

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.

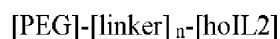
10. The hIL2 ortholog of Claim 9, wherein said hIL2 ortholog further comprises an amino acid substitution at one or more positions selected from the group consisting of L18R, L18G, L18M, L18F, L18E, L18H, L18W, L18K, L18Q, L18S, L18V, L18I, L18Y, L18H, L18D, L18T, Q22E, Q22E, Q22E, Q22E, Q22G, Q22E, 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
 LTFKFYMPKKATELKHLQCLEEELKPLEEVNLNLAQSK
 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.