptide has been separated from an environment in which it was made by either synthetic or recombinant means.

[0069] Intracellular Domain of the Orthogonal Receptor: As used herein the terms "intracellular domain of the orthogonal receptor" or "ICD-OR" refer to the portion of a transmembrane spanning orthogonal receptor that is inside of the plasma membrane of a cell expressing such transmembrane spanning orthogonal receptor. The ICD-OR may comprise one or more "proliferation signaling domain(s)" or "PSD(s)" which refers to a protein domain which signals the cell to enter mitosis and begin cell growth. Examples include the Janus kinases, including but not limited to, JAK1, JAK2, JAK3, Tyk2, Ptk-2, homologous members of the Janus kinase family from other mammalian or eukaryotic species, the IL-2 receptor β and/or γ chains and other subunits from the cytokine receptor superfamily of proteins that may interact with the Janus kinase family of proteins to transduce a signal, or portions, modifications or combinations thereof. Examples of signals include phosphorylation of one or more STAT molecules including but not limited to one or more of STAT1, STAT3, STAT5a, and/or STAT5b.

[0070] Kabat Numbering: The term "Kabat numbering" as used herein is recognized in the art and refers to a system of numbering amino acid residues which are more variable than other amino acid residues (*e.g.*, hypervariable) in the heavy and light chain regions of immunoglobulins (Kabat, *et al.*, (1971) *Ann. NY Acad. Sci.* 190:382-93; Kabat, *et al.*, (1991) *Sequences of Proteins of Immunological Interest*, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242). For purposes of the present disclosure, the positioning of CDRs in the variable region of an antibody follows Kabat numbering or simply, "Kabat."

[0071] Ligand: As used herein, the term "ligand" refers to a molecule that specifically binds a receptor and causes a change in the receptor so as to effect a change in the activity of the receptor or a response in cell that expresses that receptor. In one embodiment, the term "ligand" refers to a molecule or complex thereof that can act as an agonist or antagonist of a receptor. As used herein, the term "ligand" encompasses natural and synthetic ligands. "Ligand" also encompasses small molecules, peptide mimetics of cytokines and antibodies. The complex of a ligand and receptor is termed a "ligand-receptor complex." A ligand may comprise one domain of a polypeptide or fusion protein (*e.g.*, either domain of an antibody/ligand fusion protein).

[0072] Metastasis: As used herein the term “metastasis” describes the spread of cancer cell from the primary tumor to surrounding tissues and to distant organs.

[0073] Modified IL-2 Ortholog: As used herein the term “modified IL-2 orthologs” is used to refer to IL-2 orthologs that have been modified by one or more modifications such as pegylation, glycosylation (N- and O-linked), acylation, or polysialylation or by conjugation (either chemical or as fusion proteins) with other polypeptide carrier molecules including but not limited to albumin fusion polypeptides comprising serum albumin (e.g., human serum albumin (HSA) or bovine serum albumin (BSA) or and Fc-fusion proteins or with targeting moieties such as IgG comprising hIL2 orthogonal polypeptide fusion proteins, targeted IL-2 orthogonal polypeptides such as ScFv-hIL2 orthogonal polypeptide fusion proteins and VHH-IL-2 orthogonal polypeptide fusion proteins. Modified hIL2 orthologs may be prepared to order to enhance one or more properties for example, modulating immunogenicity; methods of increasing water solubility, bioavailability, serum half-life, and/or therapeutic half-life; and/or modulating biological activity. Certain modifications can also be useful to, for example, raise of antibodies for use in detection assays (e.g., epitope tags) and to provide for ease of protein purification.

[0074] Modulate: As used herein, the terms “modulate”, “modulation” and the like refer to the ability of a test agent to affect a response, either positive or negative or directly or indirectly, in a system, including a biological system or biochemical pathway. The term modulator includes both agonists and antagonists.

[0075] Neoplastic Disease: As used herein, the term “neoplastic disease” refers to disorders or conditions in a subject arising from cellular hyper-proliferation or unregulated (or dysregulated) cell replication. The term neoplastic disease refers to disorders arising from the presence of neoplasms in the subject. Neoplasms may be classified as: (1) benign (2) pre-malignant (or “pre-cancerous”); and (3) malignant (or “cancerous”). The term “neoplastic disease” includes neoplastic-related diseases, disorders and conditions referring to conditions that are associated, directly or indirectly, with neoplastic disease, and includes, e.g., angiogenesis and precancerous conditions such as dysplasia or smoldering multiple myeloma. Examples of benign disorders arising from dysregulated cell replication include hypertrophic scars such as keloid scars.

[0076] N-Terminus: As used herein in the context of the structure of a polypeptide, “N-terminus” (or “amino terminus”) and “C-terminus” (or “carboxyl terminus”) refer to the extreme amino and carboxyl ends of the polypeptide, respectively, while the terms “N-terminal” and “C-terminal” refer to relative positions in the amino acid sequence of the polypeptide toward the N-terminus and the C-terminus, respectively, and can include the residues at the N-terminus and C-terminus, respectively. “Immediately N-terminal” or “immediately C-terminal” refers to a position of a first amino acid residue relative to a second amino acid residue where the first and second amino acid residues are covalently bound to provide a contiguous amino acid sequence.

[0077] Nucleic Acid: The terms “nucleic acid”, “nucleic acid molecule”, “polynucleotide” and the like are used interchangeably herein to refer to a polymeric form of nucleotides of any length, either deoxyribonucleotides or ribonucleotides, or analogs thereof. Non-limiting examples of polynucleotides include linear and circular nucleic acids, messenger RNA (mRNA), complementary DNA (cDNA), recombinant polynucleotides, vectors, probes, primers and the like.

[0078] Numbered in accordance with hIL2: The term “numbered in accordance with hIL2” as used herein refers to the identification of a location of particular amino acid with reference to the position at which that amino acid normally occurs in the sequence of the mature wild type hIL2 (SEQ ID NO: 4). For example, in reference to hIL2, “R81” refers to the eighty-first (numbered from the N-terminus) amino acid, arginine, that occurs in sequence of the mature wild type hIL2. It should be noted that the amino acid sequences of IL2 molecules of different mammalian species have different numbers and sequences of amino acids. Consequently, when referencing a residue in accordance with this convention it is helpful to identify the IL2 species in question.

[0079] Numbered in accordance with hCD122: The term “numbered in accordance with hCD122” as used herein refers to the identification of a location of particular amino acid with reference to the position at which that amino acid normally occurs in the sequence of the mature wild type hCD122 molecule. In one embodiment, the hCD122 of SEQ ID NO. 3. For example, in reference to human CD122, H133 refers to the histidine at the one-hundred thirty third (numbered from the N-terminus) amino acid of the sequence of the mature wild type hCD122.

[0080] Numbered in accordance with the Extracellular Domain of hCD122: The term "numbered in accordance with extracellular domain of hCD122" or "numbered in accordance with hCD122 ECD" as used herein refers to the identification of a location of particular amino acid with reference to the position at which that amino acid normally occurs in the extracellular domain (ECD) sequence of the mature wild type hCD122 molecules (SEQ ID NO. 3). For example, in reference to human CD122 ECD, H133 refers to the histidine at the one-hundred thirty third (numbered from the N-terminus) amino acid of the sequence of the mature wild type hCD122 ECD.

[0081] Operably Linked: The term "operably linked" is used herein to refer to the relationship between molecules, typically polypeptides or nucleic acids, which are arranged in a construct such that each of the functions of the component molecules is retained although the operable linkage may result in the modulation of the activity, either positively or negatively, of the individual components of the construct. For example, the operable linkage of a polyethylene glycol (PEG) molecule to a wild-type protein may result in a construct where the biological activity of the protein is diminished relative to the to the wild-type molecule, however the two are nevertheless considered operably linked. When the term "operably linked" is applied to the relationship of multiple nucleic acid sequences encoding differing functions, the multiple nucleic acid sequences when combined into a single nucleic acid molecule that, for example, when introduced into a cell using recombinant technology, provides a nucleic acid which is capable of effecting the transcription and/or translation of a particular nucleic acid sequence in a cell. For example, the nucleic acid sequence encoding a signal sequence may be considered operably linked to DNA encoding a polypeptide if it results in the expression of a preprotein whereby the signal sequence facilitates the secretion of the polypeptide; a promoter or enhancer is considered operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is considered operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally in the context of nucleic acid molecules, the term "operably linked" means that the nucleic acid sequences being linked are contiguous, and, in the case of a secretory leader or associated subdomains of a molecule, contiguous and in reading phase. However, certain genetic elements such as enhancers may function at a distance and need not be

contiguous with respect to the sequence to which they provide their effect but nevertheless may be considered operably linked.

[0082] Orthogonal hCD122: As used herein the term “orthogonal hCD122” or “CD122 orthogonal receptor” are used interchangeably herein to refer to an hCD122 polypeptide variant comprising amino acid substitutions that result in specific binding to an hIL2 ortholog but does not specifically bind to a naturally occurring variant of hIL2. In one embodiment, the hCD122 is hCD122 having amino acid modifications at as positions 133 and 134 of Sequence ID NO:4 (the naturally occurring hCD122). In some embodiments, the orthogonal hCD122 comprises is a hCD122 molecule comprising the substitutions H133D and Y134 (Sequence ID NO:2).

[0083] Orthogonal Receptor: As used herein the term “orthogonal receptor” refers to a variant of receptor, the orthogonal receptor comprising modifications to the amino acid sequence so that the orthogonal receptor exhibits significantly reduced binding to its cognate ligand but exhibits specific binding for an orthogonal ligand engineered to interact with the orthogonal receptor. In some embodiments, the orthogonal receptor may comprise an extracellular domain that exhibits significantly reduced binding to its cognate native ligand, while an orthogonal ligand exhibits significantly reduced binding to the ECD of its cognate native receptor(s). In some embodiments, the affinity of the orthogonal ligand for the cognate orthogonal receptor exhibits affinity comparable to the affinity of the native ligand for the native receptor, e.g. having an affinity that is least about 1% of the native cytokine receptor pair affinity, at least about 5%, at least about 10%, at least about 25%, at least about 50%, at least about 75%, at least about 100%, and may be higher, e.g. 2X, 3X, 4X, 5X, 10X or more of the affinity of the native cytokine for the native receptor. An orthogonal receptor may be referred to by the parent molecule from which it was derived (e.g. orthogonal hCD122) or by the cognate ligand from which the orthogonal ligand for the orthogonal receptor was derived (e.g. orthogonal hIL2 receptor).

[0084] Ortholog: As used herein the term “ortholog” refers to a ligand component of an orthogonal ligand/receptor pair and refers to a polypeptide incorporating modifications to its primary structure to provide polypeptide variant that exhibits: (a) significantly reduced affinity to its native cognate receptor (i.e., the native receptor for the parent polypeptide from which the ortholog is derived); and (b) specific binding a engineered orthogonal receptor which is a variant

of the cognate receptor for the ortholog. Upon binding of the ortholog to the orthogonal receptor (which is expressed on surface of cell which has been modified by recombinant DNA technology to incorporate a nucleic acid sequence encoding the orthogonal receptor operably linked to control elements to effect the expression of the orthogonal receptor in the recombinantly modified cell), the activated orthogonal receptor initiates signaling that is transduced through native cellular elements to provide for a biological activity that mimics that native response of the cognate but which is specific to the recombinantly modified cell population expressing the orthogonal receptor. In some embodiments of the invention, orthologs possess significant selectivity for the orthogonal receptor relative to the cognate receptor and optionally possessing significantly reduced potency with respect to the cognate receptor. Selectivity is typically assessed by activity measured in an assay characteristic of the activity induced in response to ligand/receptor binding. In some embodiments, the ortholog possesses at least 5 fold, alternatively at least 10 fold, alternatively at least 20 fold, alternatively at least 30 fold, alternatively at least 40 fold, alternatively at least 50 fold, alternatively at least 100 fold, alternatively at least 200 fold difference in EC₅₀ increased affinity for the orthogonal receptor as compared to the as measured in the same assay.

[0085] Parent Polypeptide: As used herein, the terms "parent polypeptide" or "parent protein" are used interchangeably to designate the source of a second polypeptide (e.g. a derivative or variant) which is modified with respect to a first "parent" polypeptide. In some instances, the parent polypeptide is a wild-type or naturally occurring form of a protein. In some instance, the parent polypeptide may be a modified form a naturally occurring protein that is further modified. The term "parent polypeptide" may refer to the polypeptide itself or compositions that comprise the parent polypeptide (e.g. glycosylated or PEGylated forms and/or fusion proteins comprising the parent polypeptide).

[0086] Partial Agonist: As used herein, the term "partial agonist" refers to a molecule that specifically binds that bind to and activate a given receptor but possess only partial activation the receptor relative to a full agonist. Partial agonists may display both agonistic and antagonistic effects. For example when both a full agonist and partial agonist are present, the partial agonist acts as a competitive antagonist by competing with the full agonist for the receptor binding resulting in net decrease in receptor activation relative to the contact of the receptor with the full

agonist in the absence of the partial agonist. Partial agonists can be used to activate receptors to give a desired submaximal response in a subject when inadequate amounts of the endogenous ligand are present, or they can reduce the overstimulation of receptors when excess amounts of the endogenous ligand are present. The maximum response ($E_{\max}$) produced by a partial agonist is called its intrinsic activity and may be expressed on a percentage scale where a full agonist produced a 100% response. An partial agonist may have greater than 10% but less than 100%, alternatively greater than 20% but less than 100%, alternatively greater than 30% but less than 100%, alternatively greater than 40% but less than 100%, alternatively greater than 50% but less than 100%, alternatively greater than 60% but less than 100%, alternatively greater than 70% but less than 100%, alternatively greater than 80% but less than 100%, or alternatively greater than 90% but less than 100%, of the activity of the reference polypeptide when evaluated at similar concentrations in a given assay system.

[0087] PEG-hIL2 Ortholog: As used herein the term “PEG-hIL2 ortholog” refers to a hIL2 ortholog covalently bound to at least one polyethylene glycol (PEG) molecule, the at least one PEG molecule being covalently attached to at least one amino acid residue of an IL-2 ortholog. The PEGylated polypeptide may be further referred to as monopegylated, dipegylated, tripegylated (and so forth) to denote PEG-hIL2 orthologs comprising one, two, three (or more) PEG moieties attached to the IL-2 ortholog, respectively. In some embodiments, the PEG may be covalently attached directly to the IL-2 ortholog (e.g., through a lysine side chain, sulfhydryl group of a cysteine or N-terminal amine) or optionally employ a linker between the PEG and the IL-2 ortholog. In some embodiments the PEG-hIL2 ortholog comprises more than one PEG molecule each of which is attached to a different amino acid residue. In some embodiments, the PEG-hIL2 ortholog is derived from Sequence ID NO:4.

[0088] Polypeptide: As used herein the terms “polypeptide,” “peptide,” and “protein”, used interchangeably herein, refer to a polymeric form of amino acids of any length, which can include genetically coded and non-genetically coded amino acids, chemically or biochemically modified or derivatized amino acids, and polypeptides having modified polypeptide backbones. The terms include fusion proteins, including, but not limited to, fusion proteins with a

heterologous amino acid sequence; fusion proteins with heterologous and homologous leader sequences; fusion proteins with or without N-terminus methionine residues; fusion proteins with immunologically tagged proteins; fusion proteins of immunologically active proteins (e.g. antigenic diphtheria or tetanus toxin fragments) and the like.

[0089] Prevent: As used herein the terms “prevent”, “preventing”, “prevention” and the like refer to a course of action initiated with respect to a subject prior to the onset of a disease, disorder, condition or symptom thereof so as to prevent, suppress, inhibit or reduce, either temporarily or permanently, a subject’s risk of developing a disease, disorder, condition or the like (as determined by, for example, the absence of clinical symptoms) or delaying the onset thereof, generally in the context of a subject predisposed due to genetic, experiential or environmental factors to having a particular disease, disorder or condition. In certain instances, the terms “prevent”, “preventing”, “prevention” are also used to refer to the slowing of the progression of a disease, disorder or condition from a present its state to a more deleterious state.

[0090] Receptor: As used herein, the term “receptor” refers to a polypeptide having a domain that specifically binds a ligand that binding of the ligand results in a change to at least one biological property of the polypeptide. In some embodiments, the receptor is a cell surface receptor that comprises an extracellular domain (ECD) and a membrane associated domain which serves to anchor the ECD to the cell surface. In some embodiments of cell surface receptors, the receptor is a membrane spanning polypeptide comprising an intracellular domain (ICD) and extracellular domain (ECD) linked by a membrane spanning domain typically referred to as a transmembrane domain (TM). The binding of a cognate ligand to the receptor results in a conformational change in the receptor resulting in a measurable biological effect. In some instances, where the receptor is a membrane spanning polypeptide comprising an ECD, TM and ICD, the binding of the ligand to the ECD results in a measurable intracellular biological effect mediated by one or more domains of the ICD in response to the binding of the ligand to the ECD. In some embodiments, a receptor is a component of a multi-component complex to facilitate intracellular signaling. For example, the ligand may bind a cell surface receptor that is not associated with any intracellular signaling alone but upon ligand binding facilitates the formation of a heteromultimeric (including heterodimeric, heterotrimeric, etc.) or homomultimeric (including homodimeric, homotrimeric, homotetrameric, etc.) complex that

results in a measurable biological effect in the cell such as activation of an intracellular signaling cascade (e.g. the Jak/STAT pathway). For example, the ligand may bind a cell surface molecule having not associated with any intracellular signaling alone but upon ligand binding facilitates the formation of a heteromultimeric including heterodimeric (e.g. the intermediate affinity hCD122/CD132 hIL2 receptor), heterotrimeric (e.g. the high affinity CD25/CD122/CD132 hIL2 receptor) or homomultimeric (homodimeric, homotrimeric, homotetrameric) complex that results in the activation of an intracellular signaling cascade (e.g. the Jak/STAT pathway). In some embodiments, a receptor is a membrane spanning single chain polypeptide comprising ECD, TM and ICD domains wherein the ECD, TM and ICD domains are derived from the same or differing naturally occurring receptor variants or synthetic functional equivalents thereof.

[0091] Recombinant: As used herein, the term “recombinant” is used as an adjective to refer to the method by which a polypeptide, nucleic acid, or cell was modified using recombinant DNA technology. A “recombinant protein” is a protein produced using recombinant DNA technology and is frequently abbreviated with a lower case “r” preceding the protein name to denote the method by which the protein was produced (e.g., recombinantly produced human growth hormone is commonly abbreviated “rhGH”). Similarly a cell is referred to as a “recombinant cell” if the cell has been modified by the incorporation (e.g. transfection, transduction, infection) of exogenous nucleic acids (e.g., ssDNA, dsDNA, ssRNA, dsRNA, mRNA, viral or non-viral vectors, plasmids, cosmids and the like) using recombinant DNA technology. The techniques and protocols for recombinant DNA technology are well known in the art such as those can be found in Sambrook, *et al.* (1989) *Molecular Cloning: A Laboratory Manual* (2d ed., Cold Spring Harbor Laboratory Press, Plainview, N.Y.) and other standard molecular biology laboratory manuals.

[0092] Response: The term “response,” for example, of a cell, tissue, organ, or organism, encompasses a quantitative or qualitative change in a evaluable biochemical or physiological parameter, (e.g., concentration, density, adhesion, proliferation, activation, phosphorylation, migration, enzymatic activity, level of gene expression, rate of gene expression, rate of energy consumption, level of or state of differentiation) where the change is correlated with the activation, stimulation, or treatment, with or contact with exogenous agents or internal mechanisms such as genetic programming. In certain contexts, the terms “activation”,

“stimulation”, and the like refer to cell activation as regulated by internal mechanisms, as well as by external or environmental factors; whereas the terms “inhibition”, “down-regulation” and the like refer to the opposite effects. A “response” may be evaluated *in vitro* such as through the use of assay systems, surface plasmon resonance, enzymatic activity, mass spectroscopy, amino acid or protein sequencing technologies. A “response” may be evaluated *in vivo* quantitatively by evaluation of objective physiological parameters such as body temperature, bodyweight, tumor volume, blood pressure, results of X-ray or other imaging technology or qualitatively through changes in reported subjective feelings of well-being, depression, agitation, or pain. In some embodiments, the level of proliferation of CD3 activated primary human T-cells may be evaluated in a bioluminescent assay that generates a luminescent signal that is proportional to the amount of ATP present which is directly proportional to the number of cells present in culture as described in Crouch, *et al.* (1993) J. Immunol. Methods 160: 81–8 or through the use of commercially available assays such as the CellTiter-Glo® 2.0 Cell Viability Assay or CellTiter-Glo® 3D Cell Viability kits commercially available from Promega Corporation, Madison WI 53711 as catalog numbers G9241 and G9681 in substantial accordance with the instructions provided by the manufacturer. In some embodiments, the level of activation of T cells in response to the administration of a test agent may be determined by flow cytometric methods as described as determined by the level of STAT (e.g., STAT1, STAT3, STAT5) phosphorylation in accordance with methods well known in the art. For example, STAT5 phosphorylation may be measured using flow cytometric techniques as described in Horta, *et al. supra.*, Garcia, *et al., supra.* or commercially available kits such as the Phospho-STAT5 (Tyr694) kit (commercially available from Perkin-Elmer, Waltham MA as Part Number 64AT5PEG) in performed in substantial accordance with the instructions provided by the manufacturer.

[0093] Significantly Reduced Binding: The term “exhibits significantly reduced binding” is used with respect to a variant of a first molecule (e.g. a ligand) which exhibits a significant reduction in the affinity for a second molecule (e.g. receptor) relative the parent form of the first molecule. As used herein, the term “exhibits significantly reduced binding” is used with respect to the affinity of the binding of the orthogonal ligand to the orthogonal receptor relative to the binding of the orthogonal ligand for the naturally occurring form of its cognate receptor. An orthogonal ligand exhibits significantly reduced binding with respect to the native form of the

ligand if the orthogonal ligand binds to the native form of the receptor with less than 20%, alternatively less than about 10%, alternatively less than about 8%, alternatively less than about 6%, alternatively less than about 4%, alternatively less than about 2%, alternatively less than about 1%, alternatively less than about 0.5% of the naturally occurring ligand. Similarly and orthogonal receptor exhibits significantly reduced binding with respect to the native form of the ligand if the native form of the ligand binds to the orthogonal form of the receptor with less than 20%, alternatively less than about 10%, alternatively less than about 8%, alternatively less than about 6%, alternatively less than about 4%, alternatively less than about 2%, alternatively less than about 1%, alternatively less than about 0.5% of the naturally occurring receptor.

[0094] **Small Molecule(s):** The term “small molecules” refers to chemical compounds (typically pharmaceutically active compounds) having a molecular weight that is less than about 10kDa, less than about 2kDa, or less than about 1kDa. Small molecules include, but are not limited to, inorganic molecules, organic molecules, organic molecules containing an inorganic component, molecules comprising a radioactive atom, and synthetic molecules. The term “small molecule” is a term well understood to those of ordinary skill in the pharmaceutical arts and is typically used to distinguish organic chemical compounds from biologics.

[0095] **Specifically Binds:** As used herein the term “specifically binds” refers to the degree of selectivity or affinity for which one molecule binds to another. In the context of binding pairs (e.g. a ligand/receptor, antibody/antigen, antibody/ligand, antibody/receptor binding pairs) a first molecule of a binding pair is said to specifically bind to a second molecule of a binding pair when the first molecule of the binding pair does not bind in a significant amount to other components present in the sample. A first molecule of a binding pair is said to specifically bind to a second molecule of a binding pair when the first molecule of the binding pair when the affinity of the first molecule for the second molecule is at least two-fold greater, alternatively at least five times greater, alternatively at least ten times greater, alternatively at least 20-times greater, or alternatively at least 100-times greater than the affinity of the first molecule for other components present in the sample. In a particular embodiment, where the first molecule of the binding pair is an antibody, the antibody specifically binds to the second molecule of the binding pair (e.g. a protein, antigen, ligand, or receptor) if the equilibrium dissociation constant between antibody and to the second molecule of the binding pair is greater than about 10^6M , alternatively

greater than about 10^8 M, alternatively greater than about 10^{10} M, alternatively greater than about 10^{11} M, alternatively greater than about 10^{10} M, greater than about 10^{12} M as determined by, e.g., Scatchard analysis (Munsen, et al. 1980 *Analyt. Biochem.* 107:220-239). In one embodiment where the ligand is an hIL2 ortholog and the receptor comprises an orthogonal hCD122 ECD, the hIL2 ortholog specifically binds if the equilibrium dissociation constant of the hIL2 ortholog/orthogonal hCD122 ECD is greater than about 10^5 M, alternatively greater than about 10^6 M, alternatively greater than about 10^7 M, alternatively greater than about 10^8 M, alternatively greater than about 10^9 M, alternatively greater than about 10^{10} M, or alternatively greater than about 10^{11} M. Specific binding may be assessed using techniques known in the art including but not limited to competition ELISA assays, radioactive ligand binding assays (e.g., saturation binding, Scatchard plot, nonlinear curve fitting programs and competition binding assays); non-radioactive ligand binding assays (e.g., fluorescence polarization (FP), fluorescence resonance energy transfer (FRET); liquid phase ligand binding assays (e.g., real-time polymerase chain reaction (RT-qPCR), and immunoprecipitation); and solid phase ligand binding assays (e.g., multiwell plate assays, on-bead ligand binding assays, on-column ligand binding assays, and filter assays)) and surface plasmon resonance assays (see, e.g., Drescher *et al.*, (2009) *Methods Mol Biol* 493:323-343 with commercially available instrumentation such as the Biacore 8+, Biacore S200, Biacore T200 (GE Healthcare Bio-Sciences, 100 Results Way, Marlborough MA 01752).

[0096] Subject: The terms “recipient”, “individual”, “subject”, and “patient”, are used interchangeably herein and refer to any mammalian subject for whom diagnosis, treatment, or therapy is desired, particularly humans. “Mammal” for purposes of treatment refers to any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, horses, cats, cows, sheep, goats, pigs, etc. In some embodiments, the mammal is a human being.

[0097] Suffering From: As used herein, the term “suffering from” refers to a determination made by a physician with respect to a subject based on the available information accepted in the field for the identification of a disease, disorder or condition including but not limited to X-ray, CT-scans, conventional laboratory diagnostic tests (e.g. blood count, etc.), genomic data, protein expression data, immunohistochemistry, that the subject requires or will benefit from treatment.

The term suffering from is typically used in conjunction with a particular disease state such as “suffering from a neoplastic disease” refers to a subject which has been diagnosed with the presence of a neoplasm.

[0098] Substantially Pure: As used herein, the term “substantially pure” indicates that a component of a composition makes up greater than about 50%, alternatively greater than about 60%, alternatively greater than about 70%, alternatively greater than about 80%, alternatively greater than about 90%, alternatively greater than about 95%, of the total content of the composition. A protein that is “substantially pure” comprises greater than about 50%, alternatively greater than about 60%, alternatively greater than about 70%, alternatively greater than about 80%, alternatively greater than about 90%, alternatively greater than about 95%, of the total content of the composition.

[0099] T-cell: As used herein the term “T-cell” or “T cell” is used in its conventional sense to refer to a lymphocytes that differentiates in the thymus, possess specific cell-surface antigen receptors, and include some that control the initiation or suppression of cell-mediated and humoral immunity and others that lyse antigen-bearing cells. In some embodiments the T cell includes without limitation naïve CD8⁺ T cells, cytotoxic CD8⁺ T cells, naïve CD4⁺ T cells, helper T cells, e.g. Th1, Th2, Th9, Th11, Th22, T_{FH}; regulatory T cells, e.g. Tr1, Tregs, inducible Tregs; memory T cells, e.g. central memory T cells, effector memory T cells, NKT cells, tumor infiltrating lymphocytes (TILs) and engineered variants of such T-cells including but not limited to CAR-T cells, recombinantly modified TILs and TCR-engineered cells. .

[0100] Terminus/Terminal: As used herein in the context of the structure of a polypeptide, “N-terminus” (or “amino terminus”) and “C-terminus” (or “carboxyl terminus”) refer to the extreme amino and carboxyl ends of the polypeptide, respectively; while the terms “N-terminal” and “C-terminal” refer to relative positions in the amino acid sequence of the polypeptide toward the N-terminus and the C-terminus, respectively, and can include the residues at the N-terminus and C-terminus, respectively. “Immediately N-terminal” refers to the position of a first amino acid residue relative to a second amino acid residue in a contiguous polypeptide sequence, the first amino acid being closer to the N-terminus of the polypeptide. “Immediately C-terminal” refers to

the position of a first amino acid residue relative to a second amino acid residue in a contiguous polypeptide sequence, the first amino acid being closer to the C-terminus of the polypeptide.

[0101] Therapeutically Effective Amount: The phrase “therapeutically effective amount” as used herein in reference to the administration of an agent to a subject, either alone or as part of a pharmaceutical composition or treatment regimen, in a single dose or as part of a series of doses in an amount capable of having any detectable, positive effect on any symptom, aspect, or characteristic of a disease, disorder or condition when administered to the subject. The therapeutically effective amount can be ascertained by measuring relevant physiological effects, and it may be adjusted in connection with a dosing regimen and in response to diagnostic analysis of the subject’s condition, and the like. The parameters for evaluation to determine a therapeutically effective amount of an agent are determined by the physician using art accepted diagnostic criteria including but not limited to indicia such as age, weight, sex, general health, ECOG score, observable physiological parameters, blood levels, blood pressure, electrocardiogram, computerized tomography, X-ray, and the like. Alternatively, or in addition, other parameters commonly assessed in the clinical setting may be monitored to determine if a therapeutically effective amount of an agent has been administered to the subject such as body temperature, heart rate, normalization of blood chemistry, normalization of blood pressure, normalization of cholesterol levels, or any symptom, aspect, or characteristic of the disease, disorder or condition, biomarkers (such as inflammatory cytokines, IFN- γ , granzyme, and the like), reduction in serum tumor markers, improvement in Response Evaluation Criteria In Solid Tumors (RECIST), improvement in Immune-Related Response Criteria (irRC), increase in duration of survival, extended duration of progression free survival, extension of the time to progression, increased time to treatment failure, extended duration of event free survival, extension of time to next treatment, improvement objective response rate, improvement in the duration of response, reduction of tumor burden, complete response, partial response, stable disease, and the like that are relied upon by clinicians in the field for the assessment of an improvement in the condition of the subject in response to administration of an agent. As used herein the terms “Complete Response (CR),” “Partial Response (PR)” “Stable Disease (SD)” and

“Progressive Disease (PD)” with respect to target lesions and the terms “Complete Response (CR),” “Incomplete Response/Stable Disease (SD)” and Progressive Disease (PD) with respect to non-target lesions are understood to be as defined in the RECIST criteria. As used herein the terms “immune-related Complete Response (irCR),” “immune-related Partial Response (irPR),” “immune-related Progressive Disease (irPD)” and “immune-related Stable Disease (irSD)” as as defined in accordance with the Immune-Related Response Criteria (irRC). As used herein, the term “Immune-Related Response Criteria (irRC)” refers to a system for evaluation of response to immunotherapies as described in Wolchok, *et al.* (2009) *Guidelines for the Evaluation of Immune Therapy Activity in Solid Tumors: Immune-Related Response Criteria*, *Clinical Cancer Research* 15(23): 7412-7420. A therapeutically effective amount may be adjusted over a course of treatment of a subject in connection with the dosing regimen and/or evaluation of the subject’s condition and variations in the foregoing factors. In one embodiment, a therapeutically effective amount is an amount of an agent when used alone or in combination with another agent does not result in non-reversible serious adverse events in the course of administration to a mammalian subject.

[0102] Transmembrane Domain: The term "transmembrane domain " or "TM " refers to the domain of a membrane spanning polypeptide (e.g. a membrane spanning receptor) which, when the membrane spanning polypeptide is associated with a cell membrane, is which is embedded in the cell membrane and is in peptidyl linkage with the extracellular domain (ECD) and the intracellular domain (ICD) of a membrane spanning polypeptide. A transmembrane domain may be homologous (naturally associated with) or heterologous (not naturally associated with) with either or both of the extracellular and/or intracellular domains. In some embodiments the transmembrane domain is the transmembrane domain natively associated with the ECD domain of the cognate receptor from which the orthogonal receptor is derived. In some embodiments the transmembrane domain is the transmembrane domain natively associated with the ICD domain of the cognate receptor from which the orthogonal receptor is derived. In some embodiments the transmembrane domain is the transmembrane domain natively associated with the proliferation signaling domain. In some embodiments the transmembrane domain is the transmembrane domain natively associated with a different protein. Alternatively, the transmembrane domain of the orthogonal receptor may be an artificial amino acid sequence which spans the plasma membrane. In some embodiments, the transmembrane domain of the orthogonal receptor is the

transmembrane domain normally associated with the ICD of the cognate receptor from which the orthogonal receptor is derived.

[0103] Treat: The terms “treat”, “treating”, “treatment” and the like refer to a course of action ((such as contacting a subject with an hIL-2 ortholog, a hCR₂ T cell, a hCAR-T cell, or a pharmaceutical composition comprising same) alone or in combination with a supplementary agent) that is initiated with respect to a subject in response to a diagnosis that the subject is suffering from a disease, disorder or condition, or a symptom thereof, the course of action being initiated so as to eliminate, reduce, suppress, mitigate, or ameliorate, either temporarily or permanently, at least one of: (a) the underlying causes of such disease, disorder, or condition afflicting a subject; and/or (b) at least one of the symptoms associated with such disease, disorder, or condition. In some embodiments, treating includes a course of action taken with respect to a subject suffering from a disease where the course of action results in the inhibition (e.g., arrests the development of the disease, disorder or condition or ameliorates one or more symptoms associated therewith) of the disease in the subject.

[0104] Treg Cell or Regulatory T Cell. The terms “regulatory T cell” or “Treg cell” as used herein refers to a type of CD4⁺ T cell that can suppress the responses of other T cells including but not limited to effector T cells (Teff). Treg cells are characterized by expression of CD4, the α -subunit of the IL-2 receptor (CD25), and the transcription factor forkhead box P3 (FOXP3) (Sakaguchi, Annu Rev Immunol 22, 531-62 (2004). By “conventional CD4⁺ T cells” is meant CD4⁺ T cells other than regulatory T cells.

[0105] Variant: The terms “variant”, “protein variant” or “variant protein” or “variant polypeptide” are used interchangeably herein to refer to a polypeptide that differs from a parent polypeptide by virtue of at least one amino acid modification, substitution, or deletion. The parent polypeptide may be a naturally occurring or wild-type (WT) polypeptide or may be a modified version of a WT polypeptide. The term variant polypeptide may refer to the polypeptide itself, a composition comprising the polypeptide, or the nucleic acid sequence that encodes it. In some embodiments, the variant polypeptide comprises from about one to about ten, alternatively about one to about eight, alternatively about one to about seven, alternatively about one to about five, alternatively about one to about four, alternatively from about one to about

three alternatively from one to two amino acid modifications, substitutions, or deletions, or alternatively a single amino acid amino acid modification, substitution, or deletion compared to the parent polypeptide. A variant may be at least about 99% identical, alternatively at least about 98% identical, alternatively at least about 97% identical, alternatively at least about 95% identical, or alternatively at least about 90% identical to the parent polypeptide from which the variant is derived.

[0106] Wild Type: By "wild type" or "WT" or "native" herein is meant an amino acid sequence or a nucleotide sequence that is found in nature, including allelic variations. A WT protein, polypeptide, antibody, immunoglobulin, IgG, etc. has an amino acid sequence or a nucleotide sequence that has not been modified by the hand of man.

hIL2 Orthologs

Nomenclature:

[0107] The present disclosure provides a variety polypeptide ligands of hIL2 receptor polypeptide variants. The following nomenclature is used herein to refer to substitutions, deletions or insertions. Residues may be designated herein by the one-letter or three-letter amino acid code of the naturally occurring amino acid found in the wild-type molecule but followed by the hIL2 amino acid position of the mature hIL2 molecule, e.g., "Cys125" or "C125" refers to the cysteine residue at position 125 of the wild-type hIL2 molecule. In reference to the hIL2 orthologs, substitutions are designated herein by the one letter amino acid code followed by the hIL2 amino acid position followed by the one letter amino acid code which is substituted. The numbering of the hIL2 orthologs of the present disclosure are numbered in accordance with hIL2. For example, an hIL2 ortholog having the modification "K35A" refers to a substitution of the lysine (K) residue at position 35 of the wild-type hIL2 sequence with an alanine (A) residue at this position. A deletion of an amino acid residue is referred to as "des" followed by the amino acid residue and its position in SEQ ID NO:4. For example the term "des-Ala1" or "ΔA1" "desA1" refers to the deletion of the alanine at position 1 of the polypeptide of wild-type hIL2 sequence. Similarly, in reference to amino acid substitutions in the orthogonal hCD122, amino

acid substitutions are designated herein by the one letter amino acid code of the naturally occurring amino acid followed by the number of its position in the wild-type hIL2 sequence followed by the one letter amino acid code of the amino acid which is substituted at that position. The hCD122 modifications to hCD122 incorporated into the orthogonal receptor are numbered in accordance with hCD122. For example, the hCD122 orthogonal receptor having a substitution of the tyrosine residue at position 134 with a phenylalanine residue, the substitution is abbreviated "Y134F." The abbreviation hoRb is used synonymously with hoCD122 to refer to a human CD122 which comprises orthogonal hCD122 receptor. Similarly, the reference to an "hoRb cell" (e.g., an hoRb T cell or hoRb NKL cell) refers to a cell which expresses an orthogonal hCD122 receptor. The term SQVLKA as used herein refers to a hIL2 ortholog comprising the amino acid substitutions: E15S, H16Q, L19V, D20L, Q22K, and M23A.

hIL2 Orthologs

[0108] In some embodiments, the present disclosure provides hIL2 orthologs and methods of use thereof, that are cognate ligands of orthogonal receptors comprising a modified hCD122 ECD. In some embodiments, the term hIL2 orthologs refers to hIL2 variants that ligands for a receptor comprising the extracellular domain of human orthogonal hCD122 comprising amino acid substitutions at positions H133 and/or Y134. In some embodiments, the hIL2 orthologs are ligands for an orthogonal receptor comprising the extracellular domain of human hCD122 comprising amino acid substitutions at positions H133 and/or Y134. In some embodiments, the hIL2 orthologs are ligands for a transmembrane receptor comprising the extracellular domain of human CD122 comprising amino acid substitutions at positions H133 and/or Y134 the ICD of which comprises one or more STAT3 binding motifs. In some embodiments, the hIL2 orthologs are ligands for an orthogonal human CD122 comprising amino acid substitutions at positions H133 and Y134. In some embodiments, the hIL2 orthologs are ligands for an orthogonal hCD122 comprising the amino acid substitutions H133D and Y134F.

[0109] In various embodiments, the compositions and methods of the present disclosure comprise the use of hIL2 ortholog polypeptides having at least 80%, alternatively 85%, alternatively 90%, alternatively 95%, alternatively 97%, alternatively 99%, identity to polypeptide of the following 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)-I114-V115-E116-F117-L118-N119-R120-
 W121-I122-T123-F124-(AA125)-(AA126)-S127-I128-I129-(AA130)-T131-L132-
 T133

[1]

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, 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.

[0110] A series of hIL2 orthologs of the foregoing Formula 1 (expressed with an additional N-terminal signal peptide of the sequence MYRMQLLSICIALSLALVTNS (SEQ ID NO. 421) of the mature protein sequence) were prepared as provided in Table 2 below in accordance with the

Examples and evaluated to their ability to selectively activate NKL cells relative to NKL cells which have been engineered to express an orthogonal CD122 of SEQ ID NO: 2 T cells expressing an orthogonal IL2 receptor comprising the substitutions.

Table 2. hIL2 Orthologs				
SEQ ID NO:	Ref#	Name	Modifications Relative to wt hIL2	Mature Protein Sequence
5	001	-QVLKA	H16Q L19V D20L Q22K M23A	APTSSSTKKKTQLQLEQLLVLLKAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
6	002	S-VLKA	E15S L19V D20L Q22K M23A	APTSSSTKKKTQLQLSHLLVLLKAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
7	003	SQ-LKA	E15S H16Q D20L Q22K M23A	APTSSSTKKKTQLQLSQLLLLLKAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
8	004	SQV-KA	E15S H16Q L19V Q22K M23A	APTSSSTKKKTQLQLSQLLVDLKAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
9	005	SQVL-A	E15S H16Q L19V D20L M23A	APTSSSTKKKTQLQLSQLLVLLQAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
10	006	SQVLK-	E15S H16Q L19V D20L Q22K	APTSSSTKKKTQLQLSQLLVLLKAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
11	007	S-----	E15S	APTSSSTKKKTQLQLSHLLLDLQAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
12	008	-Q-----	H16Q	APTSSSTKKKTQLQLEQLLLDLQAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
13	009	--V---	L19V	APTSSSTKKKTQLQLEHLLVDLQAILNGINNYKN PKLTRMLTFKFYMPKKATELKHLQCLEEELKPL EEVLNLAQSKNFHLRPRDLISNINVIVLELKGSE TTFMCEYADETATIVEEFLNRWITFCQSIISTLT

Table 2. hIL2 Orthologs				
SEQ ID NO:	Ref#	Name	Modifications Relative to wt hIL2	Mature Protein Sequence
14	010	---L--	D20L	APTSSSTKKKTQLQLEHLLLLLQMILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
15	011	----K-	Q22K	APTSSSTKKKTQLQLEHLLLDLKMILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
16	012	-----A	M23A	APTSSSTKKKTQLQLEHLLLDLQAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
17	013	SQ----	E15S H16Q	APTSSSTKKKTQLQLSQLLLDLQMILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
18	014	SQVL--	E15S H16Q L19V D20L	APTSSSTKKKTQLQLSQLLVLLQMILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
19	015	SQVLKA	E15S H16Q L19V D20L Q22K M23A	APTSSSTKKKTQLQLSQLLVLLKAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
20	016	ΔA1, SQVLKA	desA1 E15S H16Q L19V D20L Q22K M23A	PTSSSTKKKTQLQLSQLLVLLKAILNGINNYKNPK LTRMLTFKFYMPKKATELKHLQCLEEELKPLEE VLNLAQSKNFHLRPRDLISNINVIVLELKGSETTF MCEYADETATIVEEFLNRWITFCQSIISTLT
21	017	ΔP2, SQVLKA	desP2 E15S H16Q L19V D20L Q22K M23A	ATSSSTKKKTQLQLSQLLVLLKAILNGINNYKNPK LTRMLTFKFYMPKKATELKHLQCLEEELKPLEE VLNLAQSKNFHLRPRDLISNINVIVLELKGSETTF MCEYADETATIVEEFLNRWITFCQSIISTLT
22	018	ΔT3, SQVLKA	desT3 E15S H16Q L19V D20L Q22K M23A	APSSSTKKKTQLQLSQLLVLLKAILNGINNYKNPK LTRMLTFKFYMPKKATELKHLQCLEEELKPLEE VLNLAQSKNFHLRPRDLISNINVIVLELKGSETTF MCEYADETATIVEEFLNRWITFCQSIISTLT
23	019	T3C, SQVLKA	T3C E15S H16Q L19V D20L Q22K M23A	APCSSSTKKKTQLQLSQLLVLLKAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
24	020	T3A, SQVLKA	T3A E15S H16Q L19V D20L Q22K M23A	APASSSTKKKTQLQLSQLLVLLKAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT

Table 2. hIL2 Orthologs				
SEQ ID NO:	Ref#	Name	Modifications Relative to wt hIL2	Mature Protein Sequence
25	021	T3G, SQVLKA	T3G E15S H16Q L19V D20L Q22K M23A	APGSSSTKKTQLQLSQLLVLLKAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
26	022	T3Q, SQVLKA	T3Q E15S H16Q L19V D20L Q22K M23A	APQSSSTKKTQLQLSQLLVLLKAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
27	023	T3E, SQVLKA	T3E E15S H16Q L19V D20L Q22K M23A	APSSSTKKTQLQLSQLLVLLKAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
28	024	T3N, SQVLKA	T3N E15S H16Q L19V D20L Q22K M23A	APNSSSTKKTQLQLSQLLVLLKAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
29	025	T3D, SQVLKA	T3D E15S H16Q L19V D20L Q22K M23A	APDSSSTKKTQLQLSQLLVLLKAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
30	026	T3R, SQVLKA	T3R E15S H16Q L19V D20L Q22K M23A	APRSSSTKKTQLQLSQLLVLLKAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
31	027	T3K, SQVLKA	T3K E15S H16Q L19V D20L Q22K M23A	APKSSSTKKTQLQLSQLLVLLKAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
32	028	T3P, SQVLKA	T3P E15S H16Q L19V D20L Q22K M23A	APPSSSTKKTQLQLSQLLVLLKAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
33	029	ΔS4, SQVLKA	desS4 E15S H16Q L19V D20L Q22K M23A	APTSSSTKKTQLQLSQLLVLLKAILNGINNYKNPK LTRMLTFKFYMPKKATELKHLQCLEEELKPLEE VLNLAQSKNFHLRPRDLISNINVIVLELKGSETTF MCEYADETATIVEEFLNRWITFCQSIISTLT
34	030	ΔS5, SQVLKA	desS5 E15S H16Q L19V D20L Q22K M23A	APTSSSTKKTQLQLSQLLVLLKAILNGINNYKNPK LTRMLTFKFYMPKKATELKHLQCLEEELKPLEE VLNLAQSKNFHLRPRDLISNINVIVLELKGSETTF MCEYADETATIVEEFLNRWITFCQSIISTLT
35	031	ΔS6, SQVLKA	desS6 E15S H16Q L19V D20L Q22K M23A	APTSSSTKKTQLQLSQLLVLLKAILNGINNYKNPK LTRMLTFKFYMPKKATELKHLQCLEEELKPLEE VLNLAQSKNFHLRPRDLISNINVIVLELKGSETTF MCEYADETATIVEEFLNRWITFCQSIISTLT

Table 2. hIL2 Orthologs				
SEQ ID NO:	Ref#	Name	Modifications Relative to wt hIL2	Mature Protein Sequence
36	032	ΔT7, SQVLKA	desT7 E15S H16Q L19V D20L Q22K M23A	APTSSSKKTQLQLSQLLVLLKAILNGINNYKNPK LTRMLTFKFYMPKKATELKHLQCLEEELKPLEE VLNLAQSKNFHLRPRDLISNINVIVLELKGSETTF MCEYADETATIVEFLNRWITFCQSIISTLT
37	033	ΔK8, SQVLKA	desK8 E15S H16Q L19V D20L Q22K M23A	APTSSSTKTQLQLSQLLVLLKAILNGINNYKNPK LTRMLTFKFYMPKKATELKHLQCLEEELKPLEE VLNLAQSKNFHLRPRDLISNINVIVLELKGSETTF MCEYADETATIVEFLNRWITFCQSIISTLT
38	034	ΔK9, SQVLKA	desK9 E15S H16Q L19V D20L Q22K M23A	APTSSSTKTQLQLSQLLVLLKAILNGINNYKNPK LTRMLTFKFYMPKKATELKHLQCLEEELKPLEE VLNLAQSKNFHLRPRDLISNINVIVLELKGSETTF MCEYADETATIVEFLNRWITFCQSIISTLT
39	035	ΔQ13, SQVLKA	desQ13 E15S H16Q L19V D20L Q22K M23A	APTSSSTKKTQLLSQLLVLLKAILNGINNYKNPK LTRMLTFKFYMPKKATELKHLQCLEEELKPLEE VLNLAQSKNFHLRPRDLISNINVIVLELKGSETTF MCEYADETATIVEFLNRWITFCQSIISTLT
40	036	Q13W SQVLKA	Q13W E15S H16Q L19V D20L Q22K M23A	APTSSSTKKTQLWLSQLLVLLKAILNGINNYKNPK KLTRMLTFKFYMPKKATELKHLQCLEEELKPLEE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEFLNRWITFCQSIISTLT
41	037	ΔL14, SQVLKA	desL14 E15S H16Q L19V D20L Q22K M23A	APTSSSTKKTQLQLSQLLVLLKAILNGINNYKNPK LTRMLTFKFYMPKKATELKHLQCLEEELKPLEE VLNLAQSKNFHLRPRDLISNINVIVLELKGSETTF MCEYADETATIVEFLNRWITFCQSIISTLT
42	038	L14M, SQVLKA	L14M E15S H16Q L19V D20L Q22K M23A	APTSSSTKKTQLQMSQLLVLLKAILNGINNYKNPK KLTRMLTFKFYMPKKATELKHLQCLEEELKPLEE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSE TFMCEYADETATIVEFLNRWITFCQSIISTLT
43	039	L14W, SQVLKA	L14W E15S H16Q L19V D20L Q22K M23A	APTSSSTKKTQLQWSQLLVLLKAILNGINNYKNPK KLTRMLTFKFYMPKKATELKHLQCLEEELKPLEE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSE TFMCEYADETATIVEFLNRWITFCQSIISTLT
44	041	KQVLKA	E15K H16Q L19V D20L Q22K M23A	APTSSSTKKTQLQLKQLLVLLKAILNGINNYKNPK KLTRMLTFKFYMPKKATELKHLQCLEEELKPLEE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEFLNRWITFCQSIISTLT
45	043	TQVLKA	E15T H16Q L19V D20L Q22K M23A	APTSSSTKKTQLQLTQLLVLLKAILNGINNYKNPK KLTRMLTFKFYMPKKATELKHLQCLEEELKPLEE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEFLNRWITFCQSIISTLT
46	047	ΔH16, S_VLKA	desH16 E15S L19V D20L Q22K M23A	APTSSSTKKTQLQLSLLVLLKAILNGINNYKNPK LTRMLTFKFYMPKKATELKHLQCLEEELKPLEE VLNLAQSKNFHLRPRDLISNINVIVLELKGSETTF MCEYADETATIVEFLNRWITFCQSIISTLT

Table 2. hIL2 Orthologs				
SEQ ID NO:	Ref#	Name	Modifications Relative to wt hIL2	Mature Protein Sequence
47	048	SNVLKA	E15S H16N L19V D20L Q22K M23A	APTSSSTKKKTQLQLSNLLVLLKAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
48	050	L18G, SQVLKA	E15S H16Q L18G L19V D20L Q22K M23A	APTSSSTKKKTQLQLSQLGVLLKAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
49	051	L18M, SQVLKA	E15S H16Q L18M L19V D20L Q22K M23A	APTSSSTKKKTQLQLSQLMVLLKAILNGINNYKNP PKLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EEVLNLAQSKNFHLRPRDLISNINVIVLELKGSE TTFMCEYADETATIVEEFLNRWITFCQSIISTLT
50	052	L18F, SQVLKA	E15S H16Q L18F L19V D20L Q22K M23A	APTSSSTKKKTQLQLSQLFVLLKAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
51	053	L18E, SQVLKA	E15S H16Q L18E L19V D20L Q22K M23A	APTSSSTKKKTQLQLSQLVLLKAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
52	054	L18H, SQVLKA	E15S H16Q L18H L19V D20L Q22K M23A	APTSSSTKKKTQLQLSQLHVLLKAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
53	055	L18W, SQVLKA	E15S H16Q L18W L19V D20L Q22K M23A	APTSSSTKKKTQLQLSQLWVLLKAILNGINNYKNP PKLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EEVLNLAQSKNFHLRPRDLISNINVIVLELKGSE TTFMCEYADETATIVEEFLNRWITFCQSIISTLT
54	056	L18K, SQVLKA	E15S H16Q L18K L19V D20L Q22K M23A	APTSSSTKKKTQLQLSQLKVLLKAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
55	057	L18Q, SQVLKA	E15S H16Q L18Q L19V D20L Q22K M23A	APTSSSTKKKTQLQLSQLQVLLKAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
56	058	L18S, SQVLKA	E15S H16Q L18S L19V D20L Q22K M23A	APTSSSTKKKTQLQLSQLSVLLKAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
57	059	L18V, SQVLKA	E15S H16Q L18V L19V D20L Q22K M23A	APTSSSTKKKTQLQLSQLVVLLKAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT

Table 2. hIL2 Orthologs				
SEQ ID NO:	Ref#	Name	Modifications Relative to wt hIL2	Mature Protein Sequence
58	060	L18I, SQVLKA	E15S H16Q L18I L19V D20L Q22K M23A	APTSSSTKKKTQLQLSQLIVLLKAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
59	061	L18Y, SQVLKA	E15S H16Q L18Y L19V D20L Q22K M23A	APTSSSTKKKTQLQLSQLYVLLKAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
60	062	L18H, SQVLKA	E15S H16Q L18H L19V D20L Q22K M23A	APTSSSTKKKTQLQLSQLHVLLKAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
61	063	L18D, SQVLKA	E15S H16Q L18D L19V D20L Q22K M23A	APTSSSTKKKTQLQLSQLDVLLKAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
62	064	L18T, SQVLKA	E15S H16Q L18T L19V D20L Q22K M23A	APTSSSTKKKTQLQLSQLTVLLKAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
63	065	ΔL19, SQ_LKA	desL19 E15S H16Q D20L Q22K M23A	APTSSSTKKKTQLQLSQLLLLKAILNGINNYKNPK LTRMLTFKFYMPKKATELKHLQCLEEELKPLEE VLNLAQSKNFHLRPRDLISNINVIVLELKGSETTF MCEYADETATIVEEFLNRWITFCQSIISTLT
64	067	SQILKA	E15S H16Q L19I D20L Q22K M23A	APTSSSTKKKTQLQLSQLLILKAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
65	068	ΔD20, SQV_KA	desD20 E15S H16Q L19V Q22K M23A	APTSSSTKKKTQLQLSQLLVKAILNGINNYKNPK LTRMLTFKFYMPKKATELKHLQCLEEELKPLEE VLNLAQSKNFHLRPRDLISNINVIVLELKGSETTF MCEYADETATIVEEFLNRWITFCQSIISTLT
66	070	SQVSKA	E15S H16Q L19V D20s Q22K M23A	APTSSSTKKKTQLQLSQLLVSLKAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
67	073	SQVLFA	E15S H16Q L19V D20L Q22F M23A	APTSSSTKKKTQLQLSQLLVLLKAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
68	074	SQVLEA	E15S H16Q L19V D20L Q22E M23A	APTSSSTKKKTQLQLSQLLVLEAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT

Table 2. hIL2 Orthologs				
SEQ ID NO:	Ref#	Name	Modifications Relative to wt hIL2	Mature Protein Sequence
69	075	SQVLGA	E15S H16Q L19V D20L Q22G M23A	APTSSSTKKTQLQLSQLLVLLGAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
70	076	SQVLAA	E15S H16Q L19V D20L Q22AM23A	APTSSSTKKTQLQLSQLLVLLAAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
71	077	SQVLLA	E15S H16Q L19V D20L Q22L M23A	APTSSSTKKTQLQLSQLLVLLAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
72	078	SQVLM A	E15S H16Q L19V D20L Q22M M23A	APTSSSTKKTQLQLSQLLVLLMAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
73	079	SQVLFA	E15S H16Q L19V D20L Q22F M23A	APTSSSTKKTQLQLSQLLVLLFAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
74	080	SQVLWA	E15S H16Q L19V D20L Q22W M23A	APTSSSTKKTQLQLSQLLVLLWAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
75	081	SQVLSA	E15S H16Q L19V D20L Q22S M23A	APTSSSTKKTQLQLSQLLVLLSAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
76	082	SQVLVA	E15S H16Q L19V D20L Q22V M23A	APTSSSTKKTQLQLSQLLVLLVAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
77	083	SQVLIA	E15S H16Q L19V D20L Q22I M23A	APTSSSTKKTQLQLSQLLVLLIAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
78	084	SQVLYA, E116G	E15S H16Q L19V D20L Q22Y M23A E116G	APTSSSTKKTQLQLSQLLVLLYAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
79	085	SQVLHA	E15S H16Q L19V D20L Q22H M23A	APTSSSTKKTQLQLSQLLVLLHAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT

Table 2. hIL2 Orthologs				
SEQ ID NO:	Ref#	Name	Modifications Relative to wt hIL2	Mature Protein Sequence
80	086	SQVLRA	E15S H16Q L19V D20L Q22R M23A	APTSSSTKKKTQLQLSQLLVLLRAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
81	087	SQVLNA	E15S H16Q L19V D20L Q22N M23A	APTSSSTKKKTQLQLSQLLVLLNAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
82	088	SQVLDA	E15S H16Q L19V D20L Q22D M23A	APTSSSTKKKTQLQLSQLLVLLDAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
83	089	SQVLTA	E15S H16Q L19V D20L Q22T M23A	APTSSSTKKKTQLQLSQLLVLLTAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
84	090	Δ M23, SQVLK-	desM23 E15S H16Q L19V D20L Q22K	APTSSSTKKKTQLQLSQLLVLLKILNGINNYKNPK LTRMLTFKFYMPKKATELKHLQCLEEELKPLEE VLNLAQSKNFHLRPRDLISNINVIVLELKGSETTF MCEYADETATIVEEFLNRWITFCQSIISTLT
85	091	SQVLKW	E15S H16Q L19V D20L Q22K M23w	APTSSSTKKKTQLQLSQLLVLLKWILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
86	092	SQVLKH	E15S H16Q L19V D20L Q22K M23H	APTSSSTKKKTQLQLSQLLVLLKHILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
87	093	SQVLKY	E15S H16Q L19V D20L Q22K M23Y	APTSSSTKKKTQLQLSQLLVLLKYILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
88	094	SQVLKF	E15S H16Q L19V D20L Q22K M23F	APTSSSTKKKTQLQLSQLLVLLKFILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
89	095	SQVLKQ	E15S H16Q L19V D20L Q22K M23Q	APTSSSTKKKTQLQLSQLLVLLKQILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
90	096	SQVLKS	E15S H16Q L19V D20L Q22K M23S	APTSSSTKKKTQLQLSQLLVLLKSIILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT

Table 2. hIL2 Orthologs				
SEQ ID NO:	Ref#	Name	Modifications Relative to wt hIL2	Mature Protein Sequence
91	097	SQVLKV	E15S H16Q L19V D20L Q22K M23V	APTSSSTKKKTQLQLSQLLVLLKVILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
92	098	SQVLKL	E15S H16Q L19V D20L Q22K M23L	APTSSSTKKKTQLQLSQLLVLLKLILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
93	099	SQVLKT	E15S H16Q L19V D20L Q22K M23T	APTSSSTKKKTQLQLSQLLVLLKTILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
94	100	Δ G27, SQVLKA	desG27 E15S H16Q L19V D20L Q22K M23A	APTSSSTKKKTQLQLSQLLVLLKAILNINNYKNPK LTRMLTFKFYMPKKATELKHLQCLEEELKPLEE VLNLAQSKNFHLRPRDLISNINVIVLELKGSETTF MCEYADETATIVEEFLNRWITFCQSIISTLT
95	101	G27K, SQVLKA	E15S H16Q L19V D20L Q22K M23A G27K	APTSSSTKKKTQLQLSQLLVLLKAILNKINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
96	102	G27S, SQVLKA	E15S H16Q L19V D20L Q22K M23A G27S	APTSSSTKKKTQLQLSQLLVLLKAILNSINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
97	103	R38W, SQVLKA	E15S H16Q L19V D20L Q22K M23A R38W	APTSSSTKKKTQLQLSQLLVLLKAILNGINNYKNP KLTWMLTFKFYMPKKATELKHLQCLEEELKPL EEVLNLAQSKNFHLRPRDLISNINVIVLELKGSE TFMCEYADETATIVEEFLNRWITFCQSIISTLT
98	104	R38G, SQVLKA	E15S H16Q L19V D20L Q22K M23A R38G	APTSSSTKKKTQLQLSQLLVLLKAILNGINNYKNP KLTGMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
99	105	M39L, SQVLKA	E15S H16Q L19V D20L Q22K M23A M39L	APTSSSTKKKTQLQLSQLLVLLKAILNGINNYKNP KLTRLLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
101	106	M39V, SQVLKA	E15S H16Q L19V D20L Q22K M23A M39V	APTSSSTKKKTQLQLSQLLVLLKAILNGINNYKNP KLTRVLTFFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
101	107	F42K, SQVLKA	E15S H16Q L19V D20L Q22K M23A F42K	APTSSSTKKKTQLQLSQLLVLLKAILNGINNYKNP KLTRMLTKKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT

Table 2. hIL2 Orthologs				
SEQ ID NO:	Ref#	Name	Modifications Relative to wt hIL2	Mature Protein Sequence
102	108	Δ T51, SQVLKA	desT51 E15S H16Q L19V D20L Q22K M23A	APTSSTKKKTQLQLSQLLVLLKAILNGINNYKNP KLTRMLTFKFYMPKKA ELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRD LISNINVIVLELKGSETTFMCEYADETATIVEEFLN RWITFCQSIISTLT
103	109	T51I, SQVLKA	E15S H16Q L19V D20L Q22K M23A T51I	APTSSTKKKTQLQLSQLLVLLKAILNGINNYKNP KLTRMLTFKFYMPKKAIELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
104	110	H55Y, SQVLKA	E15S H16Q L19V D20L Q22K M23A H55Y	APTSSTKKKTQLQLSQLLVLLKAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
105	111	Q74N, SQVLKA	E15S H16Q L19V D20L Q22K M23A Q74N	APTSSTKKKTQLQLSQLLVLLKAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
106	112	Q74H, SQVLKA	E15S H16Q L19V D20L Q22K M23A Q74H	APTSSTKKKTQLQLSQLLVLLKAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
107	113	Q74S, SQVLKA	E15S H16Q L19V D20L Q22K M23A Q74S	APTSSTKKKTQLQLSQLLVLLKAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
108	114	L80F, SQVLKA	E15S H16Q L19V D20L Q22K M23A L80F	APTSSTKKKTQLQLSQLLVLLKAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
109	115	L80V, SQVLKA	E15S H16Q L19V D20L Q22K M23A L80V	APTSSTKKKTQLQLSQLLVLLKAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
110	116	Δ R81, SQVLKA	desR81 E15S H16Q L19V D20L Q22K M23A	APTSSTKKKTQLQLSQLLVLLKAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
111	117	R81I, SQVLKA	E15S H16Q L19V D20L Q22K M23A R81I	APTSSTKKKTQLQLSQLLVLLKAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
112	118	R81D, SQVLKA	E15S H16Q L19V D20L	APTSSTKKKTQLQLSQLLVLLKAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE

Table 2. hIL2 Orthologs				
SEQ ID NO:	Ref#	Name	Modifications Relative to wt hIL2	Mature Protein Sequence
			Q22K M23A R81D	EVNLNAQSKNFHLDPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
113	119	R81Y, SQVLKA	E15S H16Q L19V D20L Q22K M23A R81Y	APTSSSTKKTQLQLSOLLVLLKAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVNLNAQSKNFHLYPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
114	121	L85V, SQVLKA	E15S H16Q L19V D20L Q22K M23A L85V	APTSSSTKKTQLQLSOLLVLLKAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVNLNAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
115	122	I86V, SQVLKA	E15S H16Q L19V D20L Q22K M23A I86V	APTSSSTKKTQLQLSOLLVLLKAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVNLNAQSKNFHLRPRDLVSININVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
116	123	Δ88N, SQVLKA	E15S H16Q L19V D20L Q22K M23A desN88	APTSSSTKKTQLQLSOLLVLLKAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVNLNAQSKNFHLRPRDLIS INVIVLELKGSETTFMCEYADETATIVEEFLNRWI TFCQSIISTLT
117	124	N88E, SQVLKA	E15S H16Q L19V D20L Q22K M23A N88E	APTSSSTKKTQLQLSOLLVLLKAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVNLNAQSKNFHLRPRDLISEINIVIVLELKGSET FMCEYADETATIVEEFLNRWITFCQSIISTLT
118	125	N88Q, SQVLKA	E15S H16Q L19V D20L Q22K M23A N88Q	APTSSSTKKTQLQLSOLLVLLKAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVNLNAQSKNFHLRPRDLISQINIVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
119	127	V91R, SQVLKA	E15S H16Q L19V D20L Q22K M23A V91R	APTSSSTKKTQLQLSOLLVLLKAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVNLNAQSKNFHLRPRDLISNINRIVIVLELKGSET FMCEYADETATIVEEFLNRWITFCQSIISTLT
120	128	V91K, SQVLKA	E15S H16Q L19V D20L Q22K M23A V91K	APTSSSTKKTQLQLSOLLVLLKAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVNLNAQSKNFHLRPRDLISNINKIVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
121	129	I92F, SQVLKA	E15S H16Q L19V D20L Q22K M23A I92F	APTSSSTKKTQLQLSOLLVLLKAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVNLNAQSKNFHLRPRDLISNINVFVLELKGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT
122	130	K97Q, SQVLKA	E15S H16Q L19V D20L Q22K M23A K97Q	APTSSSTKKTQLQLSOLLVLLKAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVNLNAQSKNFHLRPRDLISNINVIVLELQSGSET TFMCEYADETATIVEEFLNRWITFCQSIISTLT

Table 2. hIL2 Orthologs				
SEQ ID NO:	Ref#	Name	Modifications Relative to wt hIL2	Mature Protein Sequence
123	131	M104A, SQVLKA	E15S H16Q L19V D20L Q22K M23A M104A	APTSSSTKKKTQLQLSQLLVLLKAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFACEYADETATIVEFLNRWITFCQSIISTLT
124	132	D109C, SQVLKA	E15S H16Q L19V D20L Q22K M23A D109C	APTSSSTKKKTQLQLSQLLVLLKAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYACETATIVEFLNRWITFCQSIISTLT
125	133	T113N, SQVLKA	E15S H16Q L19V D20L Q22K M23A T113N	APTSSSTKKKTQLQLSQLLVLLKAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETANIVEFLNRWITFCQSIISTLT
126	134	C125A, SQVLKA	E15S H16Q L19V D20L Q22K M23A C125A	APTSSSTKKKTQLQLSQLLVLLKAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEFLNRWITFAQSIISTLT
127	135	C125S, SQVLKA	E15S H16Q L19V D20L Q22K M23A C125S	APTSSSTKKKTQLQLSQLLVLLKAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEFLNRWITFCSQSIISTLT
128	136	Q126H, SQVLKA	E15S H16Q L19V D20L Q22K M23A Q126H	APTSSSTKKKTQLQLSQLLVLLKAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEFLNRWITFCHSIISTLT
129	137	Q126M, SQVLKA	E15S H16Q L19V D20L Q22K M23A Q126M	APTSSSTKKKTQLQLSQLLVLLKAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEFLNRWITFCMSIISTLT
130	138	Q126K, SQVLKA	E15S H16Q L19V D20L Q22K M23A Q126K	APTSSSTKKKTQLQLSQLLVLLKAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEFLNRWITFCKSIISTLT
131	139	Q126C, SQVLKA	E15S H16Q L19V D20L Q22K M23A Q126C	APTSSSTKKKTQLQLSQLLVLLKAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEFLNRWITFCCSIISTLT
132	140	Q126D, SQVLKA	E15S H16Q L19V D20L Q22K M23A Q126D	APTSSSTKKKTQLQLSQLLVLLKAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEFLNRWITFCDSIISTLT
133	141	Q126E, SQVLKA	E15S H16Q L19V D20L Q22K M23A Q126E	APTSSSTKKKTQLQLSQLLVLLKAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEFLNRWITFCESIISTLT

Table 2. hIL2 Orthologs				
SEQ ID NO:	Ref#	Name	Modifications Relative to wt hIL2	Mature Protein Sequence
134	142	Q126G, SQVLKA	E15S H16Q L19V D20L Q22K M23A Q126G	APTSSSTKKKTQLQLSQLLVLLKAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCGSIISTLT
135	143	Q126I, SQVLKA	E15S H16Q L19V D20L Q22K M23A Q126I	APTSSSTKKKTQLQLSQLLVLLKAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCISIISTLT
136	144	Q126R, SQVLKA	E15S H16Q L19V D20L Q22K M23A Q126R	APTSSSTKKKTQLQLSQLLVLLKAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCRSIISTLT
137	145	Q126S, SQVLKA	E15S H16Q L19V D20L Q22K M23A Q126S	APTSSSTKKKTQLQLSQLLVLLKAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCSSIISTLT
138	146	Q126T, SQVLKA	E15S H16Q L19V D20L Q22K M23A Q126T	APTSSSTKKKTQLQLSQLLVLLKAILNGINNYKNP KLTRMLTFKFYMPKKATELKHLQCLEEEELKPLE EVLNLAQSKNFHLRPRDLISNINVIVLELKGSET TFMCEYADETATIVEEFLNRWITFCTSIISTLT

The activity of the foregoing hIL2 orthologs was evaluated for their ability to activate NKL and hoRb NKL cells in substantial accordance with the teaching herein below dilutions. The results of these experiments are provided in Figures 1 and 2 of the attached drawings and in Table 3 below:

Table 3. Proliferative Activity of hIL2 Orthologs on hoCD122 NKL cells v. NKL Cells					
SEQ ID NO	Mutant #	NKL Proliferation Signal cts/min	NKL Ortho Proliferation Signal	Relative Proliferation Fold Change	Dilution
20	16	11290	33439	2.96	1:2
		15321	27747	1.81	1:10
		14397	13824	0.96	1:50
21	17	14514	63991	4.41	1:2
		16305	61911	3.80	1:10
		15733	27785	1.77	1:50
22	18	15205	60773	4.00	1:2
		17304	64585	3.73	1:10
		16255	32287	1.99	1:50
23	19	13346	60626	4.54	1:2
		17004	70804	4.16	1:10
		17168	35301	2.06	1:50
24	20	17687	69148	3.91	1:2
		18869	79543	4.22	1:10
		17596	46983	2.67	1:50
25	21	14942	58905	3.94	1:2
		17779	66909	3.76	1:10
		16747	32200	1.92	1:50
26	22	15597	65319	4.19	1:2
		18280	73740	4.03	1:10
		18054	34763	1.93	1:50
27	23	17301	68608	3.97	1:2
		19419	75040	3.86	1:10
		18178	37407	2.06	1:50
28	24	16135	62428	3.87	1:2
		19760	65663	3.32	1:10
		17742	31375	1.77	1:50
29	25	12176	49755	4.09	1:2
		13388	53895	4.03	1:10
		11903	27904	2.34	1:50
30	26	14052	58383	4.15	1:2
		14043	40680	2.90	1:10
		13313	19342	1.45	1:50

Table 3. Proliferative Activity of hIL2 Orthologs on hoCD122 NKL cells v. NKL Cells (cont ¹)					
SEQ ID NO	Mutant #	NKL Proliferation Signal cts/min	NKL Ortho Proliferation Signal	Relative Proliferation Fold Change	Dilution
31	27	12359	42918	3.47	1:2
		14355	35526	2.47	1:10
		13265	18371	1.38	1:50
32	28	9175	55355	6.03	1:2
		11584	56868	4.91	1:10
		11286	32736	2.90	1:50
33	29	12703	51159	4.03	1:2
		13769	42300	3.07	1:10
		12021	21368	1.78	1:50
34	30	13166	58054	4.41	1:2
		15087	57368	3.80	1:10
		13754	30580	2.22	1:50
35	31	11759	54377	4.62	1:2
		13828	50211	3.63	1:10
		13162	25401	1.93	1:50
36	32	15203	55447	3.65	1:2
		15635	51908	3.32	1:10
		14368	25282	1.76	1:50
37	33	14709	52082	3.54	1:2
		15971	43484	2.72	1:10
		15099	21672	1.44	1:50
38	34	13448	55505	4.13	1:2
		13960	61288	4.39	1:10
		12991	31595	2.43	1:50
39	35	12976	26668	2.06	1:2
		14898	20740	1.39	1:10
		14846	11770	0.79	1:50
40	36	11087	13735	1.24	1:2
		15364	12919	0.84	1:10
		13859	8726	0.63	1:50
41	37	13329	31787	2.38	1:2
		14639	23481	1.60	1:10
		14126	12167	0.86	1:50

Table 3. Proliferative Activity of hIL2 Orthologs on hoCD122 NKL cells v. NKL Cells (cont')					
SEQ ID NO	Mutant #	NKL Proliferation Signal cts/min	NKL Ortho Proliferation Signal	Relative Proliferation Fold Change	Dilution
42	38	15198	57185	3.76	1:2
		15817	54533	3.45	1:10
		13528	26727	1.98	1:50
43	39	12437	50303	4.04	1:2
		15903	45080	2.83	1:10
		15069	20889	1.39	1:50
44	41	13914	71249	5.12	1:2
		15513	67638	4.36	1:10
		14815	38935	2.63	1:50
45	43	14568	50423	3.46	1:2
		17486	57880	3.31	1:10
		15855	27801	1.75	1:50
46	47	15842	20855	1.32	1:2
		16921	14245	0.84	1:10
		15861	9764	0.62	1:50
47	48	15894	46760	2.94	1:2
		18183	39738	2.19	1:10
		16805	18332	1.09	1:50
48	50	14669	15397	1.05	1:2
		16085	13421	0.83	1:10
		15206	8641	0.57	1:50
49	51	11549	42731	3.70	1:2
		16148	43886	2.72	1:10
		14258	22100	1.55	1:50
50	52	12620	48563	3.85	1:2
		15644	39424	2.52	1:10
		14855	19352	1.30	1:50
51	53	14506	8261	0.57	1:2
		16289	9063	0.56	1:10
		15779	8115	0.51	1:50
52	54	12284	32164	2.62	1:2
		16415	27614	1.68	1:10
		15166	13909	0.92	1:50

Table 3. Proliferative Activity of hIL2 Orthologs on hoCD122 NKL cells v. NKL Cells (cont ¹)					
SEQ ID NO	Mutant #	NKL Proliferation Signal cts/min	NKL Ortho Proliferation Signal	Relative Proliferation Fold Change	Dilution
53	55	11964	18701	1.56	1:2
		15427	16963	1.10	1:10
		15163	10726	0.71	1:50
54	56	14228	22200	1.56	1:2
		16729	20183	1.21	1:10
		15170	11463	0.76	1:50
55	57	12082	47459	3.93	1:2
		16495	41174	2.50	1:10
		15136	21381	1.41	1:50
56	58	11308	27160	2.40	1:2
		13796	21520	1.56	1:10
		13477	11506	0.85	1:50
57	59	13097	17769	1.36	1:2
		14608	13969	0.96	1:10
		13961	8670	0.62	1:50
58	60	11620	23840	2.05	1:2
		14893	20537	1.38	1:10
		13821	11018	0.80	1:50
59	61	11148	37259	3.34	1:2
		12930	26982	2.09	1:10
		12473	14806	1.19	1:50
60	62	11959	32614	2.73	1:2
		13404	23272	1.74	1:10
		13261	12409	0.94	1:50
61	63	10603	6383	0.60	1:2
		13815	8157	0.59	1:10
		13317	7465	0.56	1:50
62	64	10516	24970	2.37	1:2
		13432	20059	1.49	1:10
		12998	11344	0.87	1:50
63	65	13174	6436	0.49	1:2
		14721	9117	0.62	1:10
		14404	8467	0.59	1:50

Table 3. Proliferative Activity of hIL2 Orthologs on hoCD122 NKL cells v. NKL Cells (cont ¹)					
SEQ ID NO	Mutant #	NKL Proliferation Signal cts/min	NKL Ortho Proliferation Signal	Relative Proliferation Fold Change	Dilution
64	67	13796	55309	4.01	1:2
		15267	52537	3.44	1:10
		14636	24499	1.67	1:50
65	68	12926	6786	0.52	1:2
		16040	9389	0.59	1:10
		15423	8687	0.56	1:50
66	70	59557	59470	1.00	1:2
		42545	73657	1.73	1:10
		23632	45032	1.91	1:50
67	73	13546	16646	1.23	1:2
		14734	12280	0.83	1:10
		14101	8499	0.60	1:50
68	74	11816	21060	1.78	1:2
		15275	18219	1.19	1:10
		14206	9479	0.67	1:50
69	75	12255	13518	1.10	1:2
		14129	11701	0.83	1:10
		14485	8727	0.60	1:50
70	76	13851	14570	1.05	1:2
		16489	11844	0.72	1:10
		15281	9399	0.62	1:50
71	77	10941	16408	1.50	1:2
		15123	16082	1.06	1:10
		14493	8571	0.59	1:50
72	78	14622	20542	1.40	1:2
		17357	18647	1.07	1:10
		16948	11010	0.65	1:50
73	79	16218	19987	1.23	1:2
		18720	14095	0.75	1:10
		17534	10015	0.57	1:50
74	80	12257	14033	1.14	1:2
		16880	11123	0.66	1:10
		16584	8874	0.54	1:50

Table 3. Proliferative Activity of hIL2 Orthologs on hoCD122 NKL cells v. NKL Cells (cont ¹)					
SEQ ID NO	Mutant #	NKL Proliferation Signal cts/min	NKL Ortho Proliferation Signal	Relative Proliferation Fold Change	Dilution
75	81	7643	16014	2.10	1:2
		10421	12984	1.25	1:10
		10247	7839	0.77	1:50
76	82	12955	10804	0.83	1:2
		13827	8605	0.62	1:10
		12919	6586	0.51	1:50
77	83	11761	13004	1.11	1:2
		13772	10709	0.78	1:10
		12870	6782	0.53	1:50
78	84	10892	13205	1.21	1:2
		14094	11634	0.83	1:10
		13332	8394	0.63	1:50
79	85	15316	53023	3.46	1:2
		16010	26042	1.63	1:10
		15303	13269	0.87	1:50
80	86	14043	60823	4.33	1:2
		16878	68450	4.06	1:10
		15056	34281	2.28	1:50
81	87	11124	50560	4.55	1:2
		12142	36485	3.00	1:10
		12372	17317	1.40	1:50
82	88	13762	35108	2.55	1:2
		13790	22737	1.65	1:10
		12245	11592	0.95	1:50
83	89	11630	26364	2.27	1:2
		14002	19385	1.38	1:10
		12843	7649	0.60	1:50
84	90	9645	27637	2.87	1:2
		13246	18989	1.43	1:10
		12208	8951	0.73	1:50
85	91	14650	51969	3.55	1:2
		14296	35992	2.52	1:10
		13462	17860	1.33	1:50

Table 3. Proliferative Activity of hIL2 Orthologs on hoCD122 NKL cells v. NKL Cells (cont ¹)					
SEQ ID NO	Mutant #	NKL Proliferation Signal cts/min	NKL Ortho Proliferation Signal	Relative Proliferation Fold Change	Dilution
86	92	11117	44420	4.00	1:2
		14496	39187	2.70	1:10
		12461	18514	1.49	1:50
87	93	14904	52383	3.51	1:2
		16631	43832	2.64	1:10
		15863	21862	1.38	1:50
88	94	18416	65107	3.54	1:2
		17875	44692	2.50	1:10
		16292	23226	1.43	1:50
89	95	16752	74288	4.43	1:2
		17805	82001	4.61	1:10
		16276	49574	3.05	1:50
90	96	11739	56202	4.79	1:2
		14877	59385	3.99	1:10
		13750	29007	2.11	1:50
91	97	13742	63408	4.61	1:2
		15956	66142	4.15	1:10
		14936	29859	2.00	1:50
92	98	12716	59665	4.69	1:2
		15304	67053	4.38	1:10
		14498	32715	2.26	1:50
93	99	13128	61425	4.68	1:2
		14049	63448	4.52	1:10
		13347	33416	2.50	1:50
94	100	14140	46551	3.29	1:2
		15302	31408	2.05	1:10
		14020	14616	1.04	1:50
95	101	12378	36298	2.93	1:2
		15802	30577	1.94	1:10
		14743	15178	1.03	1:50
96	102	11613	48857	4.21	1:2
		13925	40967	2.94	1:10
		13560	20710	1.53	1:50

Table 3. Proliferative Activity of hIL2 Orthologs on hoCD122 NKL cells v. NKL Cells (cont')					
SEQ ID NO	Mutant #	NKL Proliferation Signal cts/min	NKL Ortho Proliferation Signal	Relative Proliferation Fold Change	Dilution
97	103	13389	36697	2.74	1:2
		15773	23763	1.51	1:10
		14922	11507	0.77	1:50
98	104	11262	20088	1.78	1:2
		15198	18115	1.19	1:10
		14644	9740	0.67	1:50
99	105	12872	57571	4.47	1:2
		15491	44060	2.84	1:10
		14627	21801	1.49	1:50
100	106	15750	42790	2.72	1:2
		16892	31264	1.85	1:10
		16607	15331	0.92	1:50
101	107	14238	11882	0.83	1:2
		17401	10462	0.60	1:10
		16713	8733	0.52	1:50
102	108	12160	12147	1.00	1:2
		14210	10083	0.71	1:10
		13405	7781	0.58	1:50
103	109	14233	50765	3.57	1:2
		14367	37908	2.64	1:10
		13262	18506	1.40	1:50
104	110	12633	47057	3.72	1:2
		15843	37066	2.34	1:10
		14553	17789	1.22	1:50
105	111	13034	55149	4.23	1:2
		14919	47442	3.18	1:10
		13569	22641	1.67	1:50
106	112	14984	58345	3.89	1:2
		15836	50345	3.18	1:10
		14399	22979	1.60	1:50
107	113	13433	54620	4.07	1:2
		15733	65670	4.17	1:10
		14336	35047	2.44	1:50

Table 3. Proliferative Activity of hIL2 Orthologs on hoCD122 NKL cells v. NKL Cells (cont ¹)					
SEQ ID NO	Mutant #	NKL Proliferation Signal cts/min	NKL Ortho Proliferation Signal	Relative Proliferation Fold Change	Dilution
108	114	14432	61200	4.24	1:2
		16348	67761	4.14	1:10
		14651	33242	2.27	1:50
109	115	14672	62456	4.26	1:2
		16489	51561	3.13	1:10
		15442	24341	1.58	1:50
110	116	12883	62380	4.84	1:2
		16845	68836	4.09	1:10
		15192	31880	2.10	1:50
111	117	13744	52806	3.84	1:2
		15045	57953	3.85	1:10
		14183	27287	1.92	1:50
112	118	19746	71838	3.64	1:2
		16964	73760	4.35	1:10
		14482	44423	3.07	1:50
113	119	16432	59763	3.64	1:2
		16784	77926	4.64	1:10
		14404	49457	3.43	1:50
114	121	14304	56313	3.94	1:2
		14834	49554	3.34	1:10
		14135	24202	1.71	1:50
115	122	10440	39923	3.82	1:2
		14232	38044	2.67	1:10
		12979	18379	1.42	1:50
116	123	10882	4610	0.42	1:2
		12687	7474	0.59	1:10
		11598	6688	0.58	1:50
117	124	13165	6411	0.49	1:2
		13474	7086	0.53	1:10
		12791	6479	0.51	1:50
118	125	11513	4981	0.43	1:2
		13037	6955	0.53	1:10
		12398	6244	0.50	1:50

Table 3. Proliferative Activity of hIL2 Orthologs on hoCD122 NKL cells v. NKL Cells (cont ¹)					
SEQ ID NO	Mutant #	NKL Proliferation Signal cts/min	NKL Ortho Proliferation Signal	Relative Proliferation Fold Change	Dilution
119	127	9932	8526	0.86	1:2
		10456	5585	0.53	1:10
		10090	4416	0.44	1:50
120	128	6963	5176	0.74	1:2
		8289	5206	0.63	1:10
		9382	4940	0.53	1:50
121	129	13891	32233	2.32	1:2
		14731	27033	1.84	1:10
		13864	12751	0.92	1:50
122	130	16092	52990	3.29	1:2
		15233	55372	3.64	1:10
		13269	25150	1.90	1:50
123	131	12261	47557	3.88	1:2
		15753	54802	3.48	1:10
		14118	29487	2.09	1:50
124	132	11394	47798	4.20	1:2
		13698	44151	3.22	1:10
		13348	20374	1.52	1:50
125	133	12764	47432	3.71	1:2
		14164	48815	3.45	1:10
		13064	21356	1.63	1:50
126	134	10628	41984	3.95	1:2
		13997	50413	3.60	1:10
		11700	22800	1.95	1:50
127	135	11227	48166	4.29	1:2
		13134	59512	4.53	1:10
		12291	27806	2.26	1:50
128	136	11988	43616	3.64	1:2
		15116	28971	1.92	1:10
		14513	15807	1.09	1:50
129	137	9890	19801	2.00	1:2
		14489	18666	1.29	1:10
		13147	9763	0.74	1:50

Table 3. Proliferative Activity of hIL2 Orthologs on hoCD122 NKL cells v. NKL Cells (cont')					
SEQ ID NO	Mutant #	NKL Proliferation Signal cts/min	NKL Ortho Proliferation Signal	Relative Proliferation Fold Change	Dilution
130	138	11958	6514	0.54	1:2
		14104	7927	0.56	1:10
		13240	6978	0.53	1:50
131	139	14812	6557	0.44	1:2
		15356	8208	0.53	1:10
		14649	7195	0.49	1:50
132	140	12048	4645	0.39	1:2
		15294	7556	0.49	1:10
		13970	6378	0.46	1:50
133	141	10476	10620	1.01	1:2
		12737	8599	0.68	1:10
		12278	6804	0.55	1:50
134	142	12806	5739	0.45	1:2
		14651	7220	0.49	1:10
		13319	6047	0.45	1:50
135	143	10571	40003	3.78	1:2
		14275	35628	2.50	1:10
		13362	16693	1.25	1:50
136	144	11784	13914	1.18	1:2
		12870	9670	0.75	1:10
		11644	6647	0.57	1:50
137	145	13359	9459	0.71	1:2
		13868	7878	0.57	1:10
		12614	6308	0.50	1:50
138	146	13448	5983	0.44	1:2
		14275	6752	0.47	1:10
		12782	5181	0.41	1:50

[0111] The IL2 orthologs were evaluated for activity in CD4 positive human T cell clone 3F8 cells as more fully described in Example 8. The resulting data from these experiments is provided in Table 4 below.

Table 4. Proliferative Activity of hIL2 Orthologs on hoCD122 3F8 cells v 3F8 Cells					
Sequence ID	Mutant #	3F8 Proliferation Signal cts/min	3F8 Ortho Proliferation Signal cts/min	Relative Proliferation Fold Change	Dilution
20	16	190	32446	170.77	1:02
		966	21870	22.64	1:10
		698	8148	11.67	1:50
21	17	1172	40086	34.2	1:02
		1058	31048	29.35	1:10
		1176	12364	10.51	1:50
22	18	1274	38872	30.51	1:02
		2308	34780	15.07	1:10
		2126	15990	7.52	1:50
23	19	948	33054	34.87	1:02
		2640	33654	12.75	1:10
		1224	14650	11.97	1:50
24	20	2416	41406	17.14	1:02
		3580	39262	10.97	1:10
		2240	19454	8.68	1:50
25	21	1556	31754	20.41	1:02
		1254	24386	19.45	1:10
		1324	9456	7.14	1:50
26	22	574	39196	68.29	1:02
		1144	32216	28.16	1:10
		824	14180	17.21	1:50
27	23	1738	36116	20.78	1:02
		796	36222	45.51	1:10
		1182	18896	15.99	1:50
28	24	16510	34194	2.07	1:02
		9126	27244	2.99	1:10
		2390	9790	4.1	1:50
29	25	952	33412	35.1	1:02
		1886	35028	18.57	1:10
		704	13374	19	1:50

Table 4. Proliferative Activity of hIL2 Orthologs on hoCD122 3F8 cells v 3F8 Cells					
Sequence ID	Mutant #	3F8 Proliferation Signal cts/min	3F8 Ortho Proliferation Signal cts/min	Relative Proliferation Fold Change	Dilution
30	26	3428	28888	8.43	1:02
		1262	16752	13.27	1:10
		1128	5984	5.3	1:50
31	27	568	28890	50.86	1:02
		2052	15082	7.35	1:10
		774	7148	9.24	1:50
32	28	1078	36522	33.88	1:02
		928	39744	42.83	1:10
		494	19040	38.54	1:50
33	29	20690	41050	1.98	1:02
		11516	29554	2.57	1:10
		1176	13948	11.86	1:50
34	30	14128	40508	2.87	1:02
		2634	39450	14.98	1:10
		582	22902	39.35	1:50
35	31	494	40440	81.86	1:02
		1404	35202	25.07	1:10
		696	15794	22.69	1:50
36	32	832	40844	49.09	1:02
		1346	29186	21.68	1:10
		698	10672	15.29	1:50
37	33	858	42550	49.59	1:02
		1182	30142	25.5	1:10
		1130	12316	10.9	1:50
38	34	1810	39790	21.98	1:02
		1076	45440	42.23	1:10
		1002	24362	24.31	1:50
39	35	370	37642	101.74	1:02
		1104	26132	23.67	1:10
		1628	12416	7.63	1:50

Table 4. Proliferative Activity of hIL2 Orthologs on hoCD122 3F8 cells v 3F8 Cells					
Sequence ID	Mutant #	3F8 Proliferation Signal cts/min	3F8 Ortho Proliferation Signal cts/min	Relative Proliferation Fold Change	Dilution
40	36	182	26986	148.27	1:02
		760	16898	22.23	1:10
		590	6424	10.89	1:50
41	37	242	30270	125.08	1:02
		924	24488	26.5	1:10
		614	8132	13.24	1:50
42	38	974	39816	40.88	1:02
		1218	41530	34.1	1:10
		1528	18514	12.12	1:50
43	39	534	35346	66.19	1:02
		1134	36034	31.78	1:10
		828	13628	16.46	1:50
44	41	7242	35180	4.86	1:02
		9488	36794	3.88	1:10
		1046	24030	22.97	1:50
45	43	11192	29098	2.6	1:02
		2238	35886	16.03	1:10
		1952	16972	8.69	1:50
46	47	678	2364	3.49	1:02
		2624	4418	1.68	1:10
		1256	1670	1.33	1:50
47	48	3412	31378	9.2	1:02
		2788	32746	11.75	1:10
		3972	19016	4.79	1:50
48	50	712	37660	52.89	1:02
		1524	25864	16.97	1:10
		2570	9752	3.79	1:50
49	51	764	41998	54.97	1:02
		2208	48472	21.95	1:10
		2246	37170	16.55	1:50

Table 4. Proliferative Activity of hIL2 Orthologs on hoCD122 3F8 cells v 3F8 Cells					
Sequence ID	Mutant #	3F8 Proliferation Signal cts/min	3F8 Ortho Proliferation Signal cts/min	Relative Proliferation Fold Change	Dilution
50	52	908	49780	54.82	1:02
		2430	40804	16.79	1:10
		2542	29982	11.79	1:50
51	53	610	6094	9.99	1:02
		2540	2354	0.93	1:10
		2576	1982	0.77	1:50
52	54	1366	32926	24.1	1:02
		4212	29950	7.11	1:10
		3328	29722	8.93	1:50
53	55	266	36764	138.21	1:02
		762	32848	43.11	1:10
		756	9568	12.66	1:50
54	56	280	45378	162.06	1:02
		862	42754	49.6	1:10
		1392	20368	14.63	1:50
55	57	368	40786	110.83	1:02
		708	57226	80.83	1:10
		948	44728	47.18	1:50
56	58	234	43268	184.91	1:02
		546	48596	89	1:10
		564	23614	41.87	1:50
57	59	262	36538	139.46	1:02
		1532	29456	19.23	1:10
		824	9918	12.04	1:50
58	60	454	36884	81.24	1:02
		1250	37306	29.84	1:10
		1758	19380	11.02	1:50
59	61	222	47570	214.28	1:02
		510	51420	100.82	1:10
		440	28182	64.05	1:50

Table 4. Proliferative Activity of hIL2 Orthologs on hoCD122 3F8 cells v 3F8 Cells					
Sequence ID	Mutant #	3F8 Proliferation Signal cts/min	3F8 Ortho Proliferation Signal cts/min	Relative Proliferation Fold Change	Dilution
60	62	238	49984	210.02	1:02
		586	51554	87.98	1:10
		686	28784	41.96	1:50
61	63	476	44038	92.52	1:02
		610	49584	81.29	1:10
		888	25250	28.43	1:50
62	64	29818	61054	2.05	1:02
		8262	51812	6.27	1:10
		1864	25654	13.76	1:50
63	65	666	736	1.11	1:02
		842	1068	1.27	1:10
		776	1538	1.98	1:50
64	67	842	38522	45.75	1:02
		1338	45738	34.18	1:10
		590	20288	34.39	1:50
65	68	670	1014	1.51	1:02
		936	1266	1.35	1:10
		822	1366	1.66	1:50
66	70	58180	47276	0.81	1:02
		46084	56580	1.23	1:10
		25656	33070	1.29	1:50
67	73	7510	65214	8.68	1:02
		7730	42872	5.55	1:10
		730	10104	13.84	1:50
68	74	1014	33384	32.92	1:02
		1120	31604	28.22	1:10
		958	12568	13.12	1:50
69	75	242	18038	74.54	1:02
		586	9166	15.64	1:10
		454	2296	5.06	1:50

Table 4. Proliferative Activity of hIL2 Orthologs on hoCD122 3F8 cells v 3F8 Cells					
Sequence ID	Mutant #	3F8 Proliferation Signal cts/min	3F8 Ortho Proliferation Signal cts/min	Relative Proliferation Fold Change	Dilution
70	76	230	15926	69.24	1:02
		724	7288	10.07	1:10
		684	2936	4.29	1:50
71	77	330	19746	59.84	1:02
		750	9734	12.98	1:10
		674	3152	4.68	1:50
72	78	328	20772	63.33	1:02
		764	11060	14.48	1:10
		642	3798	5.92	1:50
73	79	252	7800	30.95	1:02
		612	5398	8.82	1:10
		930	2176	2.34	1:50
74	80	538	7842	14.58	1:02
		1334	4472	3.35	1:10
		780	714	0.92	1:50
75	81	188	21956	116.79	1:02
		606	14492	23.91	1:10
		676	4058	6	1:50
76	82	422	8534	20.22	1:02
		582	5586	9.6	1:10
		882	1852	2.1	1:50
77	83	41410	18632	0.45	1:02
		8742	10500	1.2	1:10
		4528	3498	0.77	1:50
78	84	422	12516	29.66	1:02
		820	8026	9.79	1:10
		920	2508	2.73	1:50
79	85	406	32554	80.18	1:02
		622	35052	56.35	1:10
		740	19090	25.8	1:50

Table 4. Proliferative Activity of hIL2 Orthologs on hoCD122 3F8 cells v 3F8 Cells					
Sequence ID	Mutant #	3F8 Proliferation Signal cts/min	3F8 Ortho Proliferation Signal cts/min	Relative Proliferation Fold Change	Dilution
80	86	778	33426	42.96	1:02
		834	32330	38.76	1:10
		650	19616	30.18	1:50
81	87	406	32554	80.18	1:02
		622	35052	56.35	1:10
		740	19090	25.8	1:50
82	88	778	33426	42.96	1:02
		834	32330	38.76	1:10
		650	19616	30.18	1:50
83	89	52640	24098	0.46	1:02
		1390	15108	10.87	1:10
		1102	6552	5.95	1:50
84	90	482	6222	12.91	1:02
		554	4414	7.97	1:10
		1038	2926	2.82	1:50
85	91	1134	40826	36	1:02
		1320	32596	24.69	1:10
		596	20466	34.34	1:50
86	92	644	25662	39.85	1:02
		1184	17254	14.57	1:10
		1364	18676	13.69	1:50
87	93	1324	39110	29.54	1:02
		1014	50650	49.95	1:10
		810	24570	30.33	1:50
88	94	982	42072	42.84	1:02
		994	48458	48.75	1:10
		1206	21008	17.42	1:50
89	95	1470	45652	31.06	1:02
		3196	64050	20.04	1:10
		1122	33140	29.54	1:50

Table 4. Proliferative Activity of hIL2 Orthologs on hoCD122 3F8 cells v 3F8 Cells					
Sequence ID	Mutant #	3F8 Proliferation Signal cts/min	3F8 Ortho Proliferation Signal cts/min	Relative Proliferation Fold Change	Dilution
90	96	482	43790	90.85	1:02
		952	55494	58.29	1:10
		1098	26628	24.25	1:50
91	97	1256	45292	36.06	1:02
		1822	52956	29.06	1:10
		1014	21036	20.75	1:50
92	98	934	40160	43	1:02
		2418	36988	15.3	1:10
		1646	8934	5.43	1:50
93	99	2032	46428	22.85	1:02
		990	54366	54.92	1:10
		754	28502	37.8	1:50
94	100	270	28366	105.06	1:02
		720	21874	30.38	1:10
		662	6734	10.17	1:50
95	101	222	37776	170.16	1:02
		494	23668	47.91	1:10
		1322	10340	7.82	1:50
96	102	428	40248	94.04	1:02
		1050	34698	33.05	1:10
		620	15244	24.59	1:50
97	103	428	40386	94.36	1:02
		718	41110	57.26	1:10
		1126	24154	21.45	1:50
98	104	572	27176	47.51	1:02
		1492	32146	21.55	1:10
		1682	14170	8.42	1:50
99	105	174	31194	179.28	1:02
		320	24550	76.72	1:10
		328	12162	37.08	1:50

Table 4. Proliferative Activity of hIL2 Orthologs on hoCD122 3F8 cells v 3F8 Cells					
Sequence ID	Mutant #	3F8 Proliferation Signal cts/min	3F8 Ortho Proliferation Signal cts/min	Relative Proliferation Fold Change	Dilution
100	106	266	29250	109.96	1:02
		446	22884	51.31	1:10
		578	9634	16.67	1:50
101	107	236	10272	43.53	1:02
		454	5764	12.7	1:10
		714	1572	2.2	1:50
102	108	168	9936	59.14	1:02
		810	7068	8.73	1:10
		486	1056	2.17	1:50
103	109	288	31874	110.67	1:02
		468	20154	43.06	1:10
		744	11580	15.56	1:50
104	110	390	24590	63.05	1:02
		1258	29534	23.48	1:10
		988	16534	16.73	1:50
105	111	294	34188	116.29	1:02
		376	36390	96.78	1:10
		1252	21878	17.47	1:50
106	112	542	35072	64.71	1:02
		670	28718	42.86	1:10
		400	21180	52.95	1:50
107	113	1946	36418	18.71	1:02
		574	32526	56.67	1:10
		802	27108	33.8	1:50
108	114	36986	37234	1.01	1:02
		9570	26788	2.8	1:10
		1534	25830	16.84	1:50
109	115	1142	34936	30.59	1:02
		916	34830	38.02	1:10
		648	21180	32.69	1:50

Table 4. Proliferative Activity of hIL2 Orthologs on hoCD122 3F8 cells v 3F8 Cells					
Sequence ID	Mutant #	3F8 Proliferation Signal cts/min	3F8 Ortho Proliferation Signal cts/min	Relative Proliferation Fold Change	Dilution
110	116	616	28566	46.37	1:02
		824	32178	39.05	1:10
		916	21964	23.98	1:50
111	117	716	40050	55.94	1:02
		944	46042	48.77	1:10
		424	20930	49.36	1:50
112	118	5226	39266	7.51	1:02
		2208	30026	13.6	1:10
		902	10356	11.48	1:50
113	119	8646	35822	4.14	1:02
		2778	45996	16.56	1:10
		936	16464	17.59	1:50
114	121	1110	44280	39.89	1:02
		926	41336	44.64	1:10
		1316	18888	14.35	1:50
115	122	42504	33968	0.8	1:02
		8668	32580	3.76	1:10
		4116	15076	3.66	1:50
116	123	188	486	2.59	1:02
		730	580	0.79	1:10
		500	666	1.33	1:50
117	124	326	326	1	1:02
		490	588	1.2	1:10
		590	850	1.44	1:50
118	125	514	644	1.25	1:02
		1086	914	0.84	1:10
		1802	1074	0.6	1:50
119	127	350	12460	35.6	1:02
		512	5136	10.03	1:10
		972	2852	2.93	1:50

Table 4. Proliferative Activity of hIL2 Orthologs on hoCD122 3F8 cells v 3F8 Cells					
Sequence ID	Mutant #	3F8 Proliferation Signal cts/min	3F8 Ortho Proliferation Signal cts/min	Relative Proliferation Fold Change	Dilution
120	128	278	3724	13.4	1:02
		688	992	1.44	1:10
		644	804	1.25	1:50
120	129	370	29694	80.25	1:02
		630	36294	57.61	1:10
		1132	20880	18.45	1:50
122	130	388	35556	91.64	1:02
		1232	40094	32.54	1:10
		596	19732	33.11	1:50
123	131	1200	29296	24.41	1:02
		1126	42372	37.63	1:10
		682	27098	39.73	1:50
124	132	346	29070	84.02	1:02
		662	34022	51.39	1:10
		800	18576	23.22	1:50
125	133	798	31632	39.64	1:02
		854	33140	38.81	1:10
		1074	13616	12.68	1:50
126	134	60850	43706	0.72	1:02
		10198	44558	4.37	1:10
		4690	24848	5.3	1:50
127	135	258	36230	140.43	1:02
		676	45002	66.57	1:10
		968	26082	26.94	1:50
128	136	202	36514	180.76	1:02
		714	38610	54.08	1:10
		1294	18738	14.48	1:50
129	137	214	33064	154.5	1:02
		596	33160	55.64	1:10
		538	17086	31.76	1:50

Table 4. Proliferative Activity of hIL2 Orthologs on hoCD122 3F8 cells v 3F8 Cells					
Sequence ID	Mutant #	3F8 Proliferation Signal cts/min	3F8 Ortho Proliferation Signal cts/min	Relative Proliferation Fold Change	Dilution
130	138	296	2718	9.18	1:02
		342	2492	7.29	1:10
		640	964	1.51	1:50
131	139	198	452	2.28	1:02
		838	574	0.68	1:10
		534	748	1.4	1:50
132	140	470	294	0.63	1:02
		922	368	0.4	1:10
		896	544	0.61	1:50
133	141	162	20968	129.43	1:02
		436	14648	33.6	1:10
		416	2664	6.4	1:50
134	142	218	1536	7.05	1:02
		516	1104	2.14	1:10
		738	838	1.14	1:50
135	143	912	27352	29.99	1:02
		2388	26954	11.29	1:10
		1094	9508	8.69	1:50
136	144	45710	22440	0.49	1:02
		12602	18114	1.44	1:10
		2720	3714	1.37	1:50
137	145	494	9536	19.3	1:02
		778	2872	3.69	1:10
		516	826	1.6	1:50
139	146	12206	3382	0.28	1:02
		3484	1882	0.54	1:10
		686	1316	1.92	1:50

As demonstrated by the above data, the hIL2 orthologs of the Formula 1 selectively activate hoCD122 human T cells relative to the human T cells that do not express an orthogonal receptor (NKL cells).

Conservative Amino Acid Substitutions

[0112] In some embodiments, the hIL2 ortholog of Formula 1 may optionally comprise one or more conservative amino acid substitutions. Such conservative substitutions include those described by Dayhoff in *The Atlas of Protein Sequence and Structure* 5 (1978), and by Argos in *EMBO J.*, 8:779-785 (1989). Conservative substitutions are generally made in accordance with the following Table 4:

Table 4. Conservative Amino Acid Substitutions	
Wild type Residue	Substitution(s)
Ala	Ser
Arg	Lys
Asn	Gln, His
Asp	Glu
Cys	Ser, Ala
Gln	Asn
Glu	Asp
Gly	Pro
His	Asn, Gln
Ile	Leu, Val
Leu	Ile, Val
Lys	Arg, Gln, Glu, Met, Leu, Ile
Phe	Met, Leu, Tyr, Trp
Ser	Thr
Thr	Ser
Trp	Tyr, Phe
Tyr	Trp, Phe
Val	Ile, Leu

[0113] Substantial changes in function or immunological identity may be made by selecting amino acid substitutions that are less conservative than those indicated in Table 4. For example, substitutions may be made which more significantly affect the structure of the polypeptide.

backbone or disrupt secondary or tertiary elements including the substitution of an amino acid with a small uncharged side chain (e.g. glycine) with a large charge bulky side chain (asparagine). In particular, substitution of those hIL2 residues which are involved in the amino acids that interact with one or more of CD25, CD122 and/or CD123 as may be discerned from the crystal structure of hIL2 in association with its receptors as described in Wang, et al (2005) Science 310: 1159-1163. Modifications to the primary structure as provided above may optionally further comprise modifications including but not limited to the substitutions: N30E; K32E; N33D; P34G; T37L, M39Q, F42Y, F44Y, P47G, T51L, E52K, L53N, Q57E, M104A (see U.S. Pat. No. 5,206,344).

Cys125:

[0114] In some embodiments, the present disclosure provides hIL2 orthologs of Formula 1 containing modifications that facilitate recombinant expression in bacterial cells by eliminating the unpaired cysteine residue at position 125 by the substitution of C125A or C125S. In some embodiments, the hIL2 orthologs of the present invention comprise one of the following sets of amino acid modifications:

[E15S-H16Q-L19V-D20L-Q22K-M23A-C125S];
 [E15S-H16Q-L19V-D20L-Q22K-C125S];
 [E15S-H16Q-L19V-D20L-M23A-C125S];
 [E15S-H16Q-L19V-D20L-C125S];
 [E15S-H16Q-L19V-D20L-Q22K-M23A-C125A];
 [E15S-H16Q-L19V-D20L-M23A-C125A];
 [E15S-H16Q-L19V-D20L-Q22K-C125A];
 [E15S-H16Q-L19V-D20L-C125A];
 [desAla1-E15S-H16Q-L19V-D20L-Q22K-M23A-C125S];
 [desAla1-E15S-H16Q-L19V-D20L-Q22K-C125S];
 [desAla1-E15S-H16Q-L19V-D20L-C125S];
 [desAla1-E15S-H16Q-L19V-D20L-Q22K-M23A-C125A];
 [desAla1-E15S-H16Q-L19V-D20L-Q22K-C125A];
 [desAla1-E15S-H16Q-L19V-D20L-C125A];

[desAla1-E15S-H16Q-L19V-D20L-Q22K-M23A];

[desAla1-E15S-H16Q-L19V-D20L-M23A];

[desAla1-E15S-H16Q-L19V-D20L-Q22K]; or

[desAla1-E15S-H16Q-L19V-D20L].

Mutations to Enhance hCD122 Affinity

[0115] In some embodiments, hIL-2 orthologs of Formula 1 contain one or more mutations in positions of the hIL-2 sequence that either contact hCD122 or alter the orientation of other positions contacting hCD122, that modify the binding affinity of the hIL2 ortholog for hCD122. hIL-2 residues that have been identified as being involved in the binding of hIL2 to hCD122 include L12, Q13, H16, L19, D20, M23, R81, D84, S87, N88, V91, I92, and E95. In some embodiments, the hIL2 ortholog comprises one or more of the amino acid substitutions: Q74N, Q74H, Q74S, L80F, L80V, R81D, R81T, L85V, I86V, I89V, and/or I92F or combinations thereof. In some embodiments, the hIL2 ortholog comprises one or more of the amino acid substitutions: L80F, R81D, L85V, I86V and I92F. In some embodiments, the hIL2 ortholog comprises one or more of the amino acid substitutions: N74Q, L80F, R81D, L85V, I86V, I89V, and I92F. In some embodiments, the hIL2 ortholog comprises one or more of the amino acid substitutions: Q74N, L80V, R81T, L85V, I86V, and I92F. In some embodiments, the hIL2 ortholog comprises one or more of the amino acid substitutions: Q74H, L80F, R81D, L85V, I86V and I92F. In some embodiments, the hIL2 ortholog comprises one or more of the amino acid substitutions: Q74S, L80F, R81D, L85V, I86V and I92F. In some embodiments, the hIL2 ortholog comprises one or more of the amino acid substitutions: Q74N, L80F, R81D, L85V, I86V and I92F. In some embodiments, the hIL2 ortholog comprises one or more of the amino acid substitutions: Q74S, R81T, L85V, and I92F. In some embodiments, the hIL2 orthologs comprise the set of mutations [L80F-R81D-L85V-I86V-I92F] that have been identified as increasing affinity of hIL2 to hCD122. In some embodiments, the present disclosure provides hIL2 orthologs which are hIL2 polypeptides comprise one of the following sets of amino acid modifications:

[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].

[0116] In some embodiments, hIL-2 orthologs of Formula 1 comprise the substitution L85V that has been identified as increasing affinity of hIL2 to hCD122. In some embodiments, the present disclosure provides hIL2 orthologs which are hIL2 polypeptides comprising one of the following sets of amino acid modifications:

[E15S-H16Q-L19V-D20L-M23A-L85V];
 [E15S-H16Q-L19V-D20L-Q22K-M23A-L85V];
 [E15S-H16Q-L19V-D20L-M23A-L85V];
 [E15S-H16Q-L19V-D20L-Q22K-M23A-L85V];
 [E15S-H16Q-L19V-D20L-M23A-L85V-Q126H];
 [E15S-H16Q-L19V-D20L-Q22K-M23A-L85V-Q126H];
 [E15S-H16Q-L19V-D20L-M23A-L85V-Q126M]; or
 [E15S-H16Q-L19V-D20L-Q22K-M23A-L85V-Q126M].

Modifications to Modulate CD25 Affinity

[0117] In some embodiments, hIL-2 orthologs of Formula 1 contain one or more mutations in positions of the hIL-2 ortholog that modulate the binding affinity for hCD25. The mutations regions of hIL2 in close proximity to hCD25 as part of the trimeric IL2 receptor complex based on the crystal structure of IL2 in association with the IL2 receptor (Wang, et al (2005) Science 310:1159). In some embodiments, hIL-2 orthologs of Formula 1 contain modifications at one or more positions selected from 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, I114, I128 and T133.

Examples of amino acid substitutions which may be incorporated into the hIL2 ortholog sequence include one or more substitutions selected from 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, I114V, I128T, T133A, and T133N. In some embodiments, the hIL2 orthologs of the present disclosure comprise one or more of the point mutations 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, I114V, I128T, T133A, and T133N (Wittrup, *et al supra*), R38A, F41A and/or F42A (Suave, *et al* (1991) PNAS(USA)88:4636-4640); P65L (Chen *et al. Cell Death and Disease* (2018) 9:989); F42A/G/S/T/Q/E/N/R/K, Y45A/G/S/T/Q/E/N/D/R/K and/or L72G/A/S/T/Q/E/N/D/R/K (Ast, *et al* United States Patent Application Publication 2012/0244112 A1 published September 27, 2012; United States Patent No. 9,266,938 B2 issued February 23, 2016).

[0118] In addition to point mutations, combinations of the foregoing modifications may be employed to modulate the binding of the hIL-2 orthologs of Formula 1 to CD25. In some embodiments, the hIL2 orthologs of the present disclosure comprise one or more of the following sets of amino acid substitutions: [R38A-F42A-Y45A-E62A] (Carmenate, *et al* (2013) *J Immunol* 190:6230-6238); [F42A-Y45A-L72G] (Roche RG7461 (RO6874281); [V69A, Q74P]; [V69A, Q74, T101A]; [V69A, Q74P, I128T]; [N30D, V69A, Q74P, F103S]; [K49E, V69A, A73V, K76E]; [V69A, Q74P, T101A, T133N]; [N30S, V69A, Q74P, I128A]; [N30S, V69A, Q74P, I128T]; [K9T, Q11R, K35R, V69A, Q74P]; [A1T, M46L, K49R, E61D, V69A, H79R]; [K48E, E68D, N71T, N90H, F103S, I114V]; [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, I128T]; [N26D, N29S, N30S, K54R, E67G, V69A, Q74P, I92T]; [K8R, Q13R, N26D, N30T, K35R, T37R, V69A, Q74P, I92T] and [N29S, Y31H, K35R, T37A, K48E, V69A, N71R, Q74P, I39V] (Wittrup *et al.*, United States Patent No. 7,569,215 issued August 4, 2009); and/or [T41P-T51P] (Chang, *et al* (1995) *Molecular Pharmacology* 47:206-211). In some embodiments, the present disclosure

provides hIL-2 orthologs of Formula 1 comprising one of the following sets of amino acid modifications:

[E15S-H16Q-L19V-D20L-M23A-R38A-F42A-Y45A-E62A];
 [E15S-H16Q-L19V-D20L-M23A-R38A-F42A-Y45A-E62A];
 [E15S-H16Q-L19V-D20L-Q22K-M23A-R38A-F42A-Y45A-E62A];
 [E15S-H16Q-L19V-D20L-M23A-R38A-F42A-Y45A-E62A-Q126H];
 [E15S-H16Q-L19V-D20L-M23A-R38A-F42A-Y45A-E62A-Q126H];
 [E15S-H16Q-L19V-D20L-Q22K-M23A-R38A-F42A-Y45A-E62A-Q126H];
 [E15S-H16Q-L19V-D20L-M23A-V69A];
 [E15S-H16Q-L19V-D20L-Q22K-M23A-V69A];
 [E15S-H16Q-L19V-D20L-M23A-Q74P];
 [E15S-H16Q-L19V-D20L-Q22K-M23A-Q74P];
 [E15S-H16Q-L19V-D20L-M23A-R38A-F42A-Y45A-E62A-Q126M]; or
 [E15S-H16Q-L19V-D20L-M23A-R38A-F42A-Y45A-E62A-Q126M].

Modifications to Modulate CD132 Affinity

[0119] In some embodiments of the invention, the hIL-2 orthologs of the present disclosure contain one or more mutations that modulate the binding to hIL-2 ortholog to CD132. Exemplary hIL-2 orthologs contain one or more mutations in positions of the hIL-2 sequence that either contact CD132 or alter the orientation of other positions contacting hCD122, resulting in an altered binding to CD132. hIL-2 residues that are identified modulating the affinity of hIL2 to CD132 include Q11, L18 (e.g. L18R), Q22 (e.g. Q22E), E110, N119, T123, Q126 (e.g. Q126K/H), S127, I129, S130, and T133. In some embodiments, the present disclosure provides hIL2 orthologs which are hIL2 polypeptides comprising one 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].

Removal of Glycosylation Site

[0120] The hIL2 orthologs of the present disclosure may further or optionally provide elimination of the O-glycosylation site at position Thr3 of the to facilitate the production of an aglycosylated hIL2 ortholog variant when the ortholog expressed in mammalian cells such as CHO or HEK cells. Thus, in certain embodiments the hIL2 orthologs further comprise a modification which eliminates the O-glycosylation site of IL-2 at a position corresponding to residue 3 of human IL-2. In one embodiment said modification which eliminates the O-glycosylation site of IL-2 at a position corresponding to residue 3 of human IL-2 is an amino acid substitution. Exemplary amino acid substitutions include T3A, T3G, T3Q, T3E, T3N, T3D, T3R, T3K, and T3P which removes the glycosylation site at position 3 without eliminating biological activity (see U.S. Pat. No. 5,116,943; Weiger et al., (1989) Eur. J. Biochem., 180:295-300). In a specific embodiment, said modification is the amino acid substitution T3A. In some embodiments, the present disclosure provides hIL2 orthologs which are hIL2 polypeptides comprising one of the following sets of amino acid modifications:

[T3A-E15S-H16Q-L19V-D20L-Q22K-M23A-C125S];
 [T3A-E15S-H16Q-L19V-D20L-Q22K-C125S];
 [T3A-E15S-H16Q-L19V-D20L-M23A-C125S];
 [T3A-E15S-H16Q-L19V-D20L-C125S];
 [T3A-E15S-H16Q-L19V-D20L-Q22K-M23A-C125A];
 [T3A-E15S-H16Q-L19V-D20L-M23A-C125A];
 [T3A-E15S-H16Q-L19V-D20L-Q22K-C125A];
 [T3A-E15S-H16Q-L19V-D20L-C125A] ;
 [T3A-E15S-H16Q-L19V-D20L-Q22K-M23A];
 [T3A-E15S-H16Q-L19V-D20L-M23A];
 [T3A-E15S-H16Q-L19V-D20L-Q22K];
 [T3A-E15S-H16Q-L19V-D20L];
 [desAla1-T3A-E15S-H16Q-L19V-D20L-Q22K-M23A-C125S];
 [desAla1-T3A-E15S-H16Q-L19V-D20L-M23A-C125S];

[desAla1-T3A-E15S-H16Q-L19V-D20L-Q22K-C125S];
 [desAla1-T3A-E15S-H16Q-L19V-D20L-C125S];
 [desAla1-T3A-E15S-H16Q-L19V-D20L-Q22K-M23A-C125A];
 [desAla1-T3A-E15S-H16Q-L19V-D20L-M23A-C125A];
 [desAla1-T3A-E15S-H16Q-L19V-D20L-Q22K-C125A];
 [desAla1-T3A-E15S-H16Q-L19V-D20L-C125A];
 [desAla1-T3A-E15S-H16Q-L19V-D20L-Q22K-M23A];
 [desAla1-T3A-E15S-H16Q-L19V-D20L-M23A];
 [desAla1-T3A-E15S-H16Q-L19V-D20L-Q22K]; or
 [desAla1-T3A-E15S-H16Q-L19V-D20L].

N Terminal Deletions:

[0121] The IL-2 orthologs may further comprise elimination of N-terminal amino acids at one or more of positions 1-9, alternatively positions 1-8, alternatively positions 1-7 alternatively positions 1-6, alternatively positions 1-5, alternatively positions 1-4, alternatively positions des1-3, alternatively positions 1-2 while retaining hIL2 ortholog activity. In some embodiments, the present disclosure provides hIL2 orthologs which are hIL2 polypeptides comprising one of the following sets of amino acid modifications:

[desAla1-E15S-H16Q-L18R-L19V-D20L-Q22E-M23A];
 [desAla1-E15S-H16Q- L18R-L19V-D20L-Q22K-M23A];
 [desAla1-E15S-H16Q- L18R-L19V-D20L-M23A];
 [desAla1-E15S-H16Q- L18R-L19V-D20L-Q22K-M23A];
 [desAla1-E15S-H16Q- L18R-L19V-D20L-M23A-Q126H];
 [desAla1-E15S-H16Q- L18R-L19V-D20L-Q22K-M23A-Q126H];
 [desAla1-E15S-H16Q- L18R-L19V-D20L-M23A-Q126K];
 [desAla1-E15S-H16Q- L18R-L19V-D20L-Q22K-M23A-Q126K];
 [desAla1-E15S-H16Q-L19V-D20L-M23A-Q126H];
 [desAla1-E15S-H16Q-L19V-D20L-Q22K-M23A-Q126H];
 [desAla1-E15S-H16Q-L19V-D20L-M23A-Q126K];
 [desAla1-E15S-H16Q-L19V-D20L-Q22K-M23A-Q126K];

[desAla1-E15S-H16Q- L18R-L19V-D20L-M23A-Q126H];
 [desAla1-E15S-H16Q- L18R-L19V-D20L-Q22K-M23A-Q126H];
 [desAla1-E15S-H16Q- L18R-L19V-D20L-M23A-Q126K];
 [desAla1-desPro2-E15S-H16Q-L19V-D20L-Q22K-M23A];
 [desAla1-desPro2-E15S-H16Q-L19V-D20L-Q22K];
 [desAla1-desPro2-E15S-H16Q-L19V-D20L-M23A];
 [desAla1-desPro2-E15S-H16Q-L19V-D20L] ;
 [desAla1-desPro2-desThr3-E15S-H16Q-L19V-D20L-Q22K-M23A];
 [desAla1-desPro2-desThr3-E15S-H16Q-L19V-D20L-Q22K];
 [desAla1-desPro2-desThr3-E15S-H16Q-L19V-D20L-M23A];
 [desAla1-desPro2-desThr3-E15S-H16Q-L19V-D20L] ;
 [desAla1-desPro2-desThr3-desSer4-E15S-H16Q-L19V-D20L-Q22K-M23A];
 [desAla1-desPro2-desThr3-desSer4-E15S-H16Q-L19V-D20L-Q22K];
 [desAla1-desPro2-desThr3-desSer4-E15S-H16Q-L19V-D20L-M23A];
 [desAla1-desPro2-desThr3-desSer4-E15S-H16Q-L19V-D20L] ;
 [desAla1-desPro2-desThr3-desSer4-desSer5-E15S-H16Q-L19V-D20L-Q22K-M23A];
 [desAla1-desPro2-desThr3-desSer4-desSer5-E15S-H16Q-L19V-D20L-Q22K];
 [desAla1-desPro2-desThr3-desSer4-desSer5-E15S-H16Q-L19V-D20L-M23A];
 [desAla1-desPro2-desThr3-desSer4-desSer5-E15S-H16Q-L19V-D20L];
 [desAla1-desPro2-desThr3-desSer4-desSer5-desSer6-E15S-H16Q-L19V-D20L-Q22K-M23A];
 [desAla1-desPro2-desThr3-desSer4-desSer5-desSer6-E15S-H16Q-L19V-D20L-Q22K];
 [desAla1-desPro2-desThr3-desSer4-desSer5-desSer6-E15S-H16Q-L19V-D20L-M23A];
 or
 [desAla1-desPro2-desThr3-desSer4-desSer5-desSer6-E15S-H16Q-L19V-D20L].

[0122] In some embodiments, the hIL2 orthologs may comprise deletion of the first two amino acids (desAla1-desPro2) as well as substitution of the Thr3 glycosylation with a cysteine residue to facilitate for selective N-terminal modification, especially PEGylation of the sulfhydryl group of the cysteine (See, e.g. Katre, *et al.* United States Patent No 5,206,344 issued

April 27, 1993). In some embodiments, the present disclosure provides hIL2 orthologs which are hIL2 polypeptides comprising one of the following sets of amino acid modifications:

[desAla1-desPro2-T3C-E15S-H16Q-L19V-D20L-Q22K-M23A-C125S];
 [desAla1-desPro2-T3C-E15S-H16Q-L19V-D20L-Q22K-C125S];
 [desAla1-desPro2-T3C-E15S-H16Q-L19V-D20L-M23A-C125S];
 [desAla1-desPro2-T3C-E15S-H16Q-L19V-D20L-C125S];
 [desAla1-desPro2-T3C-E15S-H16Q-L19V-D20L-Q22K-M23A-C125A];
 [desAla1-desPro2-T3C-E15S-H16Q-L19V-D20L-Q22K-C125A];
 [desAla1-desPro2-T3C-E15S-H16Q-L19V-D20L-M23A-C125A];
 [desAla1-desPro2-T3C-E15S-H16Q-L19V-D20L-C125A];
 [desAla1-desPro2-T3C-E15S-H16Q-L19V-D20L-Q22K-M23A];
 [desAla1-desPro2-T3C-E15S-H16Q-L19V-D20L-Q22K];
 [desAla1-desPro2-T3C-E15S-H16Q-L19V-D20L-M23A]; or
 [desAla1-desPro2-T3C-E15S-H16Q-L19V-D20L].

[0123] In some embodiments, the hIL2 orthologs of the present disclosure comprise a deletion of the alanine at position 1 (“desAla1”) hIL2 orthologs. In some embodiments, the present disclosure provides des-Ala1-hIL2 orthologs comprising one of the following sets of amino acid modifications:

[desAla1-E15S-H16Q-L19V-D20L];
 [desAla1-E15S-H16Q-L19V-D20L-Q22K];
 [desAla1-E15S-H16Q-L19V-D20L-Q22K-M23A];
 [desAla1-E15S-H16Q-L19V-D20L-Q126H];
 [desAla1-E15S-H16Q-L19V-D20L-Q22K-Q126H];
 [desAla1-E15S-H16Q-L19V-D20L-Q22K-M23A-Q126H];
 [desAla1-E15S-H16Q-L19V-D20L-Q126M];
 [desAla1-E15S-H16Q-L19V-D20L-Q22K-Q126H];
 [desAla1-E15S-H16Q-L19V-D20L-Q22K-M23A-Q126H];
 [desAla1-E15S-H16Q-L19V-D20L-C125A];
 [desAla1-E15S-H16Q-L19V-D20L-Q22K-C125A];

[desAla1-E15S-H16Q-L19V-D20L-Q22K-M23A-C125A];
 [desAla1-E15S-H16Q-L19V-D20L-C125A-Q126H];
 [desAla1-E15S-H16Q-L19V-D20L-Q22K-C125A-Q126H];
 [desAla1-E15S-H16Q-L19V-D20L-Q22K-M23A-C125A-Q126H];
 [desAla1-E15S-H16Q-L19V-D20L- C125A-Q126M];
 [desAla1-E15S-H16Q-L19V-D20L-Q22K-C125A-Q126H];
 [desAla1-E15S-H16Q-L19V-D20L-Q22K-M23A-C125A-Q126H];
 [desAla1-E15S-H16Q-L19V-D20L-C125S];
 [desAla1-E15S-H16Q-L19V-D20L-Q22K-C125S];
 [desAla1-E15S-H16Q-L19V-D20L-Q22K-M23A-C125S];
 [desAla1-E15S-H16Q-L19V-D20L-C125S-Q126H];
 [desAla1-E15S-H16Q-L19V-D20L-Q22K-C125S-Q126H];
 [desAla1-E15S-H16Q-L19V-D20L-Q22K-M23A-C125S-Q126H];
 [desAla1-E15S-H16Q-L19V-D20L-C125S-Q126M];
 [desAla1-E15S-H16Q-L19V-D20L-Q22K-C125S-Q126M]; or
 [desAla1-E15S-H16Q-L19V-D20L-Q22K-M23A-C125S-Q126M].

Modifications to Minimize Vascular Leak Syndrome

[0124] In some embodiments of the disclosure, the hIL2 ortholog comprises amino acid substitutions to avoid vascular leak syndrome. Epstein, *et al.*, United States Patent No 7,514,073B2 issued April 7, 2009. Examples of such modifications which may be incorporated into the hIL2 orthologs of the present disclosure include one or more of R38W, R38G, R39L, R39V, F42K, and/or H55Y.

Oxidation Stabilized M104A:

[0125] In some embodiments of the disclosure, the hIL2 ortholog may comprise a modification at position M104. In one embodiment the substitution of methionine 104 with an alanine residue (M104A) to provide a more oxidation-resistant ortholog (Koths, *et al.* United States patent 4,752,585 issued June 21, 1988).

Affinity Maturation:

[0126] some embodiments, the hIL2 orthologs of the present disclosure may be affinity matured to enhance their activity with respect to the orthogonal hCD122. An "affinity matured" polypeptide is one having one or more alteration(s) in one or more residues which results in an improvement in the affinity of the orthogonal polypeptide for the cognate orthogonal receptor, or *vice versa*, compared to a parent polypeptide which does not possess those alteration(s). Affinity maturation can be done to increase the binding affinity of the hIL2 ortholog by at least about 10%, alternatively at least about 50%, alternatively at least about 100% alternatively at least about 150%, or from 1 to 5 fold as compared to the "parent" polypeptide. An engineered hIL2 ortholog of the present invention activates its cognate orthogonal receptor, as discussed above, but has significantly reduced binding and activation of the native receptor when assessed by ELISA and/or FACS analysis using sufficient amounts of the molecules under suitable assay conditions.

Modifications to Extend Duration of Action In Vivo

[0127] In some embodiments the hIL-2 orthologs of the present disclosure may comprise modifications to provide for an extended lifetime *in vivo* and/or extended duration of action in a subject. In some embodiments, the hIL-2 orthologs of the present disclosure possess a plasma half-life in a human subject of greater than 4 hours, 5 hours, 6 hours, 7 hours, 8 hours, 9 hours, 10 hours, 12 hours, 18 hours, 24 hours, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 10 days, 14 days, or 30 days. Such extended-life hIL2 orthologs may be achieved by primary sequence modifications and/or conjugation to carrier molecules.

Primary Sequence Modifications to Extend Duration of Action

[0128] hIL-2 orthologs of the present disclosure may comprise amino acid substitutions that result in prolonged *in vivo* lifetime. Examples of positions where amino acid substitutions may be incorporated into the hIL-2 orthologs to provide for extended *in vivo* duration include one or more of positions V111, R117 and/or T133. In some embodiments, the hIL2 orthologs of the present disclosure comprise one or more of V111R, R117K and/or T133N modifications.

Dakshinamurthi, *et al.* (2009) International Journal of Bioinformatics Research 1(2):4-13

Carrier Molecules

[0129] In some embodiments the hIL2 ortholog is modified to provide an extended duration of action in a subject which may be achieved through conjugation to carrier molecules to provide desired pharmacological properties such as extended half-life. In one embodiment, the hIL2 ortholog may comprise a functional domain of a chimeric polypeptide. In some embodiments, the hIL2 ortholog can be covalently linked to the Fc domain of IgG, albumin, or other molecules to extend its half-life, e.g. by pegylation, glycosylation, and the like as known in the art.

Fc Fusions

[0130] In some embodiments, the hIL2 orthologs of the present disclosure are operably linked to a functional domain of an Fc-fusion chimeric polypeptide molecule. Fc fusion conjugates have been shown to increase the systemic half-life of biopharmaceuticals, and thus the biopharmaceutical product can require less frequent administration. Fc binds to the neonatal Fc receptor (FcRn) in endothelial cells that line the blood vessels, and, upon binding, the Fc fusion molecule is protected from degradation and re-released into the circulation, keeping the molecule in circulation longer. This Fc binding is believed to be the mechanism by which endogenous IgG retains its long plasma half-life. More recent Fc-fusion technology links a single copy of a biopharmaceutical to the Fc region of an antibody to optimize the pharmacokinetic and pharmacodynamic properties of the biopharmaceutical as compared to traditional Fc-fusion conjugates. The "Fc region" useful in the preparation of Fc fusions can be a naturally occurring or synthetic polypeptide that is homologous to an IgG C-terminal domain produced by digestion of IgG with papain. IgG Fc has a molecular weight of approximately 50 kDa. The hIL2 orthologs may provide the entire Fc region, or a smaller portion that retains the ability to extend the circulating half-life of a chimeric polypeptide of which it is a part. In addition, full-length or fragmented Fc regions can be variants of the wild-type molecule. In a typical presentation, each monomer of the dimeric Fc carries a heterologous polypeptide, the heterologous polypeptides being the same or different.

[0131] In some embodiments, when the hIL2 ortholog is to be administered in the format of an Fc fusion, particularly in those situations when the polypeptide chains conjugated to each subunit of the Fc dimer are different, the Fc fusion may be engineered to possess a "knob-into-hole modification." The knob-into-hole modification is more fully described in Ridgway, et al.

(1996) Protein Engineering 9(7):617-621 and United States Patent No. 5,731,168, issued March 24, 1998. The knob-into-hole modification refers to a modification at the interface between two immunoglobulin heavy chains in the CH3 domain, wherein: i) in a CH3 domain of a first heavy chain, an amino acid residue is replaced with an amino acid residue having a larger side chain (e.g. tyrosine or tryptophan) creating a projection from the surface ("knob") and ii) in the CH3 domain of a second heavy chain, an amino acid residue is replaced with an amino acid residue having a smaller side chain (e.g. alanine or threonine), thereby generating a cavity ("hole") within at interface in the second CH3 domain within which the protruding side chain of the first CH3 domain ("knob") is received by the cavity in the second CH3 domain. In one embodiment, the "knob-into-hole modification" comprises the amino acid substitution T366W and optionally the amino acid substitution S354C in one of the antibody heavy chains, and the amino acid substitutions T366S, L368A, Y407V and optionally Y349C in the other one of the antibody heavy chains. Furthermore, the Fc domains may be modified by the introduction of cysteine residues at positions S354 and Y349 which results in a stabilizing disulfide bridge between the two antibody heavy chains in the Fc region (Carter, et al. (2001) Immunol Methods 248, 7-15). The knob-into-hole format is used to facilitate the expression of a first polypeptide (e.g. an hIL2 ortholog) on a first Fc monomer with a "knob" modification and a second polypeptide on the second Fc monomer possessing a "hole" modification to facilitate the expression of heterodimeric polypeptide conjugates.

[0132] The Fc region can be "lytic" or "non-lytic," but is typically non-lytic. A non-lytic Fc region typically lacks a high affinity Fc receptor binding site and a C1q binding site. The high affinity Fc receptor binding site of murine IgG Fc includes the Leu residue at position 235 of IgG Fc. Thus, the Fc receptor binding site can be inhibited by mutating or deleting Leu 235. For example, substitution of Glu for Leu 235 inhibits the ability of the Fc region to bind the high affinity Fc receptor. The murine C1q binding site can be functionally destroyed by mutating or deleting the Glu 318, Lys 320, and Lys 322 residues of IgG. For example, substitution of Ala residues for Glu 318, Lys 320, and Lys 322 renders IgG1 Fc unable to direct antibody-dependent complement lysis. In contrast, a lytic IgG Fc region has a high affinity Fc receptor binding site and a C1q binding site. The high affinity Fc receptor binding site includes the Leu residue at position 235 of IgG Fc, and the C1q binding site includes the Glu 318, Lys 320, and Lys 322

residues of IgG 1. Lytic IgG Fc has wild type residues or conservative amino acid substitutions at these sites. Lytic IgG Fc can target cells for antibody dependent cellular cytotoxicity or complement directed cytolysis (CDC). Appropriate mutations for human IgG are also known (see, e.g., Morrison *et al.*, The Immunologist 2:119-124, 1994; and Brekke *et al.*, The Immunologist 2: 125, 1994). In some embodiments, the Fc domain monomer comprises at least one mutation relative to a wild-type human IgG1, IgG2, or IgG4 Fc region as described in United States Patent No. US10,259,859 B2, the entire teaching of which is herein incorporated by reference. In some embodiments, the polypeptide exhibits a reduction of phagocytosis in a phagocytosis assay compared to a polypeptide with a wild-type human IgG Fc region. In some embodiments, the Fc domain monomer is linked to a second polypeptide comprising a second Fc domain monomer to form an Fc domain dimer.

PEGylation:

[0133] In some embodiments, the hIL2 ortholog of the present disclosure may be conjugated to one or more water-soluble polymers. Examples of water soluble polymers useful in the practice of the present invention include polyethylene glycol (PEG), poly-propylene glycol (PPG), polysaccharides (polyvinylpyrrolidone, copolymers of ethylene glycol and propylene glycol, poly(oxyethylated polyol), polyolefinic alcohol, polysaccharides), poly-alpha-hydroxy acid), polyvinyl alcohol (PVA), polyphosphazene, polyoxazolines (POZ), poly(N-acryloylmorpholine), or a combination thereof.

[0134] In some embodiments the hIL2 ortholog is operably linked to one or more polyethylene glycol molecules or "PEGylated." Although the method or site of PEG attachment to hIL2 ortholog may vary, in certain embodiments the PEGylation does not alter, or only minimally alters, the activity of the hIL2 ortholog.

[0135] In some embodiments, selective PEGylation of the hIL2 ortholog (for example by the incorporation of non-natural amino acids having side chains to facilitate selective PEG conjugation chemistries as described Ptacin, et al (PCT International Application No. PCT/US2018/045257 filed August 3, 2018 and published February 7, 2019 as International Publication Number WO 2019/028419A1 may be employed to generate an hIL2 ortholog with having reduced affinity for one or more subunits (e.g. CD25, CD132) of an hIL2 receptor

complex. For example, an hIL2 ortholog incorporating non-natural amino acids having a PEGylatable specific moiety at those sequences or residues of hIL2 identified as interacting with CD25 including amino acids 34-45, 61-72 and 105-109 provides an hIL2 ortholog having modulated binding to CD25. Similarly, an hIL2 ortholog incorporating non-natural amino acids having a PEGylatable specific moiety at those sequences or residues of hIL2 identified as interacting with hCD132 including but not limited to amino acids 18, 22, 109, 126, and/or 133 provides an hIL2 ortholog with modulated binding affinity for hCD132.

[0136] In certain embodiments, the increase in half-life is greater than any decrease in biological activity. PEGs suitable for conjugation to a polypeptide sequence are generally soluble in water at room temperature, and have the general formula $R(O-CH_2-CH_2)_nO-R$, where R is hydrogen or a protective group such as an alkyl or an alkanol group, and where n is an integer from 1 to 1000. When R is a protective group, it generally has from 1 to 8 carbons. The PEG conjugated to the polypeptide sequence can be linear or branched. Branched PEG derivatives, "star-PEGs" and multi-armed PEGs are contemplated by the present disclosure.

[0137] A molecular weight of the PEG useful in the context of the hIL2 orthologs of the present disclosure is not restricted to any particular range. The PEG component of the PEG-hIL2 ortholog can have a molecular mass greater than about 5kDa, greater than about 10kDa, greater than about 15kDa, greater than about 20kDa, greater than about 30kDa, greater than about 40kDa, or greater than about 50kDa. In some embodiments, the molecular mass is from about 5kDa to about 10kDa, from about 5kDa to about 15kDa, from about 5kDa to about 20kDa, from about 10kDa to about 15kDa, from about 10kDa to about 20kDa, from about 10kDa to about 25kDa or from about 10kDa to about 30kDa. Linear or branched PEG molecules having molecular weights from about 2,000 to about 80,000 daltons, alternatively about 2,000 to about 70,000 daltons, alternatively about 5,000 to about 50,000 daltons, alternatively about 10,000 to about 50,000 daltons, alternatively about 20,000 to about 50,000 daltons, alternatively about 30,000 to about 50,000 daltons, alternatively about 20,000 to about 40,000 daltons, alternatively about 30,000 to about 40,000 daltons. In one embodiment of the invention, the PEG is a 40kD branched PEG comprising two 20 kD arms.

[0138] The present disclosure also contemplates compositions of conjugates wherein the PEGs have different n values, and thus the various different PEGs are present in specific ratios. For example, some compositions comprise a mixture of conjugates where $n=1, 2, 3$ and 4 . In some compositions, the percentage of conjugates where $n=1$ is 18-25%, the percentage of conjugates where $n=2$ is 50-66%, the percentage of conjugates where $n=3$ is 12-16%, and the percentage of conjugates where $n=4$ is up to 5%. Such compositions can be produced by reaction conditions and purification methods known in the art. Chromatography may be used to resolve conjugate fractions, and a fraction is then identified which contains the conjugate having, for example, the desired number of PEGs attached, purified free from unmodified protein sequences and from conjugates having other numbers of PEGs attached.

[0139] PEGs suitable for conjugation to a IL2 ortholog polypeptide are generally soluble in water at room temperature, and have the general formula $R(O-CH_2-CH_2)_nO-R$, where R is hydrogen or a protective group such as an alkyl or an alkanol group, and where n is an integer from 1 to 1000. When R is a protective group, it generally has from 1 to 8 carbons.

[0140] Two widely used first generation activated monomethoxy PEGs (mPEGs) are succinimidyl carbonate PEG (SC-PEG; see, e.g., Zalipsky, et al. (1992) Biotechnol. Appl. Biochem 15:100-114) and benzotriazole carbonate PEG (BTC-PEG; see, e.g., Dolence, et al. US Patent No. 5,650,234), which react preferentially with lysine residues to form a carbamate linkage but are also known to react with histidine and tyrosine residues. Use of a PEG-aldehyde linker targets a single site on the N-terminus of a polypeptide through reductive amination.

[0141] Pegylation may occur at the α -amino group at the N-terminus of the polypeptide, the epsilon amino group on the side chain of lysine residues, and the imidazole group on the side chain of histidine residues. Since most recombinant polypeptides possess a single alpha and a number of epsilon amino and imidazole groups, numerous positional isomers can be generated depending on the linker chemistry. General pegylation strategies known in the art can be applied herein.

[0142] The PEG can be bound to an hIL2 ortholog of the present disclosure *via* a terminal reactive group (a "spacer") which mediates a bond between the free amino or carboxyl groups of one or more of the polypeptide sequences and polyethylene glycol. A PEG having the spacer

which can be bound to the free amino group includes N-hydroxysuccinylimide polyethylene glycol, which can be prepared by activating succinic acid ester of polyethylene glycol with N-hydroxysuccinylimide.

[0143] In some embodiments, the PEGylation of hIL2 orthologs is facilitated by the incorporation of non-natural amino acids bearing unique side chains to facilitate site specific PEGylation. The incorporation of non-natural amino acids into polypeptides to provide functional moieties to achieve site specific pegylation of such polypeptides is known in the art. See e.g. Ptacin, et al (PCT International Application No. PCT/US2018/045257 filed August 3, 2018 and published February 7, 2019 as International Publication Number WO 2019/028419A1. In one embodiment, the hIL2 orthologs of the present invention incorporate a non-natural amino acid at position D109 of the hIL2 ortholog. In one embodiment of the invention the hIL2 ortholog is a PEGylated at position 109 of the hIL2 ortholog to a PEG molecule having a molecular weight of about 20kD, alternatively about 30kD, alternatively about 40kD.

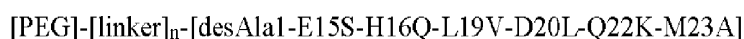
[0144] The PEG conjugated to the polypeptide sequence can be linear or branched. Branched PEG derivatives, “star-PEGs” and multi-armed PEGs are contemplated by the present disclosure. Specific embodiments PEGs useful in the practice of the present invention include a 10kDa linear PEG-aldehyde (*e.g.*, Sunbright® ME-100AL, NOF America Corporation, One North Broadway, White Plains, NY 10601 USA), 10kDa linear PEG-NHS ester (*e.g.*, Sunbright® ME-100CS, Sunbright® ME-100AS, Sunbright® ME-100GS, Sunbright® ME-100HS, NOF), a 20kDa linear PEG-aldehyde (*e.g.* Sunbright® ME-200AL, NOF), a 20kDa linear PEG- NHS ester (*e.g.*, Sunbright® ME-200CS, Sunbright® ME-200AS, Sunbright® ME-200GS, Sunbright® ME-200HS, NOF), a 20kDa 2-arm branched PEG-aldehyde the 20 kDA PEG-aldehyde comprising two 10kDA linear PEG molecules (*e.g.*, Sunbright® GL2-200AL3, NOF), a 20kDa 2-arm branched PEG-NHS ester the 20 kDA PEG-NHS ester comprising two 10kDA linear PEG molecules (*e.g.*, Sunbright® GL2-200TS, Sunbright® GL200GS2, NOF), a 40kDa 2-arm branched PEG-aldehyde the 40 kDA PEG-aldehyde comprising two 20kDA linear PEG molecules (*e.g.*, Sunbright® GL2-400AL3), a 40kDa 2-arm branched PEG-NHS ester the 40 kDA PEG-NHS ester comprising two 20kDA linear PEG molecules (*e.g.*, Sunbright® GL2-400AL3, Sunbright® GL2-400GS2, NOF), a linear 30kDa PEG-aldehyde (*e.g.*, Sunbright® ME-300AL) and a linear 30kDa PEG-NHS ester.

[0145] In one embodiment, the hIL2 ortholog of the present disclosure comprises the structure:



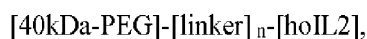
where $n = 0$ or 1 .

[0146] In embodiment, the hIL2 ortholog of the present disclosure comprises the structure:



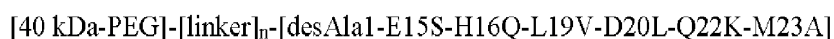
where $n = 0$ or 1 .

[0147] In one embodiment, the hIL2 ortholog of the present disclosure comprises the structure:



where $n = 0$ or 1 .

[0148] In embodiment, the hIL2 ortholog of the present disclosure comprises the structure:



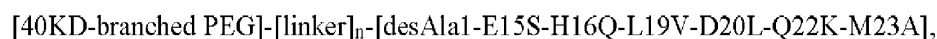
where $n = 0$ or 1 .

[0149] In one embodiment, the hIL2 ortholog of the present disclosure comprises the structure:



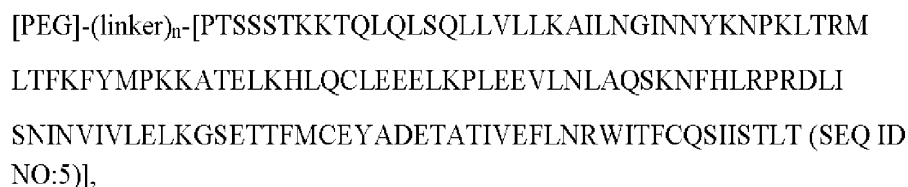
where $n = 0$ or 1 .

[0150] In embodiment, the hIL2 ortholog of the present disclosure comprises the structure:



where $n = 0$ or 1 .

[0151] In another embodiment, the hIL2 ortholog comprises the structure:



where $n = 0$ or 1 .

[0152] In another embodiment, the hIL2 ortholog comprises the structure:

[40 kDa-PEG]-(linker)_n-[PTSSSTKKTQLQLSQLLVLLKAILNGINNYK
NPKLTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSKN
FHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVEFLNRWITF
CQSIISTLT (SEQUENCE ID NO:5)],

where n = 0 or 1.

[0153] Suitable linkers include “flexible linkers” which are generally of sufficient length to permit some movement between the modified polypeptide sequences and the linked components and molecules. The linker molecules are generally from about 6-50 atoms long. The linker may also be, for example aryl acetylene, ethylene glycol oligomers containing 2-10 monomer units, diamines, diacids, amino acids or combinations thereof. Suitable linkers can be readily selected and can be of a suitable length such as 1 amino acid (e.g. glycine), 2, 3, 4, 5, 6, 7, 8, 9, 10, 10-20, 20-30, 30-50 or more than 50 amino acids.

[0154] Examples of flexible linkers include glycine polymers (G)_n, glycine-alanine polymers, alanine-serine polymers, glycine-serine polymers (for example GmSo)_n, (GSGG)_n, (GmSoGm)_n, (GmSoGmSoGm)_n, (GSGGS)_m, (GSGSmG)_n and (GGGGSm)_n and combinations thereof, where m, n and o are each independently selected from an integer of at least 1 to 10, e.g. 1-18, 2-16, 3-14, 4-12, 5-10, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10)or other flexible linkers. Glycine and glycine serine polymers are relatively unstructured, and therefore may serve as a neutral tether between components include, but are not limited to GGGSG (SEQ ID NO:139), GGSGG (SEQ ID NO:140), GSGSG (SEQ ID NO:141), GSGGG (SEQ ID NO:142), GGGGSG (SEQ ID NO:143). Additional examples of flexible linkers include glycine polymers (G)_n or glycine-serine polymers (e.g. GS)_n, GSGGS)_n, GGGS)_n andGGGGS)_n wherein n=1-50, for example, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 10-20, 20-30, 30-50 G linker. Exemplary flexible linkers include but are not limited to GGGGS (SEQ ID NO:144), GGGGS (SEQ ID NO:145), GGSG (SEQ ID NO:146), GGSGG (SEQ ID NO:147), GSGSG (SEQ ID NO:148), GSGGG (SEQ ID NO:149), GGGGSG (SEQ ID NO:150) and GSSSG (SEQ ID NO:151). A multimer (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 10-20, 20-30, 30-50) of these linker sequences may be linked together to provide flexible linkers that may be used to conjugate a therlogous amino acid sequence to the

polypeptides disclosed herein or a PEG molecule. Alternative to a polypeptide linker, the linker can be a chemical linker, e.g. a PEG-aldehyde linker.

Acetylated:

[0155] In some embodiments, the IL-2 ortholog is acetylated at the N-terminus by enzymatic reaction with N-terminal acetyltransferase and, for example, acetyl CoA. Alternatively, or in addition to N-terminal acetylation, the IL-2 ortholog can be acetylated at one or more lysine residues, e.g. by enzymatic reaction with a lysine acetyltransferase. See, for example Choudhary et al. (2009) Science 325 (5942):834-840.

Flag Tags

[0156] In some embodiments, the IL-2 ortholog is modified to include an additional polypeptide sequence that functions as an antigenic tag, such as a FLAG sequence. FLAG sequences are recognized by biotinylated, highly specific, anti-FLAG antibodies, as described herein (see e.g., Blancar et al. (1992) Science 256:1014 and LeClair, et al. (1992) PNAS-USA 89:8145). In some embodiments, the IL-2 ortholog polypeptide further comprises a C-terminal c-myc epitope tag.

Albumin Fusions:

[0157] In some embodiments, the IL-2 ortholog is conjugated to albumin referred to herein as an “IL2 ortholog albumin fusion.” The term “albumin” as used in the context hIL2 ortholog albumin fusions include albumins such as human serum albumin (HSA), cyno serum albumin, and bovine serum albumin (BSA). In some embodiments, the HSA the HSA comprises a C34S or K573P amino acid substitution relative to the wild type HSA sequence. According to the present disclosure, albumin can be conjugated to a hIL2 ortholog at the carboxyl terminus, the amino terminus, both the carboxyl and amino termini, and internally (see, e.g., USP 5,876,969 and USP 7,056,701). In the HSA-hIL2 ortholog polypeptide conjugates contemplated by the present disclosure, various forms of albumin can be used, such as albumin secretion pre-sequences and variants thereof, fragments and variants thereof, and HSA variants. Such forms generally possess one or more desired albumin activities. In additional embodiments, the present disclosure involves fusion proteins comprising a hIL2 ortholog polypeptide fused directly or

indirectly to albumin, an albumin fragment, and albumin variant, etc., wherein the fusion protein has a higher plasma stability than the unfused drug molecule and/or the fusion protein retains the therapeutic activity of the unfused drug molecule. In some embodiments, the indirect fusion is effected by a linker such as a peptide linker or modified version thereof as more fully discussed below.

[0158] Alternatively, the hIL2 ortholog albumin fusion comprises hIL2 orthologs that are fusion proteins which comprise an albumin binding domain (ABD) polypeptide sequence and an hIL2 ortholog polypeptide. As alluded to above, fusion proteins which comprise an albumin binding domain (ABD) polypeptide sequence and an hIL2 ortholog polypeptide can, for example, be achieved by genetic manipulation, such that the nucleic acid coding for HSA, or a fragment thereof, is joined to the nucleic acid coding for the one or more hIL2 ortholog sequences. In some embodiments, the albumin-binding peptide comprises the amino acid sequence: DICLPRWGCLW (SEQ ID NO:152).

His Tags

[0159] In some embodiment, the hIL2 orthologs (including fusion proteins of such IL-2 orthologs) of the present invention are expressed as a fusion protein with one or more transition metal chelating polypeptide sequences. The incorporation of such a transition metal chelating domain facilitates purification immobilized metal affinity chromatography (IMAC) as described in Smith, et al. United States Patent No. 4,569,794 issued February 11, 1986. Examples of transition metal chelating polypeptides useful in the practice of the present invention are described in Smith, et al. *supra* and Dobeli, et al. United States Patent No. 5,320,663 issued May 10, 1995, the entire teachings of which are hereby incorporated by reference. Particular transition metal chelating polypeptides useful in the practice of the present invention are peptides comprising 3-6 contiguous histidine residues such as a six-histidine peptide (His)₆ and are frequently referred to in the art as “His-tags.”

[0160] The foregoing fusion proteins may be readily produced by recombinant DNA methodology by techniques known in the art by constructing a recombinant vector comprising a nucleic acid sequence comprising a nucleic acid sequence encoding the hIL2 ortholog in frame with a nucleic acid sequence encoding the fusion partner either at the N-terminus or C-terminus

of the hIL2 ortholog, the sequence optionally further comprising a nucleic acid sequence in frame encoding a linker or spacer polypeptide.

Targeted hIL2 Ortholog Molecules

[0161] In some embodiments, the hIL2 ortholog is provided as a fusion protein with a polypeptide sequence (“targeting domain”) to facilitate selective binding to particular cell type or tissue expressing a cell surface molecule that specifically binds to such targeting domain, optionally incorporating a linker molecule between the hIL2 ortholog sequence and the sequence of the targeting domain of the fusion protein. In one embodiment, the targeting domain of the hIL2 ortholog fusion protein specifically binds to a cell surface molecule of the cell type that is targeted by the CAR-T cell expressing the orthogonal hCD122. For example, in the event that the orthogonal hCD122 CAR-T cell comprises a CAR with an ECD that specifically bind to CD-19, the targeting domain of the hIL2 ortholog fusion protein may also bind to CD-19. Examples of targeting domains would include ligands for cell surface receptors or specific binding molecules antibodies. In one embodiment, the hIL2 ortholog fusion protein comprises a molecule that specifically binds to the same cell type for which the engineered cell expressing the orthogonal ligand (e.g., and hoRb CAR-T cell) is targeted. In one embodiment wherein the ECD of the CAR of an hoRb CAR-T cell specifically binds to CD-19, the IL-2 ortholog may be provided as a fusion protein with a CD-19 targeting moiety. For example, in one embodiment wherein the ECD of the CAR of an hoRb CAR-T cell is an scFv molecule that provides specific binding to CD-19, the IL-2 ortholog is provided as a fusion protein with a CD-19 targeting moiety such as a single chain antibody (e.g., an scFv or VHH) that specifically binds to CD-19. In one embodiment, the fusion protein comprises an IL-10 ortholog and the anti-CD19 scFv FMC63 (Nicholson, et al. (1997) Mol Immunol 34: 1157–1165). Similarly, in some embodiments wherein the ECD of the CAR of an hoRb CAR-T cell specifically binds to BCMA, the IL-2 ortholog is provided as a fusion protein with a BCMA targeting moiety, such as antibody comprising the CDRs of anti-BMCA antibodies as described in Kalled, et al (United States Patent 9,034,324 issued May 9, 2015) or antibodies comprising the CDRs as described in Brogdon, et al (United States Patent No 10,174,095 issued January 8, 2019). In some embodiments, wherein the ECD of the CAR of an hoRb CAR-T cell specifically binds to GD2, the IL-2 ortholog is provided as a fusion protein with a GD2 targeting moiety, such as an

antibody comprising the CDRs of described in Cheung, et al (United States Patent No 9,315,585 issued April 19, 2016) or the CDRs derived from ME36.1 (Thurin et al (1987) Cancer Research 47:1229-1233), 14G2a, 3F8 (Cheung, et al 1985 Cancer Research 45:2642-2649), hu14.18, 8B6, 2E12, or ic9.

[0162] In some embodiments, the targeting moiety of the hIL2 ortholog fusion protein is the same as that provided by the CAR-T cell expressing an orthogonal hCD122 or it may be different, in particular it may be directed to an alternative antigen expressed on the tumor cell type targeted by the CAR. For example, in the context of a orthogonal hCD122 scfv 14G2a GD2 targeted CAR-T cell, the hIL2 ortholog may be provided in a targeted fusion construct comprising specific binding domain of another GD2 tumor antigen.

[0163] In an alternative embodiment, the targeted hIL2 orthologs of the present disclosure may be administered in combination with CAR-T cell therapy to provide targeted delivery of the hIL2 ortholog to the CAR-T cell based on an extracellular receptor of the CAR-T cell such as by and anti-FMC63 antibody to target the hIL2 activity to the CAR-T cells and rejuvenate exhausted CAR-T cells *in vivo*. Consequently, embodiments of the present disclosure include targeted delivery of hIL2 orthologs by conjugation of such hIL2 orthologs to antibodies or ligands that are designed to interact with specific cell surface molecules of CAR-T cells. An example of such a molecule would an anti-FMC63-hIL2 ortholog.

[0164] In other embodiments, the chimeric polypeptide includes the mutant IL-2 polypeptide and a heterologous polypeptide that functions to enhance expression or direct cellular localization of the mutant IL-2 polypeptide, such as the Aga2p agglutinin subunit (see, e.g., Boder and Wittrup, Nature Biotechnol. 15:553-7, 1997).

Protein Transduction Domain Fusion Proteins:

[0165] In some embodiments, the hIL2 ortholog may optionally comprise a “Protein Transduction Domain” or “PTD.” A PTD is a polypeptide, polynucleotide, carbohydrate, or organic or inorganic molecule that facilitates traversing a lipid bilayer, micelle, cell membrane, organelle membrane, or vesicle membrane. The incorporation of a PTD into an hIL2 ortholog facilitates the molecule traversing a membrane. In some embodiments, a PTD is covalently linked to the amino or carboxy terminus of an hIL2 ortholog. In some embodiments, the PTD is

incorporated as part of an PTD-IL2 ortholog fusion protein, either at the N or C terminus of the molecule.

[0166] Exemplary protein transduction domains include, but are not limited to, a minimal decapeptide protein transduction domain (corresponding to residues 47-57 of HIV-1 TAT); a polyarginine sequence comprising a number of arginine residues sufficient to direct entry into a cell (e.g., 3, 4, 5, 6, 7, 8, 9, 10, or 10-50 arginines); a VP22 domain (Zender et al. (2002) *Cancer Gene Ther.* 9(6):489-96); a *Drosophila* Antennapedia protein transduction domain (Noguchi et al. (2003) *Diabetes* 52(7):1732-1737); a truncated human calcitonin peptide (Trehin et al. (2004) *Pharm. Research* 21:1248-1256); polylysine (Wender et al. (2000) *Proc. Natl. Acad. Sci. USA* 97:13003-13008), Transportan (as described in Wierzbicki, et al., (2014) *Folia Histochemica et Cytobiologica* 52(4): 270-280 and Pooga, et al (1998) *FASEB J* 12(1):67-77 and commercially available from AnaSpec as Catalog No. AS-61256); KALA (as described in Wyman et al., (1997) *Biochemistry* 36(10) 3008-3017 and commercially available from AnaSpec as Catalog No. AS-65459); Antennapedia Peptide (as described in Pietersz et al., (2001) *Vaccine* 19:1397 and commercially available from AnaSpec as Catalog No. AS-61032); TAT 47-57 (commercially available from AnaSpec as Catalog No. AS-60023).

Preparation of hIL2 Orthologs

[0167] The hIL2 orthologs may be produced by any conventional method including recombinant or solid phase synthesis.

Solid Phase Chemical Synthesis:

[0168] In addition to generating mutant polypeptides via expression of nucleic acid molecules that have been altered by recombinant molecular biological techniques, subject hIL2 orthologs can be chemically synthesized. Chemically synthesized polypeptides are routinely generated by those of skill in the art. Chemical synthesis includes direct synthesis of a peptide by chemical means of the protein sequence encoding for an hIL2 ortholog exhibiting the properties described.

[0169] In some embodiments, the hIL2 orthologs of the present disclosure may be prepared by chemical synthesis. The chemical synthesis of the hIL2 orthologs of may proceed via liquid-phase or solid-phase. Solid-phase peptide synthesis (SPPS) allows the incorporation of unnatural amino acids and/or peptide/protein backbone modification. Various forms of SPPS are available for synthesizing hIL2 orthologs of the present disclosure are known in the art (e.g., Ganesan A. (2006) *Mini Rev. Med. Chem.* 6:3-10; and Camarero J.A. *et al.*, (2005) *Protein Pept Lett.* 12:723-8). In the course of chemical synthesis, the alpha functions and any reactive side chains may be protected with acid-labile or base-labile groups that are stable under the conditions for linking amide bonds but can readily be cleaved without impairing the peptide chain that has formed.

[0170] In the solid phase synthesis, either the N-terminal or C-terminal amino acid may be coupled to a suitable support material. Suitable support materials are those which are inert towards the reagents and reaction conditions for the stepwise condensation and cleavage reactions of the synthesis process and which do not dissolve in the reaction media being used. Examples of commercially available support materials include styrene/divinylbenzene copolymers which have been modified with reactive groups and/or polyethylene glycol; chloromethylated styrene/divinylbenzene copolymers; hydroxymethylated or aminomethylated styrene/divinylbenzene copolymers; and the like. The successive coupling of the protected amino acids can be carried out according to conventional methods in peptide synthesis, typically in an automated peptide synthesizer.

[0171] At the end of the solid phase synthesis, the peptide is cleaved from the support material while simultaneously cleaving the side chain protecting groups. The peptide obtained can be purified by various chromatographic methods including but not limited to hydrophobic adsorption chromatography, ion exchange chromatography, distribution chromatography, high pressure liquid chromatography (HPLC) and reversed-phase HPLC.

Recombinant Production:

[0172] In some embodiments, the hIL2 ortholog (or fusion proteins comprising the hIL2 ortholog) is produced by recombinant methods. A nucleic acid sequence encoding the desired

hIL2 ortholog polypeptide (optionally comprising a secretion leader sequence or signal peptide) is introduced into an expression vector into the cell to be engineered, nucleic acid sequence being operably linked to one or more expression control sequences encoding by the vector and functional in the target host cell. The recombinant hIL2 ortholog may be recovered through disruption of the host cell or from the cell medium if a secretion leader sequence (signal peptide) is incorporated into the polypeptide. The recombinant hIL2 ortholog may be purified and concentrated for further use including incorporation. The process for the recombinant production of hIL2 polypeptides is known in the art and described in Fernandes and Taforo, United States Patent No. 4,604,377 issued August 5, 1986 and hIL2 orthologs in Mark, et al., United States Patent no 4,512,584 issued May 21, 1985, Gillis, United States Patent No 4,401,756 issued August 30, 1983 the entire teachings of which are herein incorporated by reference..

[0173] DNA encoding an hIL2 ortholog may be obtained from various sources as designed during the engineering process. Amino acid sequence variants of the hIL2 polypeptides to the produce the hIL2 orthologs of the present disclosure are prepared by introducing appropriate nucleotide changes into the coding sequence, as described herein. Such variants represent insertions, substitutions, and/or specified deletions of, residues as noted. Any combination of insertion, substitution, and/or specified deletion is made to arrive at the final construct, provided that the final construct possesses the desired biological activity as defined herein.

Construction of Nucleic Acid Sequences Encoding the hIL2 ortholog

[0174] In some embodiments, the hIL2 ortholog is produced by recombinant methods using a nucleic acid sequence encoding the hIL2 ortholog (or fusion protein comprising the hIL2 ortholog). The nucleic acid sequence encoding the desired hIL2 ortholog can be synthesized by chemical means using an oligonucleotide synthesizer.

[0175] The nucleic acid molecules are not limited to sequences that encode polypeptides; some or all of the non-coding sequences that lie upstream or downstream from a coding sequence (e.g., the coding sequence of hIL2) can also be included. Those of ordinary skill in the art of molecular biology are familiar with routine procedures for isolating nucleic acid molecules. They can, for example, be generated by treatment of genomic DNA with restriction

endonucleases, or by performance of the polymerase chain reaction (PCR). In the event the nucleic acid molecule is a ribonucleic acid (RNA), molecules can be produced, for example, by in vitro transcription.

[0176] The nucleic acid molecules encoding the hIL2 ortholog (and fusions thereof) may contain naturally occurring sequences or sequences that differ from those that occur naturally, but, due to the degeneracy of the genetic code, encode the same polypeptide. These nucleic acid molecules can consist of RNA or DNA (for example, genomic DNA, cDNA, or synthetic DNA, such as that produced by phosphoramidite-based synthesis), or combinations or modifications of the nucleotides within these types of nucleic acids. In addition, the nucleic acid molecules can be double-stranded or single-stranded (i.e., either a sense or an antisense strand).

[0177] Nucleic acid sequences encoding the hIL2 ortholog may be obtained from various commercial sources that provide custom made nucleic acid sequences. Amino acid sequence variants of the hIL2 polypeptides to produce the hIL2 orthologs of the present disclosure are prepared by introducing appropriate nucleotide changes into the coding sequence based on the genetic code which is well known in the art. Such variants represent insertions, substitutions, and/or specified deletions of residues as noted. Any combination of insertion, substitution, and/or specified deletion is made to arrive at the final construct, provided that the final construct possesses the desired biological activity as defined herein.

[0178] Methods for constructing a DNA sequence encoding the hIL2 orthologs and expressing those sequences in a suitably transformed host include, but are not limited to, using a PCR-assisted mutagenesis technique. Mutations that consist of deletions or additions of amino acid residues to an hIL2 polypeptide can also be made with standard recombinant techniques. In the event of a deletion or addition, the nucleic acid molecule encoding hIL2 is optionally digested with an appropriate restriction endonuclease. The resulting fragment can either be expressed directly or manipulated further by, for example, ligating it to a second fragment. The ligation may be facilitated if the two ends of the nucleic acid molecules contain complementary nucleotides that overlap one another, but blunt-ended fragments can also be ligated. PCR-generated nucleic acids can also be used to generate various mutant sequences.

[0179] An hIL2 ortholog of the present disclosure may be produced recombinantly not only directly, but also as a fusion polypeptide with a heterologous polypeptide, *e.g.* a signal sequence or other polypeptide having a specific cleavage site at the N-terminus or C-terminus of the mature hIL2 ortholog. In general, the signal sequence may be a component of the vector, or it may be a part of the coding sequence that is inserted into the vector. The heterologous signal sequence selected preferably is one that is recognized and processed (*i.e.*, cleaved by a signal peptidase) by the host cell. In some embodiments, the signal sequence is the signal sequence that is natively associated with the hIL2 ortholog (*i.e.* the human IL2 signal sequence). The inclusion of a signal sequence depends on whether it is desired to secrete the hIL2 ortholog from the recombinant cells in which it is made. If the chosen cells are prokaryotic, it generally is preferred that the DNA sequence not encode a signal sequence. If the chosen cells are eukaryotic, it generally is preferred that a signal sequence be encoded and most preferably that the wild type IL2 signal sequence be used. Alternatively, heterologous mammalian signal sequences may be suitable, such as signal sequences from secreted polypeptides of the same or related species, as well as viral secretory leaders, for example, the herpes simplex gD signal. When the recombinant host cell is a yeast cell such as *Saccharomyces cerevisiae*, the alpha mating factor secretion signal sequence may be employed to achieve extracellular secretion of the IL2 ortholog into the culture medium as described in Singh, United States Patent No. 7,198,919 B1 issued April 3, 2007.

Codon Optimization:

[0180] In some embodiments, the nucleic acid sequence encoding the recombinant protein (IL2 ortholog, orthogonal hCD122, or CAR) may be “codon optimized” to facilitate expression in a particular host cell type. Techniques for codon optimization in a wide variety of expression systems, including mammalian, yeast and bacterial host cells, are well known in the art and there are online tools to provide for a codon optimized sequences for expression in a variety of host cell types. See *e.g.* Hawash, et al., (2017) 9:46-53 and Mauro and Chappell in Recombinant Protein Expression in Mammalian Cells: Methods and Protocols, edited by David Hacker (Human Press New York). Additionally, there are a variety of web based on-line software packages that are freely available to assist in the preparation of codon optimized nucleic acid sequences.

Expression Vectors:

[0181] Once assembled (by synthesis, site-directed mutagenesis or another method), the nucleic acid sequence encoding an hIL2 ortholog is inserted into an expression vector. A variety of expression vectors for uses in various host cells are available and are typically selected based on the host cell for expression. The vector components generally include, but are not limited to, one or more of the following: an origin of replication, one or more marker genes, an enhancer element, a promoter, and a transcription termination sequence. Vectors include viral vectors, plasmid vectors, integrating vectors, and the like. Plasmids are examples of non-viral vectors. In order to facilitate transfection of the target cells, the target cell may be exposed directly with the non-viral vector may under conditions that facilitate uptake of the non-viral vector. Examples of conditions which facilitate uptake of foreign nucleic acid by mammalian cells are well known in the art and include but are not limited to chemical means (such as Lipofectamine®, Thermo-Fisher Scientific), high salt, and magnetic fields (electroporation).

[0182] An hIL2 ortholog may be produced recombinantly not only directly, but also as a fusion polypeptide with a heterologous polypeptide, e.g. a signal sequence or other polypeptide having a specific cleavage site at the N-terminus of the mature protein or polypeptide. In general, the signal sequence may be a component of the vector, or it may be a part of the coding sequence that is inserted into the vector. The heterologous signal sequence selected preferably is one that is recognized and processed (i.e., cleaved by a signal peptidase) by the host cell. In mammalian cell expression the native signal sequence may be used, or other mammalian signal sequences may be suitable, such as signal sequences from secreted polypeptides of the same or related species, as well as viral secretory leaders, for example, the herpes simplex gD signal.

Selectable Marker

[0183] Expression vectors usually contain a selection gene, also termed a selectable marker. This gene encodes a protein necessary for the survival or growth of transformed host cells grown in a selective culture medium. Host cells not transformed with the vector containing the selection gene will not survive in the culture medium. Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxins, e.g., ampicillin, neomycin, methotrexate,

or tetracycline, (b) complement auxotrophic deficiencies, or (c) supply critical nutrients not available from complex media.

Regulatory Control Sequences:

[0184] Expression vectors for hIL2 orthologs of the present disclosure contain a regulatory sequence that is recognized by the host organism and is operably linked to nucleic acid sequence encoding the hIL2 ortholog. The terms “regulatory control sequence,” “regulatory sequence” or “expression control sequence” are used interchangeably herein to refer to promoters, enhancers, and other expression control elements (e.g., polyadenylation signals). See, for example, Goeddel (1990) in *Gene Expression Technology: Methods in Enzymology* 185 (Academic Press, San Diego CA USA). Regulatory sequences include those that directly constitute expression of a nucleotide sequence in many types of host cells and those that direct expression of the nucleotide sequence only in certain host cells (e.g., tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, and the like. In selecting an expression control sequence, a variety of factors understood by one of skill in the art are to be considered. These include, for example, the relative strength of the sequence, its controllability, and its compatibility with the actual DNA sequence encoding the subject hIL2 ortholog, particularly as regards potential secondary structures.

Promoters

[0185] In some embodiments, the regulatory sequence is a promoter, which is selected based on, for example, the cell type in which expression is sought. Expression vectors will contain a promoter that is recognized by the host organism and is operably linked to an orthogonal protein coding sequence. Promoters are untranslated sequences located upstream (5') to the start codon of a structural gene (generally within about 100 to 1000 bp) that control the transcription and translation of particular nucleic acid sequence to which they are operably linked. Such promoters typically fall into two classes, inducible and constitutive. Inducible promoters are promoters that initiate increased levels of transcription from DNA under their control in response to some change in culture conditions, e.g., the presence or absence of a nutrient or a change in temperature. A large number of promoters recognized by a variety of potential host cells are

well known. For example the T7 promoter can be used in bacteria, a polyhedrin promoter can be used in insect cells, and a cytomegalovirus or metallothionein promoter can be used in mammalian cells. Also, in the case of higher eukaryotes, tissue-specific and cell type-specific promoters are widely available. These promoters are so named for their ability to direct expression of a nucleic acid molecule in a given tissue or cell type within the body. Skilled artisans are well aware of numerous promoters and other regulatory elements which can be used to direct expression of nucleic acids.

[0186] Transcription from vectors in mammalian host cells may be controlled, for example, by promoters obtained from the genomes of viruses such as polyoma virus, fowlpox virus, adenovirus (such as human adenovirus serotype 5), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus (such as murine stem cell virus), hepatitis-B virus and most preferably Simian Virus 40 (SV40), from heterologous mammalian promoters, e.g., the actin promoter, PGK (phosphoglycerate kinase), or an immunoglobulin promoter, from heat-shock promoters, provided such promoters are compatible with the host cell systems. The early and late promoters of the SV40 virus are conveniently obtained as an SV40 restriction fragment that also contains the SV40 viral origin of replication.

Enhancers

[0187] Transcription by higher eukaryotes is often increased by inserting an enhancer sequence into the vector and operably linked to the nucleic acid sequence encoding the hIL2 ortholog. Enhancers are cis-acting elements of DNA, usually about from 10 to 300 bp, which act on a promoter to increase its transcription. Enhancers are relatively orientation and position independent, having been found 5' and 3' to the transcription unit, within an intron, as well as within the coding sequence itself. Many enhancer sequences are now known from mammalian genes (globin, elastase, albumin, alpha-fetoprotein, and insulin). Typically, however, one will use an enhancer from a eukaryotic cell virus. Examples include the SV40 enhancer on the late side of the replication origin, the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers. The enhancer may be spliced into the expression vector at a position 5' or 3' to the coding sequence but is preferably located at a site 5' from the promoter. Expression vectors used in eukaryotic host cells will also

contain sequences necessary for the termination of transcription and for stabilizing the mRNA. Such sequences are commonly available from the 5' and, occasionally 3', untranslated regions of eukaryotic or viral DNAs or cDNAs. Construction of suitable vectors containing one or more of the above-listed components employs standard techniques.

[0188] In addition to sequences that facilitate transcription of the inserted nucleic acid molecule, vectors can contain origins of replication, and other genes that encode a selectable marker. For example, the neomycin-resistance (neoR) gene imparts G418 resistance to cells in which it is expressed, and thus permits phenotypic selection of the transfected cells. Additional examples of marker or reporter genes include beta-lactamase, chloramphenicol acetyltransferase (CAT), adenosine deaminase (ADA), dihydrofolate reductase (DHFR), hygromycin-B-phosphotransferase (HPH), thymidine kinase (TK), lacZ (encoding beta-galactosidase), and xanthine guanine phosphoribosyltransferase (XGPRT). Those of skill in the art can readily determine whether a given regulatory element or selectable marker is suitable for use in a particular experimental context.

[0189] Proper assembly of the expression vector can be confirmed by nucleotide sequencing, restriction mapping, and expression of a biologically active polypeptide in a suitable host.

Host Cells for Production of hIL2 Orthologs

[0190] In one embodiment, the present disclosure further a recombinant cell comprising a nucleic acid sequence encoding an hIL2 ortholog. The cell may be a prokaryotic or eukaryotic. A cell of the present disclosure is a transfected cell, i.e., a cell into which a nucleic acid molecule, for example a nucleic acid molecule encoding a mutant hIL2 polypeptide, has been introduced by means of recombinant DNA techniques. The progeny of such a cell are also considered within the scope of the present disclosure.

[0191] Host cells for expression of the hIL2 ortholog are typically selected in accordance with their compatibility with the chosen expression vector, the toxicity of the product coded for by the DNA sequences of this invention, their secretion characteristics, their ability to fold the polypeptides correctly, their fermentation or culture requirements, and the ease of purification of the products coded for by the DNA sequences. Suitable host cells for cloning or expressing the DNA in the vectors herein are the prokaryote, yeast, or higher eukaryote cells.

[0192] In some embodiments the host cell for recombinant production of the hIL2 orthologs are eukaryotic cells, such as yeast or human cells. Suitable eukaryotic host cells include insect cells (examples of Baculovirus vectors available for expression of proteins in cultured insect cells (e.g., Sf9 cells) include the pAc series (Smith et al. (1983) *Mol. Cell Biol.* 3:2156-2165) and the pVL series (Lucklow and Summers (1989) *Virology* 170:31-39)); yeast cells (examples of vectors for expression in yeast *S. cerevisiae* include pYepSec1 (Baldari et al. (1987) *EMBO J.* 6:229-234), pMFa (Kurjan and Herskowitz (1982) *Cell* 30:933-943), pJRY88 (Schultz et al. (1987) *Gene* 54:113-123), pYES2 (Invitrogen Corporation, San Diego, Calif.), and pPicZ (Invitrogen Corporation, San Diego, Calif.)); or mammalian cells (mammalian expression vectors include pCDM8 (Seed (1987) *Nature* 329:840) and pMT2PC (Kaufman et al. (1987) *EMBO J.* 6:187:195)).

[0193] Examples of useful mammalian host cell lines are mouse L cells (L-M[TK-], ATCC#CRL-2648), monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture; baby hamster kidney cells (BHK, ATCC CCL 10); Chinese hamster ovary cells/-DHFR (CHO); mouse sertoli cells (TM4); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1 587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TRI cells; MRC 5 cells; FS4 cells; and a human hepatoma line (Hep G2).

[0194] In some embodiments, hIL2 orthologs obtained will be glycosylated or unglycosylated depending on the host organism used to produce the mutein. If bacteria are chosen as the host then the hIL2 ortholog produced will be unglycosylated. Eukaryotic cells, on the other hand, may glycosylate the hIL2 orthologs and perhaps not in the same way as native-IL2 is glycosylated.

[0195] For other additional expression systems for both prokaryotic and eukaryotic cells, see Chapters 16 and 17 of Sambrook et al. (1989) *Molecular Cloning: A Laboratory Manual* (2nd

ed., Cold Spring Harbor Laboratory Press, Plainview, N.Y.). See, Goeddel (1990) in Gene Expression Technology: Methods in Enzymology 185 (Academic Press, San Diego, Calif.).

Transfection:

[0196] The nucleic acid expression constructs encoding the hIL2 ortholog is introduced into host cells to thereby produce the hIL2 orthologs disclosed herein or to produce biologically active muteins thereof. Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. Suitable methods for transforming or transfecting host cells can be found in Sambrook et al. (1989) Molecular Cloning: A Laboratory Manual (2d ed., Cold Spring Harbor Laboratory Press, Plainview, N.Y.) and other standard molecular biology laboratory manuals.

[0197] In order to facilitate transfection of the target cells, the target cell may be exposed directly with the non-viral vector may under conditions that facilitate uptake of the non-viral vector. Examples of conditions which facilitate uptake of foreign nucleic acid by mammalian cells are well known in the art and include but are not limited to chemical means (such as Lipofectamine®, Thermo-Fisher Scientific), high salt, and magnetic fields (electroporation).

Cell Culture:

[0198] Cells may be cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences. Mammalian host cells may be cultured in a variety of media. Commercially available media such as Ham's F10 (Sigma), Minimal Essential Medium ((MEM), Sigma), RPMI 1640 (Sigma), and Dulbecco's Modified Eagle's Medium ((DMEM), Sigma) are suitable for culturing the host cells. Any of these media may be supplemented as necessary with hormones and/or other growth factors (such as insulin, transferrin, or epidermal growth factor), salts (such as sodium chloride, calcium, magnesium, and phosphate), buffers (such as HEPES), nucleosides (such as adenosine and thymidine), antibiotics, trace elements, and glucose or an equivalent energy source. Any other necessary supplements may also be included at appropriate concentrations that would be known to those skilled in the art. The culture conditions, such as temperature, pH and the like, are those previously used with the host cell selected for expression and will be apparent to the ordinarily skilled artisan.

Recovery of Recombinant Proteins:

[0199] Recombinantly produced hIL2 ortholog polypeptides can be recovered from the culture medium as a secreted polypeptide if a secretion leader sequence is employed. Alternatively, the hIL2 ortholog polypeptides can also be recovered from host cell lysates. A protease inhibitor, such as phenyl methyl sulfonyl fluoride (PMSF) may be employed during the recovery phase from cell lysates to inhibit proteolytic degradation during purification, and antibiotics may be included to prevent the growth of adventitious contaminants. In some embodiments, the hIL2 ortholog is produced in *E. coli* in which the overexpression of the hIL2 ortholog which is sequestered in inclusion bodies. Techniques for the isolation and solubilization of inclusion bodies and recovery of active proteins are well known in the art.

[0200] Various purification steps are known in the art and find use, e.g. affinity chromatography. Affinity chromatography makes use of the highly specific binding sites usually present in biological macromolecules, separating molecules on their ability to bind a particular ligand. Covalent bonds attach the ligand to an insoluble, porous support medium in a manner that overtly presents the ligand to the protein sample, thereby using natural specific binding of one molecular species to separate and purify a second species from a mixture. Antibodies are commonly used in affinity chromatography. Size selection steps may also be used, e.g. gel filtration chromatography (also known as size-exclusion chromatography or molecular sieve chromatography) is used to separate proteins according to their size. In gel filtration, a protein solution is passed through a column that is packed with semipermeable porous resin. The semipermeable resin has a range of pore sizes that determines the size of proteins that can be separated with the column.

[0201] The orthogonal hIL2 ortholog may be concentrated, filtered, dialyzed, *etc.*, using methods known in the art. For therapeutic applications, the hIL2 ortholog can be administered to a mammal comprising the appropriate engineered orthogonal receptor. Administration may be intravenous, as a bolus or by continuous infusion over a period of time. Alternative routes of administration include intramuscular, intraperitoneal, intra-cerebrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, oral, topical, or inhalation routes. The orthogonal hIL2

orthologs also are suitably administered by intratumoral, peritumoral, intralesional, or perilesional routes or to the lymph, to exert local as well as systemic therapeutic effects.

Routes of Administration of hIL2 Orthologs:

[0202] In embodiments of the therapeutic methods of the present disclosure involve the administration of a pharmaceutical formulation comprising an hIL2 ortholog (and/or nucleic acids encoding the hIL2 ortholog) to a subject in need of treatment. Administration to the subject may be achieved by intravenous, as a bolus or by continuous infusion over a period of time. Alternative routes of administration include intramuscular, intraperitoneal, intracerebrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, oral, topical, or inhalation routes. The hIL2 orthologs also are suitably administered by intratumoral, peritumoral, intralesional, intranodal or perilesional routes or to the lymph, to exert local as well as systemic therapeutic effects.

[0203] In some embodiments, subject hIL2 orthologs (and/or nucleic acids encoding the hIL2 ortholog) can be incorporated into compositions, including pharmaceutical compositions. Such compositions typically include the polypeptide or nucleic acid molecule and a pharmaceutically acceptable carrier. A pharmaceutical composition is formulated to be compatible with its intended route of administration and is compatible with the therapeutic use for which the hIL2 ortholog is to be administered to the subject in need of treatment or prophylaxis.

Formulations of hIL2 Orthologs

[0204] In some embodiments, the present disclosure provides a pharmaceutically acceptable formulation of a hIL2 ortholog. The preferred formulation depends on the intended mode of administration and therapeutic application. Pharmaceutically acceptable formulations of hIL2 orthologs comprise physiologically acceptable carriers that are inherently non-toxic and non-therapeutic. Examples of such carriers include ion exchangers, alumina, aluminum stearate, lecithin, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts, or electrolytes such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium

trisilicate, polyvinyl pyrrolidone, cellulose-based substances, and PEG. Carriers for topical or gel-based forms of polypeptides include polysaccharides such as sodium carboxymethylcellulose or methylcellulose, polyvinylpyrrolidone, polyacrylates, polyoxyethylene-polyoxypropylene-block polymers, PEG, polymeric amino acids, amino acid copolymers, and lipid aggregates (such as oil droplets or liposomes).

[0205] The formulation may also comprise pharmaceutically-acceptable, non-toxic carriers, excipients, stabilizers or diluents, which are defined as vehicles commonly used to formulate pharmaceutical compositions for animal or human administration. The diluent is selected so as not to affect the biological activity of the combination. Acceptable carriers, excipients, or stabilizers are non-toxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or 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 TWEENTM, PLURONICSTM or polyethylene glycol (PEG). Formulations to be used for in vivo administration are typically sterile. Sterilization of the compositions of the present invention may readily accomplished by filtration through sterile filtration membranes.

[0206] Typically, formulations are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection can also be prepared. The preparation also can be emulsified or encapsulated in liposomes or micro particles such as polylactide, polyglycolide, or copolymer for enhanced adjuvant effect, as discussed above. Langer, Science (1990) 249: 1527 and Hanes, Advanced Drug Delivery Reviews (1997) 28: 97-119. The agents of this invention can be administered in

the form of a depot injection or implant preparation which can be formulated in such a manner as to permit a sustained or pulsatile release of the active ingredient. The pharmaceutical compositions are generally formulated as sterile, substantially isotonic and in full compliance with all Good Manufacturing Practice (GMP) regulations of the U.S. Food and Drug Administration.

[0207] In some embodiments, the methods of the present disclosure involve the parenteral administration of a hIL2 ortholog. Examples of parenteral routes of administration include, for example, intravenous, intradermal, subcutaneous, transdermal (topical), transmucosal, and rectal administration. Parenteral formulations comprise solutions or suspensions used for parenteral application can include vehicles the carriers and buffers. Pharmaceutical formulations for parenteral administration include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic. In one embodiment, the formulation is provided in a prefilled syringe for parenteral administration

[0208] Oral formulations, if used, may include one or more an inert diluents and/or edible carriers. For the purpose of oral therapeutic administration, the a hIL2 ortholog may be incorporated with excipients and used in the form of tablets, troches, or capsules, e.g., gelatin capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the formulation. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel™, or corn starch; a lubricant such as magnesium stearate or Sterotes™; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

[0209] In the event of administration by inhalation, subject hIL2 orthologs, or the nucleic acids encoding them, are delivered in the form of an aerosol spray from pressured container or

dispenser which contains a suitable propellant, e.g., a gas such as carbon dioxide, or a nebulizer. Such methods include those described in U.S. Pat. No. 6,468,798.

[0210] Systemic administration of the subject hIL2 orthologs or nucleic acids can also be by transmucosal or transdermal formulations. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories (e.g., with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art and may incorporate permeation enhancers such as ethanol or lanolin.

[0211] In some embodiments, the hIL2 ortholog the formulation is an extended release formulation to provide extended delivery of the hIL2 ortholog agent over a period of hours or days. Examples of extended release formulations of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin. In one embodiment, the subject hIL2 orthologs or nucleic acids are prepared with carriers that will protect the mutant IL-2 polypeptides against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Such formulations can be prepared using standard techniques. Liposomal suspensions can also be used as pharmaceutically acceptable carriers.

Engineered hoCD122 Cells:

[0212] The preparation of recombinant cells useful in the practice of the present invention is achieved by transforming isolated with an expression vector comprising a nucleic acid sequence encoding an hCD122 orthogonal receptor. The hIL2 orthologs of the present invention may be employed in methods of selectively expanding such engineered hoRb cells (e.g., human T-cells) which have been engineered to express a corresponding orthogonal hCD122 receptor. T-cells

useful for engineering with the constructs described herein include naïve T-cells, central memory T-cells, effector memory T-cells or combination thereof. T cells for engineering as described above are collected from a subject or a donor may be separated from a mixture of cells by techniques that enrich for desired cells or may be engineered and cultured without separation. Alternatively, the T cells for engineering may be separated from other cells. Techniques providing accurate separation include fluorescence activated cell sorters. The cells may be selected against dead cells by employing dyes associated with dead cells (e.g., propidium iodide). The separated cells may be collected in any appropriate medium that maintains the viability of the cells, usually having a cushion of serum at the bottom of the collection tube. Various media are commercially available and may be used according to the nature of the cells, including dMEM, HBSS, dPBS, RPMI, Iscove's medium, etc., frequently supplemented with fetal calf serum (FCS). The collected and optionally enriched cell population may be used immediately for genetic modification or may be frozen at liquid nitrogen temperatures and stored, being thawed and capable of being reused. The cells will usually be stored in 10% DMSO, 50% FCS, 40% RPMI 1640 medium.

[0213] In some embodiments, the engineered cells comprise a complex mixture of immune cells, e.g., tumor infiltrating lymphocytes (TILs) isolated from an individual in need of treatment. See, for example, Yang and Rosenberg (2016) *Adv Immunol.* 130:279-94, "Adoptive T Cell Therapy for Cancer; Feldman et al (2015) *Seminars in Oncol.* 42(4):626-39 "Adoptive Cell Therapy-Tumor-Infiltrating Lymphocytes, T-Cell Receptors, and Chimeric Antigen Receptors"; Clinical Trial NCT01174121, "Immunotherapy Using Tumor Infiltrating Lymphocytes for Patients With Metastatic Cancer"; Tran et al. (2014) *Science* 344(6184):641-645, "Cancer immunotherapy based on mutation-specific CD4⁺ T cells in a patient with epithelial cancer".

CAR-T Cells

[0214] In one embodiment of the invention the hoRb cell is a T-cell (e.g., human T-cell) which has been modified to surface express a chimeric antigen receptor (a "hoCAR-T cell"). As used herein, the term antigen binding domain (ABD) refers to a polypeptide that specifically binds to an antigen expressed on the surface of a target cell. The ABD may be any polypeptide that specifically binds to one or more antigens expressed on the surface of a target cell. CARs

further comprise a transmembrane domain joining the ABD (or linker, if employed) to the intracellular cytoplasmic domain of the CAR. The transmembrane domain is comprised of any polypeptide sequence which is thermodynamically stable in a eukaryotic cell membrane. The transmembrane spanning domain may be derived from the transmembrane domain of a naturally occurring membrane spanning protein or may be synthetic. In designing synthetic transmembrane domains, amino acids favoring alpha-helical structures are preferred.

Transmembrane domains useful in construction of CARs are comprised of approximately 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 22, 23, or 24 amino acids favoring the formation having an alpha-helical secondary structure. Amino acids having a to favor alpha-helical conformations are well known in the art. See, *e.g.* Pace, *et al.* (1998) Biophysical Journal 75: 422-427. Amino acids that are particularly favored in alpha helical conformations include methionine, alanine, leucine, glutamate, and lysine. In some embodiments, the CAR transmembrane domain may be derived from the transmembrane domain from type I membrane spanning proteins, such as CD3 ζ , CD4, CD8, CD28, *etc.*

[0215] The cytoplasmic domain (ICD) of the CAR polypeptide comprises one or more intracellular signal domains. In one embodiment, the intracellular signal domains comprise the cytoplasmic sequences of the T-cell receptor (TCR) and co-receptors that initiate signal transduction following antigen receptor engagement and functional derivatives and sub-fragments thereof. A cytoplasmic signaling domain, such as those derived from the T cell receptor zeta-chain, is employed as part of the CAR in order to produce stimulatory signals for T lymphocyte proliferation and effector function following engagement of the chimeric receptor with the target antigen. Examples of cytoplasmic signaling domains include but are not limited to the cytoplasmic domain of CD27, the cytoplasmic domain S of CD28, the cytoplasmic domain of CD137 (also referred to as 4-1BB and TNFRSF9), the cytoplasmic domain of CD278 (also referred to as ICOS), p110 α , β , or δ catalytic subunit of PI3 kinase, the human CD3 ζ - chain, cytoplasmic domain of CD134 (also referred to as OX40 and TNFRSF4), Fc ϵ R1 γ and β chains, MB1 (Ig α) chain, B29 (Ig β) chain, *etc.*), CD3 polypeptides (δ , Δ and ϵ), syk family tyrosine kinases (Syk, ZAP 70, *etc.*), src family tyrosine kinases (Lck, Fyn, Lyn, *etc.*) and other molecules involved in T-cell transduction, such as CD2, CD5 and CD28.

[0216] In some embodiments, the CAR may also provide a co-stimulatory domain. The term “co-stimulatory domain”, refers to a stimulatory domain, typically an endodomain, of a CAR that provides a secondary non-specific activation mechanism through which a primary specific stimulation is propagated. The co-stimulatory domain refers to the portion of the CAR which enhances the proliferation, survival or development of memory cells. Examples of co-stimulation include antigen nonspecific T cell co-stimulation following antigen specific signaling through the T cell receptor and antigen nonspecific B cell co-stimulation following signaling through the B cell receptor. Co-stimulation, e.g., T cell co-stimulation, and the factors involved have been described in Chen & Flies. (2013) Nat Rev Immunol 13(4):227-42. In some embodiments of the present disclosure, the CSD comprises one or more of members of the TNFR superfamily, CD28, CD137 (4-1BB), CD134 (OX40), Dap10, CD27, CD2, CD5, ICAM-1, LFA-1 (CD11a/CD18), Lck, TNFR-I, TNFR-II, Fas, CD30, CD40 or combinations thereof. CARs are often referred to as first, second, third or fourth generation. The term first-generation CAR refers to a CAR wherein the cytoplasmic domain transmits the signal from antigen binding through only a single signaling domain, for example a signaling domain derived from the high-affinity receptor for IgE FcεR1γ or the CD3ζ chain. The domain contains one or three immunoreceptor tyrosine-based activating motif(s) [ITAM(s)] for antigen-dependent T-cell activation. The ITAM-based activating signal endows T-cells with the ability to lyse the target tumor cells and secrete cytokines in response to antigen binding. Second-generation CARs include a co-stimulatory signal in addition to the CD3 ζ signal. Coincidental delivery of the delivered co-stimulatory signal enhances cytokine secretion and antitumor activity induced by CAR-transduced T-cells. The co-stimulatory domain is usually be membrane proximal relative to the CD3ζ domain. Third-generation CARs include a tripartite signaling domain, comprising for example a CD28, CD3ζ, OX40 or 4-1BB signaling region. In fourth generation, or “armored car” CAR T-cells are further modified to express or block molecules and/or receptors to enhance immune activity such as the expression of IL-12, IL-18, IL-7, and/or IL-10; 4-1BB ligand, CD-40 ligand.

[0217] Examples of intracellular signaling domains comprising may be incorporated into the hoCAR-T cell useful in the practice of the present invention include (amino to carboxy): CD3ζ;

CD28 – 41BB - CD3 ζ ; CD28 – OX40 – CD3 ζ ; CD28 – 41BB – CD3 ζ ; 41BB – CD-28 -- CD3 ζ and 41BB – CD3 ζ .

[0218] The term CAR includes CAR variants including but not limited split CARs, ON-switch CARs, bispecific or tandem CARs, inhibitory CARs (iCARs) and induced pluripotent stem (iPS) CAR-T cells.

[0219] The term “Split CARs” refers to CARs wherein the extracellular portion, the ABD and the cytoplasmic signaling domain of a CAR are present on two separate molecules. CAR variants also include ON-switch CARs which are conditionally activatable CARs, e.g., comprising a split CAR wherein conditional hetero-dimerization of the two portions of the split CAR is pharmacologically controlled. CAR molecules and derivatives thereof (i.e., CAR variants) are described, e.g., in PCT Application Nos. US2014/016527, US1996/017060, US2013/063083; Fedorov et al. *Sci Transl Med* (2013) ;5(215):215ra172; Glienke et al. *Front Pharmacol* (2015) 6:21; Kakarla & Gottschalk 52 *Cancer J* (2014) 20(2):151-5; Riddell et al. *Cancer J* (2014) 20(2):141-4; Pegram et al. *Cancer J* (2014) 20(2):127-33; Cheadle et al. *Immunol Rev* (2014) 257(1):91-106; Barrett et al. *Annu Rev Med* (2014) 65:333-47; Sadelain et al. *Cancer Discov* (2013) 3(4):388-98; Cartellieri et al., *J Biomed Biotechnol* (2010) 956304; the disclosures of which are incorporated herein by reference in their entirety.

[0220] The term “bispecific or tandem CARs” refers to CARs which include a secondary CAR binding domain that can either amplify or inhibit the activity of a primary CAR.

[0221] The term “inhibitory chimeric antigen receptors” or “iCARs” are used interchangeably herein to refer to a CAR where binding iCARs use the dual antigen targeting to shut down the activation of an active CAR through the engagement of a second suppressive receptor equipped with inhibitory signaling domains of a secondary CAR binding domain results in inhibition of primary CAR activation. Inhibitory CARs (iCARs) are designed to regulate CAR-T cells activity through inhibitory receptors signaling modules activation. This approach combines the activity of two CARs, one of which generates dominant negative signals limiting the responses of CAR-T cells activated by the activating receptor. iCARs can switch off the response of the counteracting activator CAR when bound to a specific antigen expressed only by normal tissues. In this way, iCARs-T cells can distinguish cancer cells from healthy ones, and reversibly block functionalities of transduced T cells in an antigen-selective fashion. CTLA-4 or

PD-1 intracellular domains in iCARs trigger inhibitory signals on T lymphocytes, leading to less cytokine production, less efficient target cell lysis, and altered lymphocyte motility.

The term “tandem CAR” or “TanCAR” refers to CARs which mediate bispecific activation of T cells through the engagement of two chimeric receptors designed to deliver stimulatory or costimulatory signals in response to an independent engagement of two different tumor associated antigens.

[0222] Typically, the chimeric antigen receptor T-cells (CAR-T cells) are T-cells which have been recombinantly modified by transduction with an expression vector encoding a CAR in substantial accordance with the teaching above.

[0223] In some embodiments, hoCAR-T cell is allogeneic with respect to the individual that is treated. Graham et al. (2018) Cell 7(10) E155. In some embodiments an allogeneic engineered T cell is fully HLA matched. However not all patients have a fully matched donor and a cellular product suitable for all patients independent of HLA type provides an alternative.

[0224] Because the hoCAR-T cell be derived from a subject's own T-cells, the population of the cells to be administered to the subject is necessarily variable, the response to such agents can vary and thus involves the ongoing monitoring and management of therapy related toxicities which are managed with a course of pharmacologic immunosuppression or B cell depletion prior to the administration of the hoCAR-T cell product. Usually, at least 1×10^6 hoCAR-T cell/kg will be administered, at least 1×10^7 hoCAR-T cell/kg, at least 1×10^8 hoCAR-T cells/kg, at least 1×10^9 hoCAR-T cell/kg, at least 1×10^{10} hoCAR-T cells/kg, or more, usually being limited by the number of T cells that are obtained during collection. The engineered hoCAR-T cells may be infused to the subject in any physiologically acceptable medium by any convenient route of administration, normally intravascularly, although they may also be introduced by other routes, where the cells may find an appropriate site for growth

[0225] If the hoCAR-T cells are allogeneic T cells, such cells may be modified to reduce graft versus host disease. For example, the engineered cells of the present invention may be TCR $\alpha\beta$ receptor knock-outs achieved by gene editing techniques. TCR $\alpha\beta$ is a heterodimer and both alpha and beta chains need to be present for it to be expressed. A single gene codes for the alpha chain (TRAC), whereas there are 2 genes coding for the beta chain, therefore TRAC loci KO has been deleted for this purpose. A number of different approaches have been used to

accomplish this deletion, e.g. CRISPR/Cas9; meganuclease; engineered I-CreI homing endonuclease, etc. See, for example, Eyquem et al. (2017) *Nature* 543:113–117, in which the TRAC coding sequence is replaced by a CAR coding sequence; and Georgiadis et al. (2018) *Mol. Ther.* 26:1215–1227, which linked CAR expression with TRAC disruption by clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 without directly incorporating the CAR into the TRAC loci. An alternative strategy to prevent GVHD modifies T cells to express an inhibitor of TCR $\alpha\beta$ signaling, for example using a truncated form of CD3 ζ as a TCR inhibitory molecule.

[0226] In one embodiment, the present disclosure provides a method of selectively expanding a population of engineered cells expressing an orthogonal hCD122 receptor from a mixed cell population, the method comprising contacting the mixed cell population with an hIL2 ortholog of the present disclosure under conditions that facilitate the proliferation of the engineered cell. In one embodiment when the orthogonal hCD122 receptor expressing CAR-T cell, the orthogonal receptor expressing CAR-T cells may also be selectively expanded from the background or mixed population of transduced and non-transduced cells through the use of the hIL2 orthologs described herein. Expansion of the T cells for therapeutic applications typically involves culturing the cells in contact with a surface providing an agent that stimulates a CD3 TCR complex associated signal and an agent that stimulates a co-stimulatory molecule on the surface of the T-cell. In conventional practice, engineered T-cells are stimulated prior to administration of the cell therapy product by contacting with CD3/D28, particularly in the preparation of CAR-T cells for use in clinical applications. A wide variety of commercially available products are available to facilitate bead-based activation of T-cells including but not limited to the Invitrogen® CTS Dynabeads® CD3/28 (Life Technologies, Inc. Carlsbad CA) or Miltenyi MACS® GMP ExpAct Treg beads or Miltenyi MACS GMP TransAct™ CD3/28 beads (Miltenyi Biotec, Inc.). Conditions appropriate for T-cell culture are well known in the art. Lin, et al. (2009) *Cytotherapy* 11(7):912-922; Smith, et al. (2015) *Clinical & Translational Immunology* 4:e31 published online 16 January 2015. The target cells are maintained under conditions necessary to support growth, for example, an appropriate temperature (e.g., 37°C) and atmosphere (e.g., air plus 5% CO₂). Wherein the mixed cell population containing engineered T cells expressing the hCD122 orthogonal receptor is cultured in the presence of a concentration of

the hIL2 ortholog. In some embodiments for at least 2 hours, alternatively at least 3 hours, alternatively at least 4 hours, alternatively at least 6 hours, alternatively at least 8 hours, alternatively at least 12 hours, alternatively at least 24 hours, alternatively at least 48 hours, alternatively at least 72 hours, or more. The concentration of the hIL2 ortholog in such ex vivo situations is sufficient to induce cellular proliferation in the cell population. T cell proliferation can be readily assessed by microscopic methods and the determination of the optimal concentration of the hIL2 ortholog will depend upon the relative activity of the hIL2 ortholog for the orthogonal hCD122 receptor.

In one embodiment, the present disclosure provides for a method making an engineered cell product substantially enriched for engineered hoCD122 expressing cells, the method comprising the steps of: (a) obtaining a biological sample comprising T-cells; (b) contacting said biological sample with a recombinant vector encoding an hoCD122 receptor; (c) contacting the biological sample an hIL2 ortholog for a period of time sufficient to expand the engineered hoCD122 expressing cells. The duration of contact and culture may be modified depending on the degree to which the population is to be enriched.

[0227] Where the cells are to be contacted with the hIL2 ortholog in vitro, the hIL2 ortholog is added to the engineered cells in a dose and for a period of time sufficient to activate signaling from the hoCD122 receptor, which may utilize the native cellular machinery, e.g. accessory proteins, co-receptors, and the like. Any suitable culture medium may be used. The cells thus activated may be used for any desired purpose, including experimental purposes relating to determination of antigen specificity, cytokine profiling, and the like, and for delivery in vivo.

[0228] Where the contacting is performed *in vivo*, an effective dose of engineered cells, including without limitation CAR-T cells modified to express an orthogonal hoCD122 receptor, are infused to the recipient, in combination with or prior to administration of the orthogonal cytokine, e.g. IL-2 and allowed to contact T cells in their native environment, e.g. in lymph nodes, *etc.* Dosage and frequency may vary depending on the agent; mode of administration; nature of the hIL2 ortholog, and the like. It will be understood by one of skill in the art that such guidelines will be adjusted for the individual circumstances. The dosage may also be varied for route of administration, e.g. intramuscular, intraperitoneal, intradermal, subcutaneous, intravenous infusion and the like. Generally at least about 10^4 engineered cells/kg are

administered, at least about 10^5 engineered cells /kg; at least about 10^6 engineered cells /kg, at least about 10^7 engineered cells/kg, or more.

[0229] Where the engineered cells that are modified to express the hoCD122 are T cells, an enhanced immune response may be manifest as an increase in the cytolytic response of T cells towards the target cells present in the recipient, e.g. towards elimination of tumor cells, infected cells; decrease in symptoms of autoimmune disease; and the like. In some embodiments when the engineered T cell population is to be administered to a subject, the subject is provided with immunosuppressive course of therapy prior to or in combination with the administration of the engineered T cell population. Examples of such immunosuppressive regimens include but are not limited to systemic corticosteroids (e.g., methylprednisolone). Therapies for B cell depletion include intravenous immunoglobulin (IVIG) by established clinical dosing guidelines to restore normal levels of serum immunoglobulin levels. In some embodiments, prior to administration of the CAR-T cell therapy of the present invention, the subject may optionally be subjected to a lymphodepleting regimen. One example of a such lymphodepleting regimen consists of the administration to the subject of fludarabine (30 mg/m^2 intravenous daily for 4 days) and cyclophosphamide (500 mg/m^2 IV daily for 2 days starting with the first dose of fludarabine).

[0230] Engineered hoCD122 cells can be provided in pharmaceutical compositions suitable for therapeutic use, e.g. for human treatment. Therapeutic formulations comprising such cells can be frozen, or prepared for administration with physiologically acceptable carriers, excipients or stabilizers (Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980)), in the form of aqueous solutions. The hoCD122 cells will be formulated, 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.

[0231] The hoCD122 cells can be administered by any suitable means, usually parenteral. Parenteral infusions include intramuscular, intravenous (bolus or slow infusion), intraarterial, intraperitoneal, intrathecal or subcutaneous administration. In the typical practice, the engineered T cells are infused to the subject in a physiologically acceptable medium, normally

intravascularly, although they may also be introduced into any other convenient site, where the cells may find an appropriate site for growth. Usually, at least 1×10^5 cells/kg will be administered, at least 1×10^6 cells/kg, at least 1×10^7 cells/kg, at least 1×10^8 cells/kg, at least 1×10^9 cells/kg, or more, usually being limited by the number of T cells that are obtained during collection.

[0232] For example, typical ranges for the administration of cells modified to express the a orthogonal hCD122 receptor for use in the practice of the present invention range from about 1×10^5 to 5×10^8 viable cells per kg of subject body weight per course of therapy. Consequently, adjusted for body weight, typical ranges for the administration of viable cells in human subjects ranges from approximately 1×10^6 to approximately 1×10^{13} viable cells, alternatively from approximately 5×10^6 to approximately 5×10^{12} viable cells, alternatively from approximately 1×10^7 to approximately 1×10^{12} viable cells, alternatively from approximately 5×10^7 to approximately 1×10^{12} viable cells, alternatively from approximately 1×10^8 to approximately 1×10^{12} viable cells, alternatively from approximately 5×10^8 to approximately 1×10^{12} viable cells, alternatively from approximately 1×10^9 to approximately 1×10^{12} viable cells per course of therapy. In one embodiment, the dose of the cells is in the range of 2.5×10^9 viable cells per course of therapy.

[0233] A course of therapy with an hIL2 ortholog and/or hoCD122 cell may comprise a single dose or in multiple doses over a period of time. In some embodiments, the hoCD122 cells are administered in a single dose. In some embodiments, the hoCD122 cells are administered in two or more split doses administered over a period of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 21, 28, 30, 60, 90, 120 or 180 days. The quantity of engineered hoCD122 cells administered in such split dosing protocols may be the same in each administration or may be provided at different levels. Multi-day dosing protocols over time periods may be provided by the skilled artisan (e.g. physician) monitoring the administration of the cells taking into account the response of the subject to the treatment including adverse effects of the treatment and their modulation as discussed above.

[0234] The compositions and methods of the present disclosure also provide a method for the treatment of a subject with a hoCD122 cell therapy (especially CAR T cell therapy) in the absence of prior lymphodepletion. Lymphodepletion is typically performed in a subject in

conjunction with CAR T cell therapy because the subsequent administration of the mixed cell population and the administration of non-specific agents (e.g. hIL2) to expand the engineered cell population in the subject in combination with the administration of the cell therapy product acts results in significant systemic toxicity (including cytokine release syndrome or “cytokine storm”) arising from the widespread proliferation and activation of immune cells by administration of agents that result in widespread activation as well as the presence of a substantial fraction of non-engineered cells in the cell therapy product itself. The methods and compositions of the present disclosure obviate this significant hurdle by both (or either) providing a substantially purified population of engineered cells largely devoid of contamination by non-engineered cells when the foregoing *ex vivo* method is employed and/or the selective activation and expansion of the engineered T cells with the hIL2 orthologs of the present invention which provide substantially reduced off-target effects of non-specific proliferative agents such as IL2.

[0235] For example, in the current clinical practice of CAR-T cell therapy, CAR-T cells are commonly administered in combination with lymphodepletion (e.g. by administration of Alemtuzumab (monoclonal anti-CD52), purine analogs, and the like) to facilitate expansion of the CAR-T cells to prior to host immune recovery. In some embodiments, the CAR-T cells may be modified for resistance to Alemtuzumab. In one aspect of the invention, the lymphodepletion currently employed in association with CAR-T therapy may be obviated or reduced by the orthogonal ligand expressing CAR-Ts of the present invention. As noted above, the lymphodepletion is commonly employed to enable expansion of the CAR-T cells. However, the lymphodepletion is also associated with major side effects of CAR-T cell therapy. Because the orthogonal ligand provides a means to selectively expand a particular T-cell population, the need for lymphodepletion prior to administration of the orthogonal ligand expressing CAR-Ts may be reduced. The present invention enables the practice of CAR-T cell therapy without or with reduced lymphodepletion prior to administration of the orthogonal ligand expressing CAR-Ts. In one embodiment, the present disclosure provides a method of treating a subject suffering from a disease, disorder or condition amenable to treatment with CAR-T cell therapy (e.g. cancer) by the administration of a orthogonal ligand expressing CAR-Ts in the absence of lymphodepletion prior to administration of the orthogonal ligand CAR-Ts.

[0236] In one embodiment, the present disclosure provides for a method of treatment of a mammalian subject suffering from a neoplastic disease, the method comprising the steps of (a) obtaining a biological sample comprising T-cells from the individual; (b) enriching the biological sample for the presence of T-cells; (c) transfecting the T-cells with one or more expression vectors comprising a nucleic acid sequence encoding a CAR and a nucleic acid sequence encoding an orthogonal hCD122 receptor, the antigen targeting domain of the CAR being capable of binding to at least one antigen present on a neoplastic cell; (d) contacting the population cells comprising the orthogonal receptor expressing CAR-T cells ex vivo with an hIL2 ortholog such that the population of cells is enriched for the hCD122 expressing CAR T cells, (e) administering a pharmaceutically effective amount of the orthogonal receptor expressing CAR-T cells to the mammal; and (f) modulating the growth of the orthogonal hCD122 receptor expressing CAR-T cells by the administration of a therapeutically effective amount of an hIL2 ortholog that binds selectively to the orthogonal hCD122 receptor expressed on the CAR-T cell. In the foregoing method, when the nucleic acid sequences encoding the CAR and the orthogonal hCD122 receptor are provided on the same vector, the sequences may optionally be provided in polycistronic format the nucleic acid sequences separated by an intervening sequence such as IRES or T2A sequence. In one embodiment, the foregoing method is associated with lymphodepletion or immunosuppression of the mammal prior to the initiation of the course of CAR-T cell therapy. In another embodiment, the foregoing method is practiced in the absence of lymphodepletion and/or immunosuppression of the mammal.

Administration of Viral or Non-Viral Vectors Encoding the hIL2 Ortholog:

[0237] Alternative to the administration of a hIL2 ortholog protein, the hIL2 ortholog may be provided to a subject by the administration of a nucleic acid construct encoding the hIL2 ortholog to the subject to achieve continuous exposure of the subject to the selective hIL2 ortholog. The administration of a recombinant vector encoding the hIL2 ortholog provides for extended delivery of the hIL2 ortholog to the subject and prolonged activation of the corresponding cells engineered to express the cognate orthogonal receptor associated with such hIL2 ortholog.

Non-Viral Vectors:

[0238] In one embodiment, the hIL2 ortholog may be administered to a subject in the form of nucleic acid expression construct for the hIL2 ortholog in a non-viral vector may be provided in a non-viral delivery system. Non-viral delivery systems are typically complexes to facilitate transduction of the target cell with a nucleic acid cargo wherein the nucleic acid is complexed with agents such as cationic lipids (DOTAP, DOTMA), surfactants, biologicals (gelatin, chitosan), metals (gold, magnetic iron) and synthetic polymers (PLG, PEI, PAMAM). Numerous embodiments of non-viral delivery systems are well known in the art including lipidic vector systems (Lee et al. (1997) Critical Reviews of Therapeutic Drug Carrier Systems 14:173-206); polymer coated liposomes (Marin *et al.*, U.S. Pat. No. 5,213,804, issued May 25, 1993; Woodle, *et al.*, U.S. Pat. No. 5,013,556, issued May 7, 1991); cationic liposomes (Epand *et al.*, U.S. Pat. No. 5,283,185, issued Feb. 1, 1994; Jessee, J. A., U.S. Pat. No. 5,578,475, issued Nov. 26, 1996; Rose *et al.*, U.S. Pat. No. 5,279,833, issued Jan. 18, 1994; Gebeyehu *et al.*, U.S. Pat. No. 5,334,761, issued Aug. 2, 1994). In one embodiment, the nucleic acid sequence in the non-viral vector system encoding the hIL2 receptor is under control of a regulatable promoter, inducible promoter, tissue specific or tumor specific promoter, or temporally regulated promoter.

Viral Vectors:

[0239] In another embodiment, hIL2 ortholog may be administered to a subject in the form of nucleic acid expression construct in viral vector encoding the hIL2 ortholog. The terms “viral vector” and “virus” are used interchangeably herein to refer to any of the obligate intracellular parasites having no protein-synthesizing or energy-generating mechanism. The viral genome may be RNA or DNA contained with a coated structure of protein of a lipid membrane. The terms virus(es) and viral vector(s) are used interchangeably herein. The viruses useful in the practice of the present invention include recombinantly modified enveloped or nonenveloped DNA and RNA viruses, preferably selected from baculoviridae, parvoviridae, picornoviridae, herpesviridae, poxviridae, or adenoviridae. The viruses are modified by recombinant DNA techniques to include expression of exogenous transgenes (e.g. a nucleic acid sequence encoding the hIL2 ortholog) and may be engineered to be replication deficient, conditionally replicating or replication competent. Minimal vector systems in which the viral backbone contains only the

sequences need for packaging of the viral vector and may optionally include a transgene expression cassette may also be employed. The term “replication deficient” refers to vectors that are highly attenuated for replication in a wild type mammalian cell. In order to produce such vectors in quantity, a producer cell line is generally created by co-transfection with a helper virus or genomically modified to complement the missing functions. The term “replication competent viral vectors” refers to a viral vector that is capable of infection, DNA replication, packaging and lysis of an infected cell. The term “conditionally replicating viral vectors” is used herein to refer to replication competent vectors that are designed to achieve selective expression in particular cell types. Such conditional replication may be achieved by operably linking tissue specific, tumor specific or cell type specific or other selectively induced regulatory control sequences to early genes (e.g., the E1 gene of adenoviral vectors). Infection of the subject with the recombinant virus or non-viral vector can provide for long term expression of the hIL2 ortholog in the subject and provide continuous selective maintenance of the engineered T cells expressing the hCD122 orthogonal receptor. In one embodiment, the nucleic acid sequence in the viral vector system encoding the hIL2 ortholog is under control of a regulatable promoter, inducible promoter, tissue specific or tumor specific promoter, or temporally regulated promoter.

Therapeutic Combinations:

[0240] The compositions and methods of the present disclosure may be combined with additional therapeutic agents. For example, when the disease, disorder or condition to be treated is a neoplastic disease (e.g. cancer) the methods of the present disclosure may be combined with conventional chemotherapeutic agents or other biological anti-cancer drugs such as checkpoint inhibitors (e.g. PD1 or PDL1 inhibitors) or therapeutic monoclonal antibodies (e.g. Avastin, Herceptin).

[0241] Examples of chemical agents identified in the art as useful in the treatment of neoplastic disease, include without limitation, abitrexate, adriamycin, adrucil, amsacrine, asparaginase, anthracyclines, azacitidine, azathioprine, bicnu, bleomycin, busulfan, bleomycin, camptosar, camptothecins, carboplatin, carmustine, cerubidine, chlorambucil, cisplatin, cladribine, cosmegen, cytarabine, cytosar, cyclophosphamide, cytoxan, dactinomycin, docetaxel, doxorubicin, daunorubicin, ellence, elspar, epirubicin, etoposide, fludarabine, fluorouracil,

fludara, gemcitabine, gemzar, hycamtin, hydroxyurea, hydra, idamycin, idarubicin, ifosfamide, ifex, irinotecan, lanvis, leukeran, leustatin, matulane, mechlorethamine, mercaptopurine, methotrexate, mitomycin, mitoxantrone, mithramycin, mutamycin, myleran, mylosar, navelbine, nipent, novantrone, oncovin, oxaliplatin, paclitaxel, parapl原因, pentostatin, platinol, plicamycin, procarbazine, purinethol, ralitrexed, taxotere, taxol, teniposide, thioguanine, tomudex, topotecan, valrubicin, velban, vepesid, vinblastine, vindesine, vincristine, vinorelbine, VP-16, and vumon.

[0242] The compositions of the present disclosure may be administered in combination with one or more additional therapeutic agents selected from the group consisting of tyrosine-kinase inhibitors, such as Imatinib mesylate (marketed as Gleevec®, also known as STI-571), Gefitinib (Iressa®, also known as ZD1839), Erlotinib (marketed as Tarceva®), Sorafenib (Nexavar®), Sunitinib (Sutent®), Dasatinib (Sprycel®), Lapatinib (Tykerb®), Nilotinib (Tasigna®), and Bortezomib (Velcade®), Jakafi® (ruxolitinib); Janus kinase inhibitors, such as tofacitinib; ALK inhibitors, such as crizotinib; Bcl-2 inhibitors, such as obatoclax, venclaxta, and gossypol; FLT3 inhibitors, such as midostaurin (Rydapt®), IDH inhibitors, such as AG-221, PARP inhibitors, such as Iniparib and Olaparib; PI3K inhibitors, such as perifosine; VEGF Receptor 2 inhibitors, such as Apatinib; AN-152 (AEZS-108) doxorubicin linked to [D-Lys(6)]-LHRH; Braf inhibitors, such as vemurafenib, dabrafenib, and LGX818; MEK inhibitors, such as trametinib; CDK inhibitors, such as PD-0332991 and LEE011; Hsp90 inhibitors, such as salinomycin; and/or small molecule drug conjugates, such as Vintafolide; serine/threonine kinase inhibitors, such as Temsirolimus (Torisel®), everolimus (Afinitor®), Vemurafenib (Zelboraf®), Trametinib (Mekinist), and Dabrafenib (Tafinlar®).

[0243] In some embodiments, particularly where the tumor antigen binding portion of the CAR is directed against BCMA, the engineered CAR-T cell is administered in combination with a γ -Secretase Inhibitor (GSI) as described in Pont, et al. (2019) " *γ -secretase inhibition increases efficacy of BCMA-specific chimeric antigen receptor T cells in multiple myeloma*" Blood <https://doi.org/10.1182/blood.2019000050>.

[0244] Examples of biological agents identified in the art as useful in the treatment of neoplastic disease, include without limitation, cytokines or cytokine antagonists such as IL-12, INF α , or anti-epidermal growth factor receptor, radiotherapy, irinotecan; tetrahydrofolate antimetabolites such as pemetrexed; antibodies against tumor antigens, a complex of a

monoclonal antibody and toxin, a T-cell adjuvant, bone marrow transplant, or antigen presenting cells (e.g., dendritic cell therapy), anti-tumor vaccines, replication competent viruses, signal transduction inhibitors (e.g., Gleevec® or Herceptin®) or an immunomodulator to achieve additive or synergistic suppression of tumor growth, cyclooxygenase-2 (COX-2) inhibitors, steroids, TNF antagonists (e.g., Remicade® and Enbrel®), interferon- β 1a (Avonex®), and interferon- β 1b (Betaseron®) as well as combinations of one or more of the foregoing as practiced in known chemotherapeutic treatment regimens readily appreciated by the skilled clinician in the art.

[0245] Tumor specific monoclonal antibodies that can be administered in combination with an engineered cell may include, without limitation, Rituximab (marketed as MabThera or Rituxan), Alemtuzumab, Panitumumab, Ipilimumab (Yervoy), etc.

[0246] In some embodiments the compositions and methods of the present disclosure may be combined with immune checkpoint therapy. Examples of immune checkpoint therapies include inhibitors of the binding of PD1 to PDL1 and/or PDL2. PD1 to PDL1 and/or PDL2 inhibitors are well known in the art. Examples of commercially available monoclonal antibodies that interfere with the binding of PD1 to PDL1 and/or PDL2 include nivolumab (Opdivo®, BMS-936558, MDX1106, commercially available from BristolMyers Squibb, Princeton NJ), pembrolizumab (Keytruda®/MK-3475, lambrolizumab, commercially available from Merck and Company, Kenilworth NJ), and atezolizumab (Tecentriq®, Genentech/Roche, South San Francisco CA). Additional examples of PD1 inhibitory antibodies include but are not limited to durvalumab (MEDI4736, Medimmune/AstraZeneca), pidilizumab (CT-011, CureTech), PDR001 (Novartis), BMS-936559 (MDX1105, Bristol Myers Squibb), and avelumab (MSB0010718C, Merck Serono/Pfizer) and SHR-1210 (Incyte). Additional antibody PD1 pathway inhibitors are described in United States Patent No. 8,217,149 (Genentech, Inc) issued July 10, 2012; United States Patent No. 8,168,757 (Merck Sharp and Dohme Corp.) issued May 1, 2012, United States Patent No. 8,008,449 (Medarex) issued August 30, 2011, United States Patent No. 7,943,743 (Medarex, Inc) issued May 17, 2011. Additionally, small molecule PD1 to PDL1 and/or PDL2 inhibitors are known in the art. See, e.g. Sasikumar, *et al* as WO2016142833A1 and Sasikumar, *et al*. WO2016142886A2, BMS-1166 and BMS-1001 (Skalniak, et al (2017) Oncotarget 8(42): 72167–72181).

[0247] The invention now being fully described, it will be apparent to one of ordinary skill in the art that various changes and modifications can be made without departing from the spirit or scope of the invention.

EXAMPLES

[0248] The following examples are presented in order to more fully illustrate the preferred embodiments of the invention. They should in no way, however, be construed as limiting the broad scope of the invention.

Example 1. Generation of the human IL2 expression vector pcDNA3.1/hygro(+)-huIL2

[0249] The human IL2 DNA ORF (Genbank NM_000586.3) was synthesized (Life Technologies GeneArt Service, Carlsbad, CA), and amplified via PCR using Platinum SuperFi II DNA polymerase kit (item #12361050, ThermoFisher) following the manufacturer's protocol, and using primers:

5' TATAGTCAGCGCCACcCATGTACAGGATGCAACTCCTGTC 3' (SEQ ID NO:153)

which incorporates and NheI restriction site, and

5' TATAGGGCCCTATCAAGTCAGTGTGAGATG 3' (SEQ ID NO:154)

which incorporates an ApaI restriction site. The PCR fragment was visualized on a 1% agarose gel (item #54803, Lonza, Rockland, ME), excised from the gel and purified using a QIAquick PCR Purification kit (item #28106, Qiagen, Germany) according to the manufacturer's protocol. The purified PCR fragment and mammalian expression vector pcDNA 3.1/Hygro(+) (#V87020, ThermoFisher) were digested with NheI and ApaI (#R0111S and #R0114L, New England Biolabs, Ipswich, MA) restriction enzymes. The expression vector was further treated with a Quick Dephosphorylation kit (#M0508L, New England Biolabs) according to the manufacturer's protocol. The PCR fragment was ligated into pcDNA 3.1/Hygro(+) using the Rapid DNA Ligation Kit (#11635379001, Sigma Aldrich, St. Louis, MO) following the manufacturer's protocol, transformed into One Shot TOP10 Chemically Competent E. coli (#C404006, Life Technologies, Carlsbad, CA), plated onto LB Agar plates containing 100ug/ml carbenicillin (#L1010, Teknova, Hollister, CA), and grown overnight at 37C.

[0250] The following day individual bacterial colonies were picked and used to start a 3ml bacterial culture in LB Broth (#10855-001, Life Technologies) with 100ug/ml ampicillin (#A9626, Teknova). The cultures were grown overnight at 37C.

The following day the E. coli were pelleted (6,000rpm, 10 minutes, tabletop centrifuge #5424, Eppendorf, Hauppauge, NY), and the DNA expression vector isolated using QIAprep Spin Miniprep Kit (#27106, Qiagen). The plasmid DNA was sequence verified (MCLab, South San Francisco, CA).

Example 2. Generation of the human IL2 ORTHO expression vector pcDNA3.1/hygro(+)-huIL2-ORTHO:

[0251] An expression vector which introduced six mutations into the human IL2 ORF (E35S, H36Q, L39V, D40L, Q42K and M43A; all numbering is based on the full length human IL2 ORF NM_000586.3 numbering) was assembled in substantial accordance with the teaching of Example 1 for the human IL2 expression vector in pcDNA3.1/Hygro(+), with the following exceptions: The initial template DNA used for PCR was synthesized with the E35S, H36Q, L39V, D40L, Q42K and M43A mutations.

Example 3. Introduction of mutations into pcDNA3.1/hygro(+)-huIL2 or back-mutations into pcDNA3.1/hygro(+)-huIL2 ORTHO expression vectors.

[0252] All mutations or back-mutations (reverting a mutation in *pcDNA3.1/hygro(+)-huIL2-ORTHO* back to match the wild type human IL2 ORF) were introduced into the *pcDNA3.1/Hygro(+)-huIL2* or *pcDNA3.1/Hygro(+)-huIL2-ORTHO* expression vectors using a Quik Change II Site Directed Mutagenesis Kit (#200524, Agilent Technologies, Santa Clara, CA) in substantial accordance with the manufacturer's protocol.

[0253] Table 5 lists the mutations generated, the template into which the mutation was introduced, and the primer sets used to introduce the mutation. The transformation of the Quik Change PCR reactions into E. coli, as well as the isolation and sequence analysis of the plasmid DNA, was performed using substantially the same protocol as in the generation of the pcDNA3.1/Hygro-huIL2 expression vector. The abbreviations for the templates are: Template 1 = pcDNA3.1/hygro(+)-huIL2; Template 2 = pcDNA3.1/Hygro(+)-huIL2 ORTHO.

Table 5. QuikChange Mutagenesis and Sequence Information Regarding Mutations

Ref#	Name	Modifications Relative wthIL2	Primer Set Sequences 5'→ 3'	Tem plate
5	-QVLKA	H16Q L19V D20L Q22K M23A	CAAAGAAAACACAGCTACAACCTGGAGCAGTTACTGG TGCTCTTAAAGGC (SEQ ID NO: 155) GCCTTTAAGAGCACCAGTAACTGCTCCAGTTGTAGCT GTGTTTTCTTTG (SEQ ID NO:156)	2
6	S-VLKA	E15S L19V D20L Q22K M23A	CAGCTACAACCTGAGCCATTTACTGGTGCTCTTAAA(SE Q ID NO:157) TTTAAGAGCACCAGTAAATGGCTCAGTTGTAGCTG(S EQ ID NO:158)	2
7	SQ-LKA	E15S H16Q D20L Q22K M23A	CTGAGCCAGTTACTGCTGCTCTTAAAGGCC (SEQ ID NO:159) GGCCTTTAAGAGCAGCAGTAACTGGCTCAG (SEQ ID NO:160)	2
8	SQV-KA	E15S H16Q L19V Q22K M23A	AACTGAGCCAGTTACTGGTGGATTTAAAGGCCATTTT GAATG (SEQ ID NO:161) CATTCAAAATGGCCTTTAAATCCACCAGTAACTGGCT CAGTT (SEQ ID NO:162)	2
9	SQVL-A	E15S H16Q L19V D20L M23A	TGAGCCAGTTACTGGTGCTCTTACAGGCCATTTTGA (SEQ ID NO:163) TCAAAATGGCCTGTAAGAGCACCAGTAACTGGCTCA (SEQ ID NO:164)	2
10	SQVLK-	E15S H16Q L19V D20L Q22K	CTGGTGCTCTTAAAGATGATTTTGAATGGAATTAA (SEQ ID NO:165) TTAATTCATTCAAAATCATCTTTAAGAGCACCAG (SEQ ID NO:166)	2
11	S-----	E15S	CACAGCTACAACCTGTCGATTTACTGCTGG (SEQ ID NO:167) CCAGCAGTAAATGCGACAGTTGTAGCTGTG (SEQ ID NO:168)	1
12	-Q-----	H16Q	GCTACAACCTGGAGCAGTTACTGCTGGATTAC (SEQ ID NO:169) GTAAATCCAGCAGTAACTGCTCCAGTTGTAGC (SEQ ID NO:170)	1
13	--V---	L19V	CTGGAGCATTTACTGGTGGATTTACAGATGATTTTG (SEQ ID NO:171) CAAAATCATCTGTAAATCCACCAGTAAATGCTCCAG (SEQ ID NO:172)	1

Table 5. QuikChange Mutagenesis and Sequence Information Regarding Mutations				
Ref#	Name	Modifications Relative wthIL2	Primer Set Sequences 5'→3'	Tem plate
14	---L--	D20L	GAGCATTACTGCTGCTATTACAGATGATTTTG (SEQ ID NO: 173) CAAAATCATCTGTAATAGCAGCAGTAAATGCTC (SEQ ID NO: 174)	1
15	---K-	Q22K	CATTACTGCTGGATTTAAAGATGATTTTGAATGG (SEQ ID NO:175) CCATTCAAAATCATCTTTAAATCCAGCAGTAAATG (SEQ ID NO:176)	1
16	-----A	M23A	CTGGAGCATTACTGCTGGATTTACAGGCGATTTTGAATGGAATTAATAATTACA (SEQ ID NO:177) TGTAATTATTAATTCCATTCAAAATCGCCTGTAAATCAGCAGTAAATGCTCCAG (SEQ ID NO:178)	1
17	SQ----	E15S H16Q	CAAAGAAAACACAGCTACAACCTGAGCCAGTTACTGCTGGATTTACAGATG (SEQ ID NO:179) CATCTGTAAATCCAGCAGTAACTGGCTCAGTTGTAGCTGTGTTTCTTTG (SEQ ID NO:180)	1
18	SQVL--	E15S H16Q L19V D20L	CCATTCAAAATCATCTGTAAGAGCACCAGTAACTGGCTCAGTTGTAGCTG (SEQ ID NO:181) CAACTGAGCCAGTTACTGGTGCTCTTAAAGGCCATTTGAATGGAATTAATAATTACAAG (SEQ ID NO:182)	1
20	ΔA1, SQVLKA	desA1 E15S H16Q L19V D20L Q22K M23A	CTTGTCACAAACAGTCCTACTTCAAGTTC (SEQ ID NO:183) GAACTTGAAGTAGGACTGTTTGTGACAAG (SEQ ID NO:184)	2
21	ΔP2, SQVLKA	desP2 E15S H16Q L19V D20L Q22K M23A	CACAAACAGTGCAACTTCAAGTTCTAC (SEQ ID NO:185) GTAGAAGTTGAAGTTGCACTGTTTGTG (SEQ ID NO:186)	2
018	ΔT3, SQVLKA	desT3 E15S H16Q L19V D20L Q22K M23A	CAAACAGTGCACCTTCAAGTTCTACAAAG (SEQ ID NO:187) CTTTGTAGAAGTTGAAGGTGCACTGTTTG (SEQ ID NO:188)	2
019	T3C, SQVLKA	T3C E15S H16Q L19V D20L Q22K M23A	CAAACAGTGCACCTTGTTCAGTTCTACA (SEQ ID NO:189) TGTAGAAGTTGAACAAGGTGCACTGTTTG (SEQ ID NO:190)	2
020	T3A, SQVLKA	T3A E15S H16Q L19V D20L Q22K M23A	CAAACAGTGCACCTGCTTCAAGTTCTAC (SEQ ID NO:191) GTAGAAGTTGAAGCAGGTGCACTGTTTG (SEQ ID NO:192)	2

Table 5. QuikChange Mutagenesis and Sequence Information Regarding Mutations				
Ref#	Name	Modifications Relative wthIL2	Primer Set Sequences 5'→ 3'	Tem plate
021	T3G, SQVLKA	T3G E15S H16Q L19V D20L Q22K M23A	CAAACAGTGCACCTGGTTCAAGTTCTAC (SEQ ID NO:193) GTAGAAGTTGAACACAGGTGCACTGTTTG (SEQ ID NO:194)	2
022	T3Q, SQVLKA	T3Q E15S H16Q L19V D20L Q22K M23A	CAAACAGTGCACCTCAGTCAAGTTCTACAAAG (SEQ ID NO:195) CTTTGTAGAAGTTGACTGAGGTGCACTGTTTG (SEQ ID NO:196)	2
023	T3E, SQVLKA	T3E E15S H16Q L19V D20L Q22K M23A	CAAACAGTGCACCTGAGTCAAGTTCTACAAAG (SEQ ID NO:197) CTTTGTAGAAGTTGACTCAGGTGCACTGTTTG (SEQ ID NO:198)	2
024	T3N, SQVLKA	T3N E15S H16Q L19V D20L Q22K M23A	CAAACAGTGCACCTAATTCAAGTTCTACAAAG (SEQ ID NO:199) CTTTGTAGAAGTTGAATTAGGTGCACTGTTTG (SEQ ID NO:200)	2
025	T3D, SQVLKA	T3D E15S H16Q L19V D20L Q22K M23A	AAACAGTGCACCTGATTCAAGTTCTACAAAG (SEQ ID NO:201) CTTTGTAGAAGTTGAATCAGGTGCACTGTTTG (SEQ ID NO:202)	2
026	T3R, SQVLKA	T3R E15S H16Q L19V D20L Q22K M23A	CAAACAGTGCACCTAGGTCAAGTTCTACAAAG (SEQ ID NO:203) CTTTGTAGAAGTTGACCTAGGTGCACTGTTTG (SEQ ID NO:204)	2
027	T3K, SQVLKA	T3K E15S H16Q L19V D20L Q22K M23A	CAAACAGTGCACCTAAGTCAAGTTCTACAAAG (SEQ ID NO:205) CTTTGTAGAAGTTGACTTAGGTGCACTGTTTG (SEQ ID NO:206)	2
028	T3P, SQVLKA	T3P E15S H16Q L19V D20L Q22K M23A	CAAACAGTGCACCTCCTTCAAGTTCTAC (SEQ ID NO:207) GTAGAAGTTGAAGGAGGTGCACTGTTTG (SEQ ID NO:208)	2
029	ΔS4, SQVLKA	desS4 E15S H16Q L19V D20L Q22K M23A	CAGTGCACCTACTAGTTCTACAAAGA (SEQ ID NO:209) TCTTTGTAGAAGTTAGTAGGTGCACTG (SEQ ID NO:210)	2
030	ΔS5, SQVLKA	desS5 E15S H16Q L19V D20L Q22K M23A	GTGCACCTACTTCATCTACAAAGAAAAC (SEQ ID NO:211) GTTTTCTTTGTAGATGAAGTAGGTGCAC (SEQ ID NO:212)	2

Table 5. QuikChange Mutagenesis and Sequence Information Regarding Mutations				
Ref#	Name	Modifications Relative wthIL2	Primer Set Sequences 5'→ 3'	Tem plate
031	ΔS6, SQVLKA	desS6 E15S H16Q L19V D20L Q22K M23A	CACCTACTTCAAGTACAAAGAAAACACAG (SEQ ID NO:213) CTGTGTTTTCTTTGFACTTGAAGTAGGTG (SEQ ID NO:214)	2
032	ΔT7, SQVLKA	desT7 E15S H16Q L19V D20L Q22K M23A	CTACTTCAAGTTCTAAGAAAACACAGCT (SEQ ID NO:215) AGCTGTGTTTTCTTAGAACTTGAAGTAG (SEQ ID NO:216)	2
033	ΔK8, SQVLKA	desK8 E15S H16Q L19V D20L Q22K M23A	CTTCAAGTTCTACAAAAACACAGCTACAAC (SEQ ID NO:217) GTTGTAGCTGTGTTTTGTAGAACTTGAAG (SEQ ID NO:218)	2
034	ΔK9, SQVLKA	desK9 E15S H16Q L19V D20L Q22K M23A	CAAGTTCTACAAAGACACAGCTACAACTG (SEQ ID NO:219) CAGTGTAGCTGTGTCTTTGTAGAACTTG (SEQ ID NO:220)	2
035	ΔQ13, SQVLKA	desQ13 E15S H16Q L19V D20L Q22K M23A	GAAAACACAGCTACTGAGCCAGTTACTG (SEQ ID NO:221) CAGTAACTGGCTCAGTAGCTGTGTTTTC (SEQ ID NO:222)	2
036	Q13W SQVLKA	Q13W E15S H16Q L19V D20L Q22K M23A	GAAAACACAGCTATGGCTGAGCCAGTTAC (SEQ ID NO:223) GTAAGTGGCTCAGCCATAGCTGTGTTTTC (SEQ ID NO:224)	2
037	ΔL14, SQVLKA	desL14 E15S H16Q L19V D20L Q22K M23A	AAACACAGCTACAAAGCCAGTTACTGGTGC (SEQ ID NO:225) GCACCAGTAACTGGCTTTGTAGCTGTGTTT (SEQ ID NO:226)	2
038	L14M, SQVLKA	L14ME15S H16Q L19V D20L Q22K M23A	AAACACAGCTACAAATGAGCCAGTTACTG (SEQ ID NO:227) CAGTAACTGGCTCATTGTAGCTGTGTTT (SEQ ID NO:228)	2
039	L14W, SQVLKA	L14WE15S H16Q L19V D20L Q22K M23A	AAACACAGCTACAATGGAGCCAGTTACTGG (SEQ ID NO:229) CCAGTAACTGGCTCCATTGTAGCTGTGTTT (SEQ ID NO:230)	2
041	KQVLKA	E15K H16Q L19V D20L Q22K M23A	CACAGCTACAACTGAAGCAGTTACTGGTGC (SEQ ID NO:231) GCACCAGTAACTGCTTCAGTTGTAGCTGTG (SEQ ID NO:232)	2

Table 5. QuikChange Mutagenesis and Sequence Information Regarding Mutations				
Ref#	Name	Modifications Relative with IL2	Primer Set Sequences 5'→3'	Tem plate
043	TQVLKA	E15T H16Q L19V D20L Q22K M23A	CACAGCTACAACTGACCCAGTTACTGGTGCTC (SEQ ID NO:233) GAGCACCAGTAACTGGGTCAGTTGTAGCTGTG (SEQ ID NO:234)	2
047	ΔH16, S_VLKA	desH16 E15S L19V D20L Q22K M23A	GCTACAACTGAGCTTACTGGTGCTCTTA (SEQ ID NO:235) TAAGAGCACCAGTAAGCTCAGTTGTAGC (SEQ ID NO:236)	2
048	SNVLKA	E15S H16N L19V D20L Q22K M23A	GCTACAACTGAGCAACTTACTGGTGCTC (SEQ ID NO:237) GAGCACCAGTAAGTTGCTCAGTTGTAGC (SEQ ID NO:238)	2
050	L18G, SQVLKA	E15S H16Q L18G L19V D20L Q22K M23A	AACTGAGCCAGTTAGGGGTGCTCTTAAAGG (SEQ ID NO:239) CCTTTAAGAGCACCCCTAACTGGCTCAGTT (SEQ ID NO:240)	2
051	L18M, SQVLKA	E15S H16Q L18M L19V D20L Q22K M23A	CAACTGAGCCAGTTAATGGTGCTCTTAAAGGC (SEQ ID NO:241) GCCTTTAAGAGCACCATTAAGTGGCTCAGTTG (SEQ ID NO:242)	2
052	L18F, SQVLKA	E15S H16Q L18F L19V D20L Q22K M23A	AACTGAGCCAGTTATTCGTGCTCTTAAAGGC (SEQ ID NO:243) GCCTTTAAGAGCACGAATAAGTGGCTCAGTT (SEQ ID NO:244)	2
053	L18E, SQVLKA	E15S H16Q L18E L19V D20L Q22K M23A	CAACTGAGCCAGTTAGAGGTGCTCTTAAAGGC (SEQ ID NO:245) GCCTTTAAGAGCACCTCTAACTGGCTCAGTTG (SEQ ID NO:246)	2
054	L18H, SQVLKA	E15S H16Q L18H L19V D20L Q22K M23A	ACTGAGCCAGTTACACGTGCTCTTAAAGGC (SEQ ID NO:247) GCCTTTAAGAGCACGTGTAAGTGGCTCAGT (SEQ ID NO:248)	2
055	L18W, SQVLKA	E15S H16Q L18W L19V D20L Q22K M23A	AACTGAGCCAGTTATGGGTGCTCTTAAAGG (SEQ ID NO:249) CCTTTAAGAGCACCCATAACTGGCTCAGTT (SEQ ID NO:250)	2
056	L18K, SQVLKA	E15S H16Q L18K L19V D20L Q22K M23A	CAACTGAGCCAGTTAAAGGTGCTCTTAAAGGC (SEQ ID NO:251) GCCTTTAAGAGCACCTTTAACTGGCTCAGTTG (SEQ ID NO:252)	2

Table 5. QuikChange Mutagenesis and Sequence Information Regarding Mutations				
Ref#	Name	Modifications Relative wthIL2	Primer Set Sequences 5'→ 3'	Tem plate
057	L18Q, SQVLKA	E15S H16Q L18Q L19V D20L Q22K M23A	CTGAGCCAGTTACAGGTGCTCTTAAAG (SEQ ID NO:253) CTTTAAGAGCACCTGTAAGTGGCTCAG (SEQ ID NO:254)	2
058	L18S, SQVLKA	E15S H16Q L18S L19V D20L Q22K M23A	CAACTGAGCCAGTTAAGCGTGCTCTTAAAGGC (SEQ ID NO:255) GCCTTTAAGAGCACGCTTAAGTGGCTCAGTTG (SEQ ID NO:256)	2
059	L18V, SQVLKA	E15S H16Q L18V L19V D20L Q22K M23A	CAACTGAGCCAGTTAGTGGTGCTCTTAAAGG (SEQ ID NO:257) CCTTTAAGAGCACCACTAAGTGGCTCAGTTG (SEQ ID NO:258)	2
060	L18I, SQVLKA	E15S H16Q L18I L19V D20L Q22K M23A	CAACTGAGCCAGTTAATCGTGCTCTTAAAGGC (SEQ ID NO:259) GCCTTTAAGAGCACGATTAAGTGGCTCAGTTG (SEQ ID NO:260)	2
061	L18Y, SQVLKA	E15S H16Q L18Y L19V D20L Q22K M23A	CAACTGAGCCAGTTATACGTGCTCTTAAAGGC (SEQ ID NO:261) GCCTTTAAGAGCACGTATAAGTGGCTCAGTTG (SEQ ID NO:262)	2
062	L18H, SQVLKA	E15S H16Q L18H L19V D20L Q22K M23A	CTGAGCCAGTTACACGTGCTCTTAAAGG (SEQ ID NO:263) CCTTTAAGAGCACGTGTAAGTGGCTCAG (SEQ ID NO:264)	2
063	L18D, SQVLKA	E15S H16Q L18D L19V D20L Q22K M23A	CAACTGAGCCAGTTAGACGTGCTCTTAAAGGC (SEQ ID NO:265) GCCTTTAAGAGCACGTCTAAGTGGCTCAGTTG (SEQ ID NO:266)	2
064	L18T, SQVLKA	E15S H16Q L18T L19V D20L Q22K M23A	CAACTGAGCCAGTTAACGGTGCTCTTAAAGGC (SEQ ID NO:267) GCCTTTAAGAGCACCGTTAAGTGGCTCAGTTG (SEQ ID NO:268)	2
065	ΔL19, SQ_LKA	desL19 E15S H16Q D20L Q22K M23A	CTGAGCCAGTTACTGCTCTTAAAGGCCATT (SEQ ID NO:269) AATGGCCTTTAAGAGCAGTAAGTGGCTCAG (SEQ ID NO:270)	2
067	SQILKA	E15S H16Q L19I D20L Q22K M23A	CTGAGCCAGTTACTGATCCTCTTAAAGGCCAT (SEQ ID NO:271) ATGGCCTTTAAGAGGATCAGTAAGTGGCTCAG (SEQ ID NO:272)	2

Table 5. QuikChange Mutagenesis and Sequence Information Regarding Mutations				
Ref#	Name	Modifications Relative wthIL2	Primer Set Sequences 5'→ 3'	Tem plate
068	ΔD20, SQV_KA	desD20 E15S H16Q L19V Q22K M23A	GCCAGTTACTGGTGTTAAAGGCCATTTTG (SEQ ID NO:273) CAAAATGGCCTTTAACACCAGTAACTGGC (SEQ ID NO:274)	2
070	SQVSKA	E15S H16Q L19V D20s Q22K M23A	GCCAGTTACTGGTGTCCTTAAAGGCCATTTTG (SEQ ID NO:275) CAAAATGGCCTTTAAGGACACCAGTAACTGGC (SEQ ID NO:276)	2
073	SQVLFA	E15S H16Q L19V D20L Q22F M23A	TACTGGTGCTCTTATTCGCCATTTTGAATGG (SEQ ID NO:277) CCATTCAAAATGGCGAATAAGAGCACCAGTA (SEQ ID NO:278)	2
074	SQVLEA	E15S H16Q L19V D20L Q22E M23A	TACTGGTGCTCTTAGAGGCCATTTTGAATGG (SEQ ID NO:279) CCATTCAAAATGGCCTCTAAGAGCACCAGTA (SEQ ID NO:280)	2
075	SQVLGA	E15S H16Q L19V D20L Q22G M23A	TTACTGGTGCTCTTAGGGGCCATTTTGAATGG (SEQ ID NO:281) CCATTCAAAATGGCCCCTAAGAGCACCAGTAA (SEQ ID NO:282)	2
076	SQVLAA	E15S H16Q L19V D20L Q22AM23A	TACTGGTGCTCTTAGCGGCCATTTTGAATG (SEQ ID NO:283) CATTCAAAATGGCCGCTAAGAGCACCAGTA (SEQ ID NO:284)	2
077	SQVLLA	E15S H16Q L19V D20L Q22L M23A	TACTGGTGCTCTTACTGGCCATTTTGAATG (SEQ ID NO:285) CATTCAAAATGGCCAGTAAGAGCACCAGTA (SEQ ID NO:286)	2
078	SQVLMMA	E15S H16Q L19V D20L Q22MM23A	TACTGGTGCTCTTAATGGCCATTTTGAATG (SEQ ID NO:287) CATTCAAAATGGCCATTAAGAGCACCAGTA (SEQ ID NO:288)	2
079	SQVLFA	E15S H16Q L19V D20L Q22F M23A	TACTGGTGCTCTTATTCGCCATTTTGAATGG (SEQ ID NO:289) CCATTCAAAATGGCGAATAAGAGCACCAGTA (SEQ ID NO:290)	2
080	SQVLWA	E15S H16Q L19V D20L Q22W M23A	TACTGGTGCTCTTATGGGCCATTTTGAATG (SEQ ID NO:291) CATTCAAAATGGCCCATAAGAGCACCAGTA (SEQ ID NO:292)	2

Table 5. QuikChange Mutagenesis and Sequence Information Regarding Mutations				
Ref#	Name	Modifications Relative wthIL2	Primer Set Sequences 5'→ 3'	Tem plate
081	SQVLSA	E15S H16Q L19V D20L Q22S M23A	TACTGGTGCTCTTAAGCGCCATTTGAATGG (SEQ ID NO:293) CCATTCAAAATGGCGCTTAAGAGCACCAGTA (SEQ ID NO:294)	2
082	SQVLVA	E15S H16Q L19V D20L Q22V M23A	TACTGGTGCTCTTAGTGGCCATTTGAATG (SEQ ID NO:295) CATTCAAAATGGCCACTAAGAGCACCAGTA (SEQ ID NO:296)	2
083	SQVLIA	E15S H16Q L19V D20L Q22I M23A	TACTGGTGCTCTTAATCGCCATTTGAATGG (SEQ ID NO:297) CCATTCAAAATGGCGATTAAGAGCACCAGTA (SEQ ID NO:298)	2
084	SQVLYA, E116G	E15S H16Q L19V D20L Q22Y M23A E116G	TACTGGTGCTCTTATACGCCATTTGAATGG (SEQ ID NO:299) CCATTCAAAATGGCGTATAAGAGCACCAGTA (SEQ ID NO:300)	2
085	SQVLHA	E15S H16Q L19V D20L Q22H M23A	TACTGGTGCTCTTACACGCCATTTGAATGG (SEQ ID NO:301) CCATTCAAAATGGCGTGTAAGAGCACCAGTA (SEQ ID NO:302)	2
086	SQVLRA	E15S H16Q L19V D20L Q22R M23A	TACTGGTGCTCTTACGGGCCATTTGAATG (SEQ ID NO:303) CATTCAAAATGGCCCGTAAGAGCACCAGTA (SEQ ID NO:304)	2
087	SQVLNA	E15S H16Q L19V D20L Q22N M23A	CTGGTGCTCTTAAACGCCATTTGAATGG (SEQ ID NO:305) CCATTCAAAATGGCGTTTAAGAGCACCAG (SEQ ID NO:306)	2
088	SQVLDA	E15S H16Q L19V D20L Q22D M23A	TACTGGTGCTCTTAGACGCCATTTGAATGG (SEQ ID NO:307) CCATTCAAAATGGCGTCTAAGAGCACCAGTA (SEQ ID NO:308)	2
089	SQVLTA	E15S H16Q L19V D20L Q22T M23A	TACTGGTGCTCTTAACGGGCCATTTGAATGG (SEQ ID NO:309) CCATTCAAAATGGCCGTTAAGAGCACCAGTA (SEQ ID NO:310)	2
090	ΔM23, SQVLK-	desM23 E15S H16Q L19V D20L Q22K	CTGGTGCTCTTAAAGATTTGAATGGAATTAA (SEQ ID NO:311) TTAATTCATTCAAAATCTTTAAGAGCACCAG (SEQ ID NO:312)	2

Table 5. QuikChange Mutagenesis and Sequence Information Regarding Mutations				
Ref#	Name	Modifications Relative wthIL2	Primer Set Sequences 5'→3'	Tem plate
091	SQVLKW	E15S H16Q L19V D20L Q22K M23w	CTGGTGCTCTTAAAGTGGATTTGAATGGAAT (SEQ ID NO:313) ATTCCATTCAAAATCCACTTTAAGAGCACCAG (SEQ ID NO:314)	2
092	SQVLKH	E15S H16Q L19V D20L Q22K M23H	TGGTGCTCTTAAAGCACATTTTGAATGGAA (SEQ ID NO:315) TTCCATTCAAAATGTGCTTTAAGAGCACCA (SEQ ID NO:316)	2
093	SQVLKY	E15S H16Q L19V D20L Q22K M23Y	GGTGCTCTTAAAGTACATTTTGAATGGAA (SEQ ID NO:317) TTCCATTCAAAATGTACTTTAAGAGCACC (SEQ ID NO:318)	2
094	SQVLKF	E15S H16Q L19V D20L Q22K M23F	CTGGTGCTCTTAAAGTTCATTTTGAATGGAAT (SEQ ID NO:319) ATTCCATTCAAAATGAACTTTAAGAGCACCAG (SEQ ID NO:320)	2
095	SQVLKQ	E15S H16Q L19V D20L Q22K M23Q	TGGTGCTCTTAAAGCAGATTTTGAATGG (SEQ ID NO:321) CCATTCAAAATCTGCTTTAAGAGCACCA (SEQ ID NO:322)	2
096	SQVLKS	E15S H16Q L19V D20L Q22K M23S	GGTGCTCTTAAAGAGCATTTTGAATGGA (SEQ ID NO:323) TCCATTCAAAATGCTCTTTAAGAGCACC (SEQ ID NO:324)	2
097	SQVLKV	E15S H16Q L19V D20L Q22K M23V	GGTGCTCTTAAAGGTCATTTTGAATGGAA (SEQ ID NO:325) TTCCATTCAAAATGACCTTTAAGAGCACC (SEQ ID NO:326)	2
098	SQVLKL	E15S H16Q L19V D20L Q22K M23L	CTGGTGCTCTTAAAGCTCATTTTGAATGGAAT (SEQ ID NO:327) ATTCCATTCAAAATGAGCTTTAAGAGCACCAG (SEQ ID NO:328)	2
099	SQVLKT	E15S H16Q L19V D20L Q22K M23T	GGTGCTCTTAAAGACCATTTTGAATGG (SEQ ID NO:329) CCATTCAAAATGGTCTTTAAGAGCACC (SEQ ID NO:330)	2
100	ΔG27, SQVLKA	desG27 E15S H16Q L19V D20L Q22K M23A	GGCCATTTTGAATATTAATAATTACAAG (SEQ ID NO:331) CTTGTAATTATTAATATTCAAAATGGCC (SEQ ID NO:332)	2

Table 5. QuikChange Mutagenesis and Sequence Information Regarding Mutations				
Ref#	Name	Modifications Relative wthIL2	Primer Set Sequences 5'→ 3'	Tem plate
101	G27K, SQVLKA	E15S H16Q L19V D20L Q22K M23A G27K	GGCCATTTTGAATAAAATTAATAATTAC (SEQ ID NO:333) GTAATTATTAAATTTTATTCAAATGGCC (SEQ ID NO:334)	2
102	G27S, SQVLKA	E15S H16Q L19V D20L Q22K M23A G27S	GGCCATTTTGAATTCAATTAATAATTAC (SEQ ID NO:335) GTAATTATTAAATTGAATTCAAATGGCC (SEQ ID NO:336)	2
103	R38W, SQVLKA	E15S H16Q L19V D20L Q22K M23A R38W	ATCCCAAACCTCACCTGGATGCTCACATTT (SEQ ID NO:337) AAATGTGAGCATCCAGGTGAGTTTGGGAT (SEQ ID NO:338)	2
104	R38G, SQVLKA	E15S H16Q L19V D20L Q22K M23A R38G	ATCCCAAACCTCACCGGGATGCTCACATTT (SEQ ID NO:339) AAATGTGAGCATCCCGGTGAGTTTGGGAT (SEQ ID NO:340)	2
105	M39L, SQVLKA	E15S H16Q L19V D20L Q22K M23A M39L	CCAAACTCACCAGGCTGCTCACATTTAAG (SEQ ID NO:341) CTTAAATGTGAGCAGCCTGGTGAGTTTGG (SEQ ID NO:342)	2
106	M39V, SQVLKA	E15S H16Q L19V D20L Q22K M23A M39V	CCAAACTCACCAGGGTGCTCACATTTAAG (SEQ ID NO:343) CTTAAATGTGAGCACCCCTGGTGAGTTTGG (SEQ ID NO:344)	2
107	F42K, SQVLKA	E15S H16Q L19V D20L Q22K M23A F42K	CCAGGATGCTCACAAAGAAGTTTACATGCC (SEQ ID NO:345) GGCATGTAAACTTCTTTGTGAGCATCCTGG (SEQ ID NO:346)	2
108	ΔT51, SQVLKA	desT51 E15S H16Q L19V D20L Q22K M23A	TGCCCCAAGAAGGCCGAAGTAAACATCT (SEQ ID NO:347) AGATGTTTCAGTTCGGCCTTCTTGGGCA (SEQ ID NO:348)	2
109	T51I, SQVLKA	E15S H16Q L19V D20L Q22K M23A T51I	GCCCAAGAAGGCCATAGAACTGAAACATC (SEQ ID NO:349) GATGTTTCAGTTCATGGCCTTCTTGGGC (SEQ ID NO:350)	2
110	H55Y, SQVLKA	E15S H16Q L19V D20L Q22K M23A H55Y	CCACAGAACTGAAATATCTTCAGTGTCTA (SEQ ID NO:351) TAGACACTGAAGATATTTTCAGTTCGTGG (SEQ ID NO:352)	2

Table 5. QuikChange Mutagenesis and Sequence Information Regarding Mutations				
Ref#	Name	Modifications Relative wthIL2	Primer Set Sequences 5'→ 3'	Tem plate
111	Q74N, SQVLKA	E15S H16Q L19V D20L Q22K M23A Q74N	GCTAAATTTAGCTAACAGCAAAAACCTTC (SEQ ID NO:353) GAAAGTTTTTGTCTGTAGCTAAATTTAGC (SEQ ID NO:354)	2
112	Q74H, SQVLKA	E15S H16Q L19V D20L Q22K M23A Q74H	CTAAATTTAGCTCACAGCAAAAACCTTC (SEQ ID NO:355) GAAAGTTTTTGTCTGTGAGCTAAATTTAG (SEQ ID NO:356)	2
113	Q74S, SQVLKA	E15S H16Q L19V D20L Q22K M23A Q74S	GTGCTAAATTTAGCTTCAAGCAAAAACCTTC (SEQ ID NO:357) GAAAGTTTTTGTCTGAAGCTAAATTTAGCAC (SEQ ID NO:358)	2
114	L80F, SQVLKA	E15S H16Q L19V D20L Q22K M23A L80F	CAAAAACCTTTCACCTCAGACCCAGGGACTT (SEQ ID NO:359) AAGTCCCTGGGTCTGAAGTGAAAGTTTTTG (SEQ ID NO:360)	2
115	L80V, SQVLKA	E15S H16Q L19V D20L Q22K M23A L80V	CAAAAACCTTTCACGTAAGACCCAGGGAC (SEQ ID NO:361) GTCCCTGGGTCTTACGTGAAAGTTTTTG (SEQ ID NO:362)	2
116	ΔR81, SQVLKA	desR81 E15S H16Q L19V D20L Q22K M23A	AAAACTTTCACCTTACCCAGGGACTTAATC (SEQ ID NO:363) GATTAAGTCCCTGGGTAAAGTGAAAGTTTTT (SEQ ID NO:364)	2
117	R81I, SQVLKA	E15S H16Q L19V D20L Q22K M23A R81I	AACTTTCACCTTAATACCCAGGGACTTA (SEQ ID NO:365) TAAGTCCCTGGGTATTAAGTGAAAGTT (SEQ ID NO:366)	2
118	R81D, SQVLKA	E15S H16Q L19V D20L Q22K M23A R81D	AAAACCTTTCACCTTAGACCCAGGGACTTAAT (SEQ ID NO:367) ATTAAGTCCCTGGGTCTAAGTGAAAGTTTT (SEQ ID NO:368)	2
119	R81Y, SQVLKA	E15S H16Q L19V D20L Q22K M23A R81Y	AAAACCTTTCACCTTATACCCAGGGACTTAAT (SEQ ID NO:369) ATTAAGTCCCTGGGTATAAGTGAAAGTTTT (SEQ ID NO:370)	2
121	L85V, SQVLKA	E15S H16Q L19V D20L Q22K M23A L85V	TAAGACCCAGGGACGTAATCAGCAATATC (SEQ ID NO:371) GATATGCTGATTACGTCCCTGGGTCTTA (SEQ ID NO:372)	2

Table 5. QuikChange Mutagenesis and Sequence Information Regarding Mutations				
Ref#	Name	Modifications Relative wthIL2	Primer Set Sequences 5'→ 3'	Tem plate
122	I86V, SQVLKA	E15S H16Q L19V D20L Q22K M23A I86V	GACCCAGGGACTTAGTCAGCAATATCAAC (SEQ ID NO:373) GTTGATAATTGCTGACTAAGTCCCTGGGTC (SEQ ID NO:374)	2
123	A88N, SQVLKA	E15S H16Q L19V D20L Q22K M23A desN88	GGGACTTAATCAGCATCAACGTAATAGT (SEQ ID NO:375) ACTATTACGTTGATGCTGATTAAGTCCC (SEQ ID NO:376)	2
124	N88E, SQVLKA	E15S H16Q L19V D20L Q22K M23A N88E	GGGACTTAATCAGCGAGATCAACGTAATAG (SEQ ID NO:377) CTATTACGTTGATCTCGCTGATTAAGTCCC (SEQ ID NO:378)	2
125	N88Q, SQVLKA	E15S H16Q L19V D20L Q22K M23A N88Q	GGGACTTAATCAGCCAGATCAACGTAATAG (SEQ ID NO:379) CTATTACGTTGATCTGGCTGATTAAGTCCC (SEQ ID NO:380)	2
127	V91R, SQVLKA	E15S H16Q L19V D20L Q22K M23A V91R	CAGCAATATCAACCGAATAGTTCTGGAAC (SEQ ID NO:381) GTTCCAGAACTATTCGGTTGATATTGCTG (SEQ ID NO:382)	2
128	V91K, SQVLKA	E15S H16Q L19V D20L Q22K M23A V91K	TCAGCAATATCAACAAGATAGTTCTGGAAC (SEQ ID NO:383) AGTTCCAGAACTATCTTGTTGATATTGCTGA (SEQ ID NO:384)	2
129	I92F, SQVLKA	E15S H16Q L19V D20L Q22K M23A I92F	GCAATATCAACGTATTCGTTCTGGAACATAAG (SEQ ID NO:385) CTTTAGTTCCAGAACGAATACGTTGATATTGC (SEQ ID NO:386)	2
130	K97Q, SQVLKA	E15S H16Q L19V D20L Q22K M23A K97Q	TAGTTCTGGAACACAGGGATCTGAAACA (SEQ ID NO:387) TGTTTCAGATCCCTGTAGTTCCAGAACTA (SEQ ID NO:388)	2
131	M104A, SQVLKA	E15S H16Q L19V D20L Q22K M23A M104A	CTGAAACAACATTCGCGTGTGAATATGCTG (SEQ ID NO:389) CAGCATATTCACACGCGAATGTTGTTTCAG (SEQ ID NO:390)	2
132	D109C, SQVLKA	E15S H16Q L19V D20L Q22K M23A D109C	GTGTGAATATGCTTGTGAGACAGCAACC (SEQ ID NO:391) GGTTGCTGTCTCACAAGCATATTCACAC (SEQ ID NO:392)	2

Table 5. QuikChange Mutagenesis and Sequence Information Regarding Mutations				
Ref#	Name	Modifications Relative wthIL2	Primer Set Sequences 5'→ 3'	Tem plate
133	T113N, SQVLKA	E15S H16Q L19V D20L Q22K M23A T113N	GATGAGACAGCAAACATTGTAGAATTTC (SEQ ID NO:393) GAAATTCTACAATGTTTGCTGTCTCATC (SEQ ID NO:394)	2
134	C125A, SQVLKA	E15S H16Q L19V D20L Q22K M23A C125A	GATGGATTACCTTTGCTCAAAGCATCATC (SEQ ID NO:395) GATGATGCTTTGAGCAAAGGTAATCCATC (SEQ ID NO:396)	2
135	C125S, SQVLKA	E15S H16Q L19V D20L Q22K M23A C125S	ATGGATTACCTTTTCTCAAAGCATCATCTC (SEQ ID NO:397) GAGATGATGCTTTGAGAAAAGGTAATCCAT (SEQ ID NO:398)	2
136	Q126H, SQVLKA	E15S H16Q L19V D20L Q22K M23A Q126H	TTACCTTTTGTACAGCATCATCTCAAC (SEQ ID NO:399) GTTGAGATGATGCTGTGACAAAAGGTAA (SEQ ID NO:400)	2
137	Q126M, SQVLKA	E15S H16Q L19V D20L Q22K M23A Q126M	GGATTACCTTTTGTATGAGCATCATCTCAAC (SEQ ID NO:401) GTTGAGATGATGCTCATACAAAAGGTAATCC (SEQ ID NO:402)	2
138	Q126K, SQVLKA	E15S H16Q L19V D20L Q22K M23A Q126K	GGATTACCTTTTGTAAAAGCATCATCTC (SEQ ID NO:403) GAGATGATGCTTTTACAAAAGGTAATCC (SEQ ID NO:404)	2
139	Q126C, SQVLKA	E15S H16Q L19V D20L Q22K M23A Q126C	GGATTACCTTTTGTGTCAGCATCATCTCAAC (SEQ ID NO:405) GTTGAGATGATGCTGCAACAAAAGGTAATCC (SEQ ID NO:406)	2
140	Q126D, SQVLKA	E15S H16Q L19V D20L Q22K M23A Q126D	GGATTACCTTTTGTGACAGCATCATCTCAAC (SEQ ID NO:407) GTTGAGATGATGCTGTACAAAAGGTAATCC (SEQ ID NO:408)	2
141	Q126E, SQVLKA	E15S H16Q L19V D20L Q22K M23A Q126E	GGATTACCTTTTGTGAAAGCATCATCTCA (SEQ ID NO:409) TGAGATGATGCTTTTACAAAAGGTAATCC (SEQ ID NO:410)	2
142	Q126G, SQVLKA	E15S H16Q L19V D20L Q22K M23A Q126G	GGATTACCTTTTGTGGAAGCATCATCTCAAC (SEQ ID NO:411) GTTGAGATGATGCTTCCACAAAAGGTAATCC (SEQ ID NO:412)	2

Table 5. QuikChange Mutagenesis and Sequence Information Regarding Mutations				
Ref#	Name	Modifications Relative wthIL2	Primer Set Sequences 5'→ 3'	Tem plate
143	Q126I, SQVLKA	E15S H16Q L19V D20L Q22K M23A Q126I	GGATTACCTTTTGTATAAGCATCATCTCAAC (SEQ ID NO:413) GTTGAGATGATGCTTATACAAAAGGTAATCC (SEQ ID NO:414)	2
144	Q126R, SQVLKA	E15S H16Q L19V D20L Q22K M23A Q126R	GGATTACCTTTTGTGCGAAGCATCATCTCAAC (SEQ ID NO:415) GTTGAGATGATGCTTCGACAAAAGGTAATCC (SEQ ID NO:416)	2
145	Q126S, SQVLKA	E15S H16Q L19V D20L Q22K M23A Q126S	GGATTACCTTTTGTTCAGCATCATCTCAAC (SEQ ID NO:417) GTTGAGATGATGCTTGAACAAAAGGTAATCC (SEQ ID NO:418)	2
146	Q126T, SQVLKA	E15S H16Q L19V D20L Q22K M23A Q126T	GGATTACCTTTTGTACAAGCATCATCTCAAC (SEQ ID NO:419) GTTGAGATGATGCTTGTACAAAAGGTAATCC (SEQ ID NO:420)	2

Example 4. Transient Transfections in HEK293 cells

[0254] All expression vectors were transiently transfected into HEK293 cells (#CRL-1573, ATCC, Manassas, VA). ~1E6 HEK293 cells were plated into each well of a 6 well tissue culture plate in 2ml of DMEM (#10569044, Life Technologies) supplemented with 10% Fetal Bovine serum (#SH30071.03, Fisher Scientific, Chicago, IL), and grown overnight at 37C and 5% CO₂.

[0255] The next day the cells were transfected using Lipofectamine 3000 Reagent (#L3000150, Life Technologies) following the manufacturer's protocol, using 2.5ug DNA, 5ul P3000 reagent, and 7.5ul Lipofectamine 3000 per transfection. The transfected cells were grown at 37C, 5% CO₂ for 48 – 72 hours and then the conditioned media was harvested.

Example 5. Analysis of protein expression

[0256] Protein expression for some mutant proteins was measured by ELISA using the Human IL2 V-PLEX ELISA kit (#K151QQD-4, Mesoscale Diagnostics, Baltimore, MD) following the manufacturer's protocol (transfected media was diluted 1:4 initially, then 1:2 serially). The plate was read on a Meso Quickplex SQ120 (Mesoscale Diagnostics) using the manufacture's preprogrammed setting for this ELISA kit. The human IL2 standard in the kit was used to

compute an approximate expression level in the conditioned media samples. Table 6 below details the approximate expression levels for the proteins expressed.

Table 6. Expression Levels of Human IL2 Orthologs							
Full ORF #	35	36	39	40	42	43	Expression Level in Transient Transfection (ng/ml)
Mature Peptide #	15	16	19	20	22	23	
Wild Type human IL2 Residue	E	H	L	D	Q	M	
WT IL2	E	H	L	D	Q	M	1000
SQVLKA	S	Q	V	L	K	A	325
-QVLKA	E	Q	V	L	K	A	75
S-VLKA	S	H	V	L	K	A	325
SQ-LKA	S	Q	L	L	K	A	350
SQV-KA	S	Q	V	D	K	A	325
SQVL-A	S	Q	V	L	Q	A	225
SQVLK-	S	Q	V	L	K	M	325
S----	S	H	L	D	Q	M	650
-Q----	E	Q	L	D	Q	M	1000
--V---	E	H	V	D	Q	M	650
---L--	E	H	L	L	Q	M	650
---K-	E	H	L	D	K	M	900
-----A	E	H	L	D	Q	A	1400
SQ----	S	Q	L	D	Q	M	625
SQVL--	S	Q	V	L	Q	M	225

Example 7. Evaluation of Activity of Orthologs In Cell Lines Expressing hoCD122

[0257] The IL2 orthologs were evaluated for activity in NKL cells (Robertson, et al (1996) Experimental Hematology 24(3):406-15). To generate the cell line that expresses the human orthogonal hCD122 (hoNKL hoRB), NKL cells were infected with a retrovirus encoding the hoRB hCD122 and co-expressing YFP (MSCV-hoRb-IRES-YFP) in accordance with procedures known in the art.

[0258] NKL and NKL hoRB cells were contacted with supernatants from 293 cells transfected with hIL2 orthologs as follows: Cells were seeded in growth medium consisting of RPMI 1640 (ThermoFisher), 10 percent fetal bovine serum (ThermoFisher), 1 percent penicillin/streptomycin (ThermoFisher), 1 percent glutamax (ThermoFisher) at 0.5 million cells per ml. After two days of culture, cells were seeded into 96-well plates (Falcon) at 25 thousand cells per well in 100 μ l growth medium. Two-fold or five-fold serial dilutions of transfected 293 cell supernatants were made in growth medium and 100 μ l of each dilution was added in duplicate to plates of NKL and NKL hoRB cells at final titrations ranging from 1:2 to 1:31,250. Plates were transferred to a humidified incubator (ThermoFisher) and incubated at 37 degrees centigrade, 5 percent carbon dioxide for three days.

[0259] Plates were removed from the incubator and kept at room temperature for 30 minutes. Plates were centrifuged 5 minutes at 400 x g and supernatants discarded. Cells were lysed by adding 50 μ l per well of a 1:1 dilution of Celltiterglo (Promega) in PBS. Cell lysates were mixed on an orbital shaker (VWR Scientific) for two minutes at 600 rpm then held at room temperature for 10 minutes. Lysates were transferred to black, clear bottom 96 well plates (Costar) and luminescence for NKL cell lysates (Figure 1) and NKL hoRB cell lysates (Figure 2) were read as counts per second in an Envision 2103 Multilabel Plate Reader (Perkin Elmer).

[0260] To compare the effect of each IL-2 variant upon NKL cell and NKL hoRB cell proliferation, celltiterglo values for cells treated with the supernatants were compared to those obtained for control cells treated with growth medium alone, with 293 supernatant from empty-vector transfection, wild-type IL-2 transfection, or supernatant from human orthogonal IL-2 transfection. The data from these experiments is presented in Figures 1 and 2 of the accompanying drawings.

Example 8. Evaluation of Activity of Orthologs In Cell Lines Expressing hoCD122

[0261] The IL2 orthologs were evaluated for activity in CD4 positive human T cell clone 3F8 cells. The CD4 positive T cell clone 3F8 was generated by activation of PBMC of a healthy donor with the EBV transformed B cell line JY in two successive rounds of Mixed Leukocyte Reactions followed by single cell cloning by limited dilution as described (Yssel and Spits (2002) Current Protocols in Immunology 7.19.1 – 7.19.12). The CD4 positive T cell clone 3F8

expresses CD25 and CD122 and proliferates in response to IL-2. To generate the cell line that expresses the human orthogonal hCD122 (ho3F8 hoRB), 3F8 cells were infected with a retrovirus encoding the hoRB hCD122 and co-expressing YFP (MSCV-hoRb-IRES-YFP) in accordance with procedures known in the art.

[0262] 3F8 and 3F8 hoRb cells were contacted with supernatants from 293 cells transfected with hIL2 orthologs as follows: Cells were grown in growth medium consisting of Yssel's medium (Iscove's modified Dulbecco's Medium (ThermoFisher), 0.25% w/v percent human albumin (Sigma), 1 percent penicillin/streptomycin (ThermoFisher), 1 percent ITS-X Insulin, Transferrin, Selenium (Gibco), 30 mg/L Transferrin (Roche), 2 mg/L Palmitic Acid (Sigma), 1 percent LA-OA-Albumin Linoleic Acid, Oleic Acid (Sigma), 1 percent human serum (Gemini) (Yssel et al (1984) J Immunol Methods 72: 219 – 227) at 0.2 million cells per ml with 50 Gy irradiated JY cells at 0.1 million cells per well and 40 Gy irradiated allogeneic PBMC at 1 million cells per mL. After ten days of culture and expansion with human IL-2 at 100 pM, cells were washed and seeded into black, clear bottom 96 well plates (Costar) at 50 thousand cells per well in 50 µl growth medium. Two-fold serial dilutions of transfected 293 cell supernatants were made in growth medium and 50 µl of each dilution was added to plates of 3F8 and 3F8 hoRB cells at final titrations ranging from 1:2 to 1:31250. Plates were transferred to a humidified incubator (ThermoFisher) and incubated at 37 degrees centigrade, 5 percent carbon dioxide for three days.

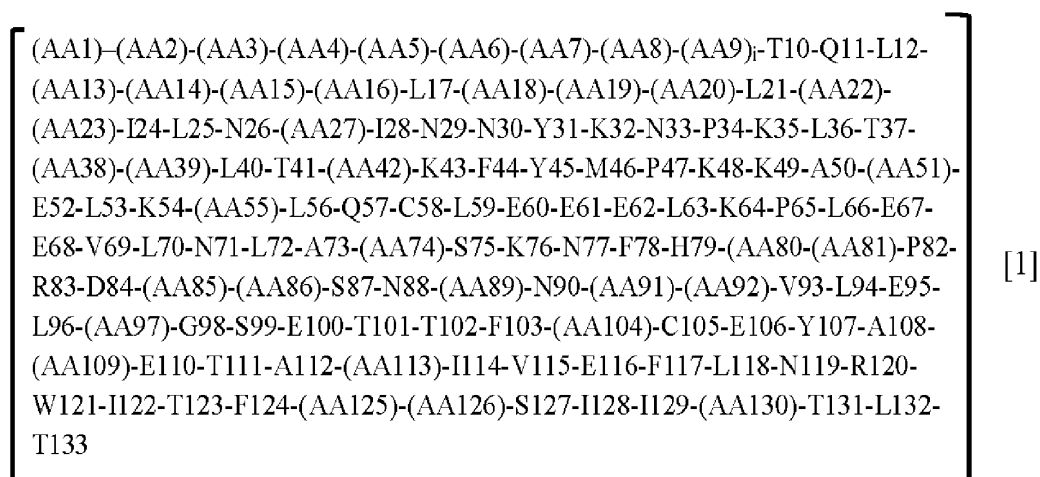
[0263] Plates were removed from the incubator and kept at room temperature for 30 minutes. Cells were lysed by adding 100 µl per well of Celltiterglo (Promega). Cell lysates were mixed on an orbital shaker (VWR Scientific) for two minutes at 600 rpm then held at room temperature for 10 minutes. Luminescence for 3F8 cell lysates (Figure 1) and 3F8 hoRB cell lysates (Figure 2) were read as counts per second in an Envision 2103 Multilabel Plate Reader (Perkin Elmer).

[0264] To compare the effect of each IL-2 variant upon 3F8 cell and 3F8 hoRB cell proliferation, celltiterglo values for cells treated with the supernatants were compared to those obtained for control cells treated with growth medium alone, with 293 supernatant from empty-vector transfection, wild-type IL-2 transfection, or supernatant from human orthogonal IL-2 transfection. The data from these experiments is presented in Table 4.

CLAIMS

WE CLAIM:

1. An hIL2 ortholog, the amino acid sequence of which has at least 90% identity to polypeptide of the Formula #1:



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, I114, I128 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, I114V, I128T, 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, I128T]; [N30D, V69A, Q74P, F103S]; [K49E, V69A, A73V, K76E]; [V69A, Q74P, T101A, T133N]; [N30S, V69A, Q74P, I128A]; [N30S, V69A, Q74P, I128T]; [K9T, Q11R, K35R, V69A, Q74P]; [A1T, M46L, K49R, E61D, V69A, H79R]; [K48E, E68D, N71T, N90H, F103S, I114V]; [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, I128T]; [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, I129, 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, Q22L, Q22Y, Q22H, Q22R, Q22N, Q22D, Q22T, Q22F, Q126H, Q126M, Q126K, Q126C, Q126D, Q126E, Q126G, Q126L, 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:



wherein where $n = 0$ or 1 and hoIL2 is a human orthogonal IL2 polypeptide variant.

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

PTSSSTKKTQLQLSQLLVLLKAILNGINNYKNPKLTRM
 LTFKFYMPKKATELKHLQCLEEELKPLEEVNLNAQSK
 NFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIV
 EFLNRWITFCQSIISTLT (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 hoCD122 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 hoCD122 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.