

CHIMERIC ORTHOGONAL RECEPTOR PROTEINS AND METHODS OF USE

CROSS REFERENCE

- [0001] This application claims priority to U.S. Provisional Application No. 62/898,917, filed September 11, 2019 which is incorporated herein in its entirety for all purposes.

BACKGROUND

- [0002] The ability to manipulate receptors to bind and respond to modified ligands in a manner independent, or orthogonal, from the influence of the native ligands, is a significant challenge in protein engineering. Synthetic ligand-ortholog receptor pairs have been created that are orthogonal to the analogous natural interaction. Manipulation of the intracellular signaling pathways activated by orthogonal ligands is of great interest and is addressed herein.
- [0003] Orthogonal ligands and receptors are disclosed in US Patent publication 2018/0228842A, and in the international patent application US2019/021451; each herein specifically incorporated by reference.

SUMMARY

- [0004] Engineered chimeric orthogonal receptors, and methods of use thereof, are provided herein. In the chimeric orthogonal receptors, an orthogonal ligand binding domain (oLBD) derived from a first receptor is operably linked to an intracellular domain (ICD) derived from a second receptor.
- [0005] The oLBD comprises a modified extracellular domain (ECD) of a receptor such as, e.g., the extracellular domain of the CD122 IL-2 receptor. The ECD is modified to comprise sequence modifications that alter its binding specificity, such that the modified ECD binds to an orthogonal ligand, a modified counterpart of the native ligand for the receptor. Binding of the orthogonal counterpart ligand to the oLBD activates signaling via the ICD of the receptor and provides specificity for extracellular interactions with ligand. The ICD transmits the activation signal to cytoplasmic components of signaling pathways, and provides signaling specificity for these intracellular interactions, e.g., through activation of specific signal transduction pathways such as, e.g., JAK, STAT, etc. This modular approach allows an orthogonal cytokine and oLBD pair to be used in combination with a variety of different ICDs, activating signaling pathways in accordance with the ICD and providing flexibility in engineering cells for a desired response.
- [0006] An orthogonal ligand specifically binds to its counterpart oLBD. The oLBD exhibits significantly reduced binding to its endogenous ligand, including to the native counterpart of the orthogonal ligand. The orthogonal ligand exhibits significantly reduced binding to its endogenous receptors, including to the native counterpart of the orthogonal receptor. In some embodiment examples, the affinity of the orthogonal ligand for the orthogonal receptor is comparable to the affinity of the native ligand for the native receptor.

[0007] In one aspect, the present disclosure provides a chimeric orthogonal receptor polypeptide comprising: (a) an extracellular orthogonal ligand binding domain (oLBD), the oLBD comprising a modified extracellular domain (ECD) of a first receptor, the modified ECD having (i) reduced binding to its native ligand, and (ii) specific binding to an orthogonal ligand; (b) an intracellular domain (ICD) of a second receptor, the second receptor comprising one or more intracellular signaling domains; and (c) a transmembrane domain (TMD) that operably joins the oLBD and the ICD. In some embodimentexamples, engineered chimeric orthogonal receptors comprise an oLBD derived from a first receptor operably linked to the ICD of a second receptor through a transmembrane domain. In some embodimentexamples the oLBD is fused to the transmembrane domain derived from the second receptor. In other embodimentexamples the transmembrane domain is provided by the receptor from which the oLBD is derived. In other embodimentexamples, the transmembrane may be an artificial amino acid sequence derived. In other embodimentexamples, the transmembrane domain is derived from a third transmembrane protein.

[0008] In some embodimentexamples the ICD of the chimeric orthogonal receptor is a functional fragment derived from a receptor, for example derived from a cytokine receptor. In some such embodimentexamples the ICD is a functional fragment derived from a receptor and is substantially or entirely the ICD of the native receptor. In some embodimentexamples the ICD comprises one or more amino acid substitutions relative to the ICD of the native receptor. In some embodimentexamples the ICD of the chimeric receptor comprises binding sites for one or more STAT signaling proteins, *e.g.* STAT1, STAT2, STAT3, STAT4, STAT5A, STAT5B, STAT6, *etc.* In some embodimentexamples the ICD of the chimeric receptor comprises one or more amino acid residues, *e.g.* a tyrosine residue, that is phosphorylated by an intracellular kinase (*e.g.* a JAK kinase).

[0009] The intracellular signaling pathway(s) activated in response to the binding of the orthogonal ligand to the oLBD of the chimeric orthogonal receptor results in an intracellular signaling pattern characteristic of the signaling pathway(s) invoked by activation of the parent receptor from which the ICD of the chimeric receptor is derived in response to the binding of native ligand to such parent receptor. For example, the specificity and/or pattern for activation of one or more STAT signaling proteins in response to the binding of the orthogonal ligand to the chimeric orthogonal receptor may be substantially the same as for activation of the native receptor from which the ICD is derived.

[0010] In some embodimentexamples the orthogonal ligand is derived from a cytokine protein and the orthogonal receptor is derived from a cytokine receptor. In some embodimentexamples the orthogonal cytokine is an orthogonal IL-2 protein. In some embodimentexamples the orthogonal ligand is derived from the human IL-2 protein. In some such embodimentexamples the orthogonal receptor is an orthogonal IL-2 receptor beta protein, also referred to as an orthogonal CD122 protein. In some embodimentexamples, the extracellular domain of the chimeric orthogonal receptor is derived from human CD122. In some embodimentexamples, the ECD of the chimeric orthogonal receptor comprises a polypeptide of SEQ ID NO: (insert number for orthoCD122). In some embodimentexamples the ICD of the chimeric receptor comprises a polypeptide sequence derived from an ICD of a common gamma chain receptor (CD132) family member other than CD122. In some embodimentexamples the ICD of the chimeric orthogonal receptor comprises the ICD of a common gamma chain receptor family member selected from group consisting essentially of the IL-4 receptor (IL4R, IL-4R α , CD124), the IL-7 receptor (IL7R, IL-7R α , CD127), the IL-9 receptor (IL-9R, CD129), the IL-15R α

(CD215), and the IL-21 receptor (IL-21R, CD360). In some embodimentexamples, the ICD of the chimeric orthogonal receptor is derived from the ICD of the erythropoietin receptor (EpoR).

[0011] In one aspect, the present disclosure provides a nucleic acid sequence encoding a chimeric orthogonal receptor polypeptide disclosed herein. In another aspect, the present disclosure provides a recombinant expression vector comprising a nucleic acid sequence disclosed herein in operable association with one or more expression control sequences. In yet another aspect, the present disclosure provides an engineered cell transformed with a recombinant expression vector disclosed herein. In some embodimentexamples, an engineered cell is provided, in which the engineered cell has been modified by introduction of a chimeric orthogonal receptor of the inventiondisclosure, the chimeric orthogonal receptor comprising an oLBD from a first receptor operably linked through a transmembrane domain to an ICD derived from a second receptor. Any cell can be used for this purpose. In some embodimentexamples the cell is a mammalian cell. In some embodimentexamples the cell is a human cell. In some embodimentexamples, the cell is a mammalian immune cell. In some embodimentexamples the cell is a T cell, including without limitation naïve CD8⁺ T cells, cytotoxic CD8⁺ T cells, naïve CD4⁺ T cells, helper T cells, e.g. T_{H1}, T_{H2}, T_{H9}, T_{H11}, T_{H22}, T_{FH}; regulatory T cells, e.g. T_{R1}, natural T_{Reg}, inducible T_{Reg}; memory T cells, e.g. central memory T cells, effector memory T cells, NKT cells, αβ T cells, γδ T cells and engineered variants of such T cells including CAR T cells; tumor infiltrating lymphocytes (TILs), etc. In other embodimentexamples the engineered cell is a stem cell, including but not limited to a hematopoietic stem cell, an NK cell, a macrophage, or a dendritic cell. In some embodimentexamples the cell is genetically modified in an *ex vivo* procedure, prior to transfer into a subject, to introduce a coding sequence for the chimeric receptor. Genetically engineered to express a chimeric orthogonal receptor and an engineered T cell receptor. Examples of engineered T cell receptors include but are not limited to chimeric antigen receptors, engineered TCR and the like. In some embodimentexamples, the present inventiondisclosure provides a method of preparing a cell comprising a cell comprising a chimeric orthogonal receptor and an engineered T cell receptor, the method comprising the isolation of a cell from a subject and introducing into the isolated cell a nucleic acid sequence encoding an engineered T cell receptor, chimeric antigen receptor, etc. In some embodimentexamples, the disclosure provides an engineered cell expressing a chimeric orthogonal receptor a cell (or population of cells) is obtained from a subject and genetically modified *ex vivo* to introduce a vector, the vector comprising a nucleic acid encoding a chimeric orthogonal receptor of the present disclosure and a engineered T cell receptor including but not limited to a chimeric antigen receptor (CAR). The engineered cell expressing the chimeric orthogonal receptor can be provided in a unit dose for therapy, and can be allogeneic, autologous, *etc.* with respect to an intended recipient.

[0012] In some embodimentexamples, a vector comprising a polynucleotide coding sequence that encodes a chimeric orthogonal receptor of the inventiondisclosure is provided, where the coding sequence is operably linked to a promoter active in the desired cell for expression of the chimeric orthogonal receptor, where an active promoter may be constitutively active or may be regulated. Various vectors are known in the art and can be used for this purpose, *e.g.* replication competent, replication deficient or conditionally replicating viral vectors, plasmid vectors, minicircle vectors. In some embodimentexamples, the vector may be integrated into the target cell genome or can be episomally maintained.

[0013] The vectors provided herein may be provided in a kit, optionally combined with a orthogonal ligand or vector encoding an orthogonal ligand that binds to and activates the chimeric orthogonal receptor. In some embodimentexamples the vector containing the coding sequence for the orthogonal ligand is operably linked to a high expression promoter active in a target cell. In other embodimentexamples, a kit is provided in which the vector encoding the orthogonal chimeric receptor is provided with a purified composition of the orthogonal ligand, e.g. in a unit dose, packaged for administration to a patient (e.g. a prefilled syringe). In still some other embodimentexamples, a kit is provided in which the vector encoding the chimeric orthogonal receptor is provided with a vector encoding the orthogonal ligand to enable expression of the chimeric orthogonal receptor in a cell and also expression of the orthogonal ligand intended for secretion by the same cell (or other cell) to enable autocrine, endocrine, or paracrine ligand/receptor signaling.

[0014] In one aspect, the present disclosure provides a method of inducing intracellular signaling in an engineered cell disclosed herein, the method comprising contacting the engineered cell with an effective amount of an orthogonal ligand that specifically binds to the orthogonal ligand binding domain of the chimeric orthogonal receptor polypeptide. In another aspect, the present disclosure provides a pharmaceutical formulation comprising an engineered cell disclosed herein and one or more pharmaceutically acceptable physiologically acceptable carriers, excipients or stabilizers. In yet another aspect, the present disclosure provides a kit comprising a pharmaceutical formulation disclosed herein and a pharmaceutical formulation comprising an orthogonal ligand and instructions for use. In some embodimentexamples a therapeutic method is provided, the method comprising introducing into a subject in need thereof a therapeutically effective quantity of an engineered cell population, wherein all or a part of the cell population has been modified by introduction of a nucleic acid sequence encoding a chimeric orthogonal receptor of the inventiondisclosure. The cell population may be engineered *ex vivo*, and may be autologous or allogeneic with respect to the subject. In some embodimentexamples, the introduced cell population is contacted with the cognate orthogonal ligand *in vivo* following administration of the engineered cells. In some embodimentexamples the engineered cell is a T cell. In some embodimentexamples the engineered cell is a CAR T cell.

BRIEF DESCRIPTION OF THE DRAWINGS

[0015] The invention may be 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.

[0016] Figure 1, panel A provides a schematic of the crystal structure of IL2-IL2R complex and schematic diagrams of murine orthogonal IL-2R β (mIL2R β) chimeric proteins illustrating one embodiment of the present disclosure, in particular illustrating (a) a murine ortho IL-2R β and IL2R β transmembrane and intracellular domains ("moR β (full length)", SEQ ID NO:2), (b) a chimeric orthogonal recepto9r comprising the extracellular domain of a murine orthogonal IL-2R β (moRB ECD) and the transmembrane (TM) and intracellular domains of murine IL-7 receptor mil7ICD (SEQ ID NO:4), and (c) a chimeric orthogonal receptor comprising the extracellular, transmembrane and a sequence partial intracellular domain of the murine ortho IL-2R β and "mIL-7RpYtail"(SEQ ID NO:6). Additionally, partial protein sequences of the chimeric receptors are provided illustrating that the C-termini with STAT5 signaling protein binding site (boxed), which binding site includes a tyrosine target residue (pY) for phosphorylation. Figure 1, Panel B provides data from an experiment where T cells isolated from BL6 mice were activated by

WHAT IS CLAIMED IS:

1. A chimeric orthogonal ~~chimeric~~-receptor polypeptide, comprising:
 - (a) an extracellular orthogonal ligand binding domain (oLBD), the oLBD comprising a modified extracellular domain (ECD) of a first receptor, the modified ECD having of an orthogonal receptor which
 - (i) ~~has significantly~~ reduced binding to its native ligand; ~~and which~~
 - (ii) ~~comprises at least one amino acid substitution relative to the sequence of the native protein~~specific binding to an orthogonal ligand;
 - (b) an intracellular domain (ICD) of a second receptor ~~which binds to one or more JAK/STAT proteins and is not the orthogonal receptor, the second receptor comprising one or more intracellular signaling domains~~; and
 - (c) a transmembrane domain (TMD) that operably joins the oLBD and the ICD.
2. The chimeric orthogonal ~~chimeric~~-receptor polypeptide of claim 1, wherein both the TMD and the ICD are derived from the second receptor.
3. The chimeric orthogonal ~~chimeric~~-receptor polypeptide of claim 1 or claim 2, wherein the second receptor is a cytokine receptor.
4. The chimeric orthogonal ~~chimeric~~-receptor polypeptide of claim 3, wherein the cytokine ~~second~~-receptor is selected from the group consisting of CD121 α ; CDw121 β ; IL-18R α ; IL-18R β ; CD122; CD25; CD124; CD213; CD127; IL-9R; CD21 α 1; CD213 α 2; IL-15R α ; CD131; CD125; CD131; CD126; CD130; IL-11R α ; CD114; CD212; LIFR; OSMR; CD210; IL-20R α , IL-20R β ; IL-14R; CD4; CD217; CD118; CD119; CD40; LT β R; CD120 α ; CD120 β ; CD137 (4-1BB); BCMA, TACI; CD27; CD30; CD95 (Fas); GITR; LT β R; HVEM; OX40; BCMA, TACI; TRAILR1-4; Apo3; RANK, OPG; TGF- β R1; TGF- β R2; TGF- β R3; EpoR; TpoR; Flt-3; CD117; CD115; and CDw136;.
5. The chimeric orthogonal ~~chimeric~~-receptor polypeptide of claim 3, wherein the cytokine ~~second~~ receptor is a cytokine receptor associated with the common gamma chain (CD132).
6. The chimeric orthogonal ~~chimeric~~-receptor polypeptide of claim 5, wherein the cytokine ~~second~~ receptor associated with the common gamma chain is selected from the group consisting of the IL-4 receptor (IL-4R, IL-4R α , CD124), the IL-7 receptor (IL-7R, IL-7R α , CD127), the IL-9 receptor (IL-9R, CD129), the IL-15R α receptor (CD215), and the IL-21 receptor (IL-21R α , CD360).

7. The chimeric orthogonal ~~chimeric~~-receptor polypeptide of claim 3, wherein the ~~cytokine~~~~second~~ receptor is the erythropoietin receptor (EpoR).

8. The chimeric orthogonal ~~chimeric~~-receptor polypeptide of any of claims 1-7, wherein the ~~first receptor isoLBD is an orthogonal variant of~~ the human CD122 ~~ligand-binding domain~~receptor.

9. The chimeric orthogonal ~~chimeric~~-receptor polypeptide of claim 8, wherein the CD122 receptor is human CD122 ~~modified comprising~~ at least one amino acid substitution or deletion ~~one or more~~at residues ~~selected from~~ R41, R42, Q70, K71, T73, T74, V75, S132, H133, Y134, F135, E136, and Q214.

10. The chimeric orthogonal ~~chimeric~~-receptor polypeptide of claim 9, wherein the CD122 receptor comprises an amino acid substitutions at residues H133 and Y134.

~~11. The orthogonal chimeric receptor polypeptide of claim 8 wherein the CD122 receptor is CD122 modified at one or more residues selected from R42, F67, Q71, S72, T74, S75, V76, S133, H134, Y135, I136, E137, R215.~~

~~11. The chimeric orthogonal receptor polypeptide of claim 10, wherein the amino acid substitution at position H133 is selected from the group consisting of H133D, H133E and H133K and the amino acid substitution at position Y134 is selected from the group consisting of Y134F, Y134E and Y134R.~~

~~12. The chimeric orthogonal receptor polypeptide of claim 11, wherein the amino acid substitution at position H133 is H133D and the amino acid substitution at position Y134 is Y134F.~~

~~13. The chimeric orthogonal receptor polypeptide of claim 12, wherein the human CD122 receptor is a polypeptide having at least 90% sequence identity to SEQ ID NO:16.~~

~~14. The chimeric orthogonal receptor polypeptide of claim 12, wherein the human CD122 receptor is a polypeptide having at least 95% sequence identity to SEQ ID NO:16.~~

~~15. The chimeric orthogonal ~~chimeric~~-receptor polypeptide of claim 1, comprising an amino acid sequence with having at least 75~~90~~% sequence identity to a chimeric orthogonal receptor selected from the group consisting any of SEQ ID NO:4, ~~6, 8, 10, 12, 14, 18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, and SEQ ID NO:28.~~~~

~~13~~16. The chimeric orthogonal ~~chimeric~~-receptor polypeptide of claim 1, comprising an amino acid sequence ~~with~~having at least 95% sequence identity to a chimeric orthogonal receptor selected from the group consisting any of SEQ ID NO:4, ~~6, 8, 10, 12, 14, 18~~, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, and SEQ ID NO:28.

17. The chimeric orthogonal receptor polypeptide of any one of claims 1-16, further comprising an N-terminally linked signal sequence.

~~14. A system for selective activation of a receptor in a cell, the system comprising:~~

~~(a) an orthogonal-chimeric receptor according to any of claims 1-13; and~~

~~(b) an engineered orthogonal ligand.~~

~~15. The system of claim 14, wherein the orthogonal-chimeric receptor is expressed by a mammalian cell.~~

~~16. The system of claim 15, wherein the cell is an immune cell or a stem cell.~~

~~17. The system of claim 16, wherein the immune cell is a T cell.~~

~~18. The system of claim 17, wherein the T cell is a CAR T cell.~~

~~19. The system of any of claims 14-18, wherein the orthogonal ligand is IL-2.~~

~~20. The system of claim 19, wherein the orthologous IL-2 is human IL-2 modified at one or more residues selected from Q13, L14, E15, H16, L19, D20, Q22, M23, G27, and N88.~~

~~21. The system of claim 19, wherein the human IL-2 is modified at one or more residues selected from E15, H16, L19, D20, Q22, and M23.~~

~~22. The system of claim 19, wherein the orthologous IL-2 is mouse IL-2, modified at one or more residues selected from H27, L28, E29, Q30, M33, D34, Q36, E37, R41, and N103.~~

~~23. The system of Claim 19, wherein the mouse IL-2 is modified at one or more residues selected from E29, Q30, M33, D34, Q36, and E37.~~

2418. A nucleic acid ~~sequence~~ encoding ~~a chimeric orthogonal~~ ~~the orthologous chimeric~~ receptor ~~polypeptide~~ of any ~~one~~ of claims 1-~~13~~17.

2519. ~~An~~ ~~A recombinant~~ expression vector comprising ~~the~~ ~~a~~ nucleic acid ~~sequence~~ of claim 2418 in operable association with one or more expression control sequences.

20. The recombinant expression vector of claim 19, wherein the vector is a viral vector.

21. The recombinant expression vector of claim 20, wherein the viral vector is a lentiviral vector or a retroviral vector.

2622. An ~~engineered~~ cell ~~transformed with a recombinant expression~~ ~~genetically engineered to comprise the~~ vector of ~~any one of~~ claims ~~2519-21~~.

23. The engineered cell of claim 22, wherein the cell is a mammalian immune cell or a mammalian stem cell.

24. The engineered cell of claim 23, wherein the immune cell is a T cell.

25. The engineered cell of claim 24, wherein the T cell is a naïve CD8 T cells, cytotoxic CD8 T cells, naïve CD4 T cells, helper T cells, e.g. TH1, TH2, TH9, TH11, TH22, TFH; regulatory T cells, e.g. TR1, natural TReg, inducible TReg; memory T cells, e.g. central memory T cells, effector memory T cells, NKT cells, abT cells, bg T cells and tumor infiltrating lymphocytes (TILs).

26. The engineered cell of claim 24 or 25, wherein the T cell is modified to express a chimeric antigen receptor (CAR).

27. The engineered cell of claim 26, wherein the ABD of the CAR specifically binds to one or more of CD19, CD20, CD30, HER2, IL-11Ra, PSCA, NCAM, NY-ESO-1, MUC1, CD123, FLT3, B7-H3, CD33, IL1RAP, CLL1 (CLEC12A) PSA, CEA, VEGF, VEGF-R2, CD22, ROR1, mesothelin, c-Met, Glycolipid F77, FAP, EGFRvIII, MAGE A3, 5T4, WT1, KG2D ligand, a folate receptor (FRa), GD2, PSMA, BCMA, and Wnt1 antigen.

28. The engineered cell of claim 27, wherein the ABD of the CAR comprises an anti-CD19 scFv.

~~27. A method of treating an individual, the method comprising introducing an immune effector cell expressing an orthogonal chimeric receptor according to any of claim 1-13 and selectively activating the cell by contacting with an orthologous ligand.~~

~~28. The method of claim 27, wherein the immune effector cell is a T cell.~~

~~29. The method of claim 28, wherein the T cell is a CAR T cell.~~

~~30. The method of any of claims 27-29, wherein the individual is treated for cancer.~~

~~31. The method of any of claims 27-29, wherein the individual is treated for autoimmune disease.~~

~~32. The method of any of claims 27-29, wherein the individual is treated for infection.~~

29. A method of inducing intracellular signaling in an engineered cell of any one of claims 22-28, the method comprising contacting the engineered cell with an effective amount of an orthogonal ligand that specifically binds to the orthogonal ligand binding domain of the chimeric orthogonal receptor polypeptide.

30. The method of claim 29, wherein the contacting occurs *in vivo*.

31. The method of claim 29, wherein the contacting occurs *in vitro*.

32. The method of any one of claims 29-31, wherein the ECD of the chimeric orthogonal receptor polypeptide has at least 95% sequence identity to SEQ ID NO:16 and the orthogonal ligand is variant human IL-2 is comprising a substitution or deletion at amino acid residues Q13, L14, E15, H16, L19, D20, Q22, M23, G27 and N88.

33. The method of claim 32, wherein the orthogonal ligand is human IL2 variant comprising one or more amino acid substitutions selected from: [Q13W], [L14M, L14W], [E15D, E15T, E15A, E15S, E15Q, E15H], [H16N, H16Q], [L19V, L19I, L19A], [D20L, D20M, D20T, D20S], [Q22S, Q22N, Q22T, Q22E, Q22K, Q22E], [M23L, M23S, M23V, M23T, M23A, M23W, M23H, M23Y, M23F, M23Q, M23Y], [G27K, G27S], [R81D, R81Y], [N88E, N88Q], and [T51I].

34. The method of claim 32, wherein the orthogonal ligand is human IL2 variant comprising a set of amino acid substitutions selected from the group consisting of: [E15S, H16Q,

L19V, D20L, Q22K, M23A]; [E15S, H16Q, L19V, D20L, Q22K, M23Q]; [E15S, H16Q, L19V, D20L, Q22K, M23T]; [E15S, H16Q, L19V, D20L, M23Q, R81Y]; [E15S, H16Q, L19V, D20L, M23Q, R81D, T51I]; [E15S, H16Q, L19V, D20L, M23Q]; [E15S, H16Q, L19V, D20L, M23A]; [E15S, H16Q, L19V, D20M; Q22K, M23L]; [E15S, H16Q, L19V, D20M; Q22K; M23S]; [E15S, H16Q, L19V, D20S; Q22K; M23S]; [E15S, H16Q, L19V, D20T; Q22K, M23L]; [E15S, H16Q, L19V, D20T; Q22K; M23S]; [E15D, H16N, L19V, D20L, Q22K, M23A]; [E15D, H16N, L19V, D20L, Q22T, M23H]; [E15D, H16N, L19V, D20L, Q22T, M23A]; [E15H, H16Q, L19I, D20L, M23T]; [E15H; H16Q; L19I; D20L; Q22K; M23T]; [E15H; H16Q; L19I; D20S; Q22K; M23L]; [L19V, D20M, Q22N, M23S]; [H16N, L19V, D20N, Q22T, M23H, G27K]; [E15Q, H16Q, L19V, D20T, Q22K, M23V]; [E15S; H16Q; L19I; D20S; Q22K; M23L]; [E15Q, L19V, D20M, Q22K, M23S]; [E15S; L19V; D20M; Q22K; M23S]; and [E15T; H16Q; L19V; D20S; M23S].

35. The method of claim 32, wherein the orthogonal ligand is a human IL2 variant comprising a set of amino acid substitutions selected from the group consisting of: [E15S, H16Q, L19V, D20L, Q22K, M23A].

36. The method of claim 32, wherein the orthogonal ligand is a polypeptide having at least 95% sequence identity to SEQ ID NO 33.

37. The method of any one of claims 32-36, wherein the orthogonal ligand is PEGylated.

38. The method of claim 37, wherein the PEG has a molecular weight of approximately 40kDa.

39. A pharmaceutical formulation comprising an engineered cell of any one of claims 22-28 and one or more pharmaceutically acceptable physiologically acceptable carriers, excipients or stabilizers.

~~33~~40. A kit comprising a pharmaceutical formulation of claim 39 and a pharmaceutical formulation comprising an orthogonal ligand and instructions for usethe system of claim 14.