

SUMMARY

[0006] Engineered orthogonal cytokine receptor/ligand pairs, and methods of use thereof, are provided. An engineered (orthogonal) cytokine specifically binds to a counterpart engineered (orthogonal) receptor. Upon binding, the orthogonal receptor activates signaling that is transduced through native cellular elements to provide for a biological activity that mimics that native response, but which is specific to an engineered cell expressing the orthogonal receptor. The orthogonal receptor exhibits significantly reduced binding to the endogenous counterpart cytokine, including the native counterpart of the orthogonal cytokine, while the orthogonal cytokine exhibits significantly reduced binding to any endogenous receptors, including the native counterpart of the orthogonal receptor. In some examples, the affinity of the orthogonal cytokine for the orthogonal receptor is comparable to the affinity of the native cytokine for the native receptor.

[0007] The process for engineering an orthogonal cytokine receptor pair may comprise the steps of: (a) engineering amino acid changes into a native receptor to disrupt binding to the native cytokine; (b) generating multiple cytokine analogs that possess selective amino acid changes into the native cytokine at contact residues for receptor binding, (c) selecting for cytokine orthologs that bind to the ortholog receptor; (d) discarding ortholog cytokines that bind significantly to the native receptor, or alternatively to steps (c) and (d); (e) selecting for receptor orthologs that bind the ortholog cytokine; (f) discarding ortholog receptors that bind to the native cytokine. In preferred examples, knowledge of the structure of the cytokine/receptor complex is used to select amino acid positions for site-directed or error prone mutagenesis. Conveniently a yeast display system can be used for the selection process, although other display and selection methods are also useful.

[0007a] In one aspect, the present disclosure refers to an engineered human IL-2 polypeptide, which (i) has significantly reduced binding to native human CD122; and which (ii) comprises at least one amino acid substitution with an amino acid other than that of the native protein at residue T51, R81, or comprises amino acid substitution M23A; and (iii) comprises amino acid substitutions at each of E15, H16, L19, D20.

[0007b] In one aspect, the present disclosure refers to nucleic acid encoding an engineered human IL-2 polypeptide of the disclosure.

[0007c] In one aspect, the present disclosure refers to an expression vector comprising the nucleic acid of the disclosure.

[0007d] In one aspect, the present disclosure refers to cell genetically engineered to comprise the vector of the disclosure.

[0008] In some examples, an engineered cell is provided, in which the cell has been modified by introduction of an orthogonal receptor of the disclosure. Any cell can be used for this purpose. In some examples 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, γδ T cells and engineered variants of such T-cells including CAR-T cells; etc. In other examples the engineered cell is a stem cell, e.g. a hematopoietic stem cell, an NK cell, a macrophage, or a dendritic cell. In some examples the cell is genetically modified in an *ex vivo* procedure, prior to transfer into a subject. The engineered cell can be provided in a unit dose for therapy, and can be allogeneic, autologous, *etc.* with respect to an intended recipient.

[0009] In some examples a vector comprising a polynucleotide coding sequence that encodes the orthogonal receptor is provided, where the coding sequence is operably linked to a promoter active in the desired cell. Various vectors are known in the art and can be used for this

purpose, e.g. viral vectors, plasmid vectors, minicircle vectors, which vectors can be integrated into the target cell genome, or can be episomally maintained. The receptor encoding vector may be provided in a kit, combined with a vector encoding an orthogonal cytokine that binds to and activates the receptor. In some examples the coding sequence for the orthogonal cytokine is operably linked to a high expression promoter and may be optimized for production. In other examples, a kit is provided in which the vector encoding the orthogonal receptor is provided with a purified composition of the orthogonal cytokine, e.g. in a unit dose, packaged for administration to a patient (e.g. a prefilled syringe). In still some other examples, a kit is provided in which the vector encoding the orthogonal receptor is provided with a vector encoding the orthogonal cytokine to enable expression of the orthogonal receptor in a cell and also expression of the orthogonal cytokine intended for secretion by the same cell to enable autocrine orthogonal cytokine-receptor signaling.

[0009a] In one aspect, the present disclosure refers to a pharmaceutical composition comprising an engineered human IL-2 polypeptide of the disclosure; and a pharmaceutically acceptable carrier.

[0009b] In one aspect, the present disclosure refers to a pre-filled syringe comprising the pharmaceutical composition of the disclosure.

[0009c] In one aspect, the present disclosure refers to a method for the recombinant preparation of composition comprising a substantially pure polypeptide of the disclosure, the method comprising: culturing a recombinant host cell of the disclosure under conditions so as to result in expression of the recombinant human IL2 polypeptide; and isolating the recombinant IL2 polypeptide.

[0009d] In one aspect, the present disclosure refers to a method for the preparation of a PEGylated IL2 polypeptide of the disclosure, the method comprising: culturing a recombinant host cell of the disclosure under conditions so as to result in expression of the recombinant human IL2 polypeptide; isolating the recombinant IL2 polypeptide; reacting the isolated recombinant IL2 polypeptide in the presence of a molar excess of a PEG molecule under conditions so as to chemically conjugate the PEG molecule the IL2 polypeptide; and isolating the PEGylated IL2 polypeptide.

[0009e] In one aspect, the present disclosure refers to a method for activation of a mammalian cell, the method comprising: (a) transfecting a mammalian cell with a vector comprising a nucleic acid sequence encoding an orthogonal human CD122 receptor, the orthogonal human CD 122 receptor comprising amino acid substitutions at H133 and Y134, the nucleic acid operably linked to expression control sequences functional in such mammalian cell such that the orthogonal human CD122 receptor is expressed in such cell; and (b) contacting the mammalian cell with an engineered human IL-2 polypeptide of the disclosure.

[0009f] In one aspect, the present disclosure refers to a use of a therapeutically effective amount of immune effector cells, the immune effector cells expressing an orthogonal human CD122 receptor comprising amino acid substitutions at H133 and Y134, in the manufacture of a medicament for use in combinatorial therapy for treating a mammalian subject suffering from a disease, disorder or condition, wherein said medicament is to be administered in combination with a therapeutically amount of an engineered human IL-2 polypeptide of the disclosure, or a recombinant vector of the disclosure.

[0009g] In one aspect, the present disclosure refers to use of a therapeutically effective amount of the population of T cells of step (d) in the manufacture of a medicament for use in combinatorial therapy for treating a mammalian subject suffering from a neoplastic disease, disorder or condition, wherein said medicament is to be administered in combination with a therapeutically effective amount of an engineered human IL-2 polypeptide of the disclosure or a recombinant vector of the disclosure, and wherein the population of T cells of step (d) is prepared by a method comprising the steps of:

(a) obtaining a quantity of blood from said subject;
(b) isolating a population of T cells from said quantity of blood;
(c) contacting said population of isolated T cells with a recombinant viral vector comprising:

a nucleic acid sequence encoding an orthogonal human CD122 receptor comprising amino acid substitutions at H133 and Y134 and a nucleic acid sequence encoding a chimeric antigen receptor the extracellular domain of said chimeric antigen receptor exhibits specific binding to a tumor antigen, such that a fraction of the population of isolated T cells are transduced by said viral vector and expresses both the chimeric antigen receptor and the orthogonal human CD122 receptor; and

(d) culturing said isolated population of cells from step (c) under T cell activating conditions, optionally in the presence of an orthogonal IL2 polypeptide of the disclosure.

[0010] In some examples a therapeutic method is provided, the method comprising introducing into a recipient in need thereof of an engineered cell population, wherein the cell population has been modified by introduction of a sequence encoding an orthogonal receptor of the disclosure. The cell population may be engineered ex vivo, and is usually autologous or allogeneic with respect to the recipient. In some examples, the introduced cell population is contacted with the cognate orthogonal cytokine *in vivo*, following administration of the engineered cells. An advantage of the present disclosure is a lack of cross-reactivity between the orthogonal cytokine and the native receptor.

BRIEF DESCRIPTION OF THE DRAWINGS.

[0011] 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.

[0012] **FIG. 1-1(ii).** Orthogonal IL-2/IL-2 receptor pairs to control T cell expansion.

[0013] **FIG. 2.** Work flow for engineering orthogonal IL-2/IL-2R β pairs.

[0014] **FIG. 3.** Sequences of orthogonal mouse IL-2R β variants.

[0015] **FIG. 4.** mIL-2R β H134D Y135F mutations abrogate wt mIL-2 binding.

[0016] **FIG. 5.** Work flow for engineering orthogonal IL-2/IL-2R β pairs.

[0017] **FIG. 6.** Sequences of characterized orthogonal mouse IL-2 variants.

[0018] **FIG. 7.** *Ortho*IL-2 variants bind *ortho*IL-2R β with affinity similar to or greater than the wild-type IL-2 and IL-2R β interaction.

[0019] **FIG. 8.** *ortho*IL-2 variants exhibit blunted activity (phosphoSTAT5) on wild-type CD25 positive and CD25 negative Splenocytes

WHAT IS CLAIMED IS:

1. An engineered human IL-2 polypeptide, which (i) has significantly reduced binding to native human CD122; and which (ii) comprises at least one amino acid substitution with an amino acid other than that of the native protein at residue T51, R81, or comprises amino acid substitution M23A; and (iii) comprises amino acid substitutions at each of E15, H16, L19, D20.

2. The engineered human IL-2 polypeptide of claim 1, wherein the polypeptide comprises one or more amino acid substitutions selected from: [E15D, E15T, E15A, E15S], [H16N, H16Q], [L19V, L19I, L19A], [D20L, D20M], [Q22S, Q22T, Q22E, Q22K, Q22E], [M23A, M23W, M23H, M23Y, M23F, M23Q, M23Y], [G27K, G27S], [R81D, R81Y], [N88E, N88Q], [T51I].

3. The engineered human IL-2 polypeptide of claim 1 or claim 2, wherein the polypeptide comprises amino acid substitutions E15S; H16Q; L19V; and D20L.

4. The engineered human IL-2 polypeptide of any of claims 1-3, further comprising amino acid substitution Q22K.

5. The engineered human IL-2 polypeptide of any of claims 1-4, comprising amino acid substitution T51I.

6. The engineered human IL-2 polypeptide of any of claims 1-5, comprising amino acid substitution R81D or R81Y.

7. The engineered human IL-2 polypeptide of claim 1 comprising a set of amino acid substitutions [E15D, H16N, L19V, D20L, Q22T, M23A].

8. The engineered human IL-2 polypeptide of claim 1 comprising a set of amino acid substitutions [E15D, H16N, L19V, D20L, Q22K, M23A].

9. The engineered human IL-2 polypeptide of claim 1 comprising a set of amino acid substitutions [E15S, H16Q, L19V, D20L, M23Q, R81D, T51I].

10. The engineered human IL-2 polypeptide of claim 1 comprising a set of amino acid substitutions [E15S, H16Q, L19V, D20L, M23Q, R81Y].

11. The engineered human IL-2 polypeptide of any of claims 1-10 or 30-41, wherein the polypeptide binds to and activates an orthogonal human CD122 protein.

12. The engineered human IL-2 polypeptide of claim 11, wherein the orthogonal human CD122 protein is modified at one or more residues selected from R41, R42, Q70, K71, T73, T74, V75, S132, H133, Y134, F135, E136, and Q214.

13. The engineered human IL-2 polypeptide of claim 12, wherein the orthogonal human CD122 protein is modified at H133 and Y134.

14. The engineered human IL-2 polypeptide of claim 13, wherein the orthogonal human CD122 protein comprises amino acid substitutions H133D and Y134F.

15. A method for activation of a mammalian cell, the method comprising:

(a) transfecting a mammalian cell with a vector comprising a nucleic acid sequence encoding an orthogonal human CD122 receptor, the orthogonal human CD 122 receptor comprising amino acid substitutions at H133 and Y134, the nucleic acid operably linked to expression control sequences functional in such mammalian cell such that the orthogonal human CD122 receptor is expressed in such cell; and

(b) contacting the mammalian cell with an engineered human IL-2 polypeptide of any of claims 1-10 or 30-41.

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

17. The method of claim 16, wherein the cell is an immune cell.

18. The method of claim 17, wherein the immune cell is a T cell.

19. The method of claim 18, wherein the T cell is a CAR-T cell.

20. A pharmaceutical composition comprising an engineered human IL-2 polypeptide of any of claims 1-10 or 30-41; and a pharmaceutically acceptable carrier.

21. A nucleic acid encoding an engineered human IL-2 polypeptide of any of claims 1-10 or 30-33.

22. An expression vector comprising the nucleic acid of claim 21.

23. A cell genetically engineered to comprise the vector of any one of claims 22, 50 or 51.

24. Use of a therapeutically effective amount of immune effector cells, the immune effector cells expressing an orthogonal human CD122 receptor comprising amino acid substitutions at H133 and Y134, in the manufacture of a medicament for use in combinatorial therapy for treating a mammalian subject suffering from a disease, disorder or condition, wherein said medicament is to be administered in combination with a therapeutically amount of an engineered human IL-2 polypeptide according to any of claims 1-10 or 30-41, or a recombinant vector of any of claims 22, 50 or 51.

25. The use of claim 24, wherein the immune effector cell is a T cell.

26. The use of claim 25, wherein the T cell is a CAR-T cell.

27. The use of any of claims 24, 25, 26, 54 or 55 wherein the disease, disorder or condition is a neoplastic disease.

28. The use of any of claims 24, 25, 26, 54 or 55, wherein the disease, disorder or condition is autoimmune disease.

29. The use of any of claims 24, 25, 26, 54 or 55, wherein the disease, disorder or condition is infection.

30. The engineered human IL-2 polypeptide of claim 1, comprising a set of amino acid substitutions: [E15S; H16Q; L19V; D20L; M23A; and Q22K.]

31. The engineered human IL-2 polypeptide of claim 1, comprising a set of amino acid substitutions: [E15S; H16Q; L19V; D20L; M23Q; R81D; and T51I.]

32. The engineered human IL-2 polypeptide of claim 1, comprising a set of amino acid substitutions: E15S; H16Q; L19V; D20L; M23Q; and R81Y.]

33. The engineered human IL-2 polypeptide of claim 1, comprising a set of amino acid substitutions: [E15S; H16Q; L19V; D20L; and M23A.]

34. The engineered human IL-2 polypeptide of any of claims 1-10 or 30-33, wherein the engineered human IL-2 polypeptide is pegylated.

35. The engineered human IL-2 polypeptide of claim 34, wherein the PEG has a molecular weight of from about 5kD to about 80kD.

36. The engineered human IL-2 polypeptide of claim 34, wherein the PEG has a molecular weight of from about 5kD to about 40kD.

37. The engineered human IL-2 polypeptide of claim 34, wherein the PEG has a molecular weight of about 40kD.

38. The engineered human IL-2 polypeptide of any of claims 34-37, wherein the PEG is a branched PEG.

39. The engineered human IL-2 polypeptide of any of claims 34-37, wherein the PEG is a linear PEG.

40. The engineered human IL-2 polypeptide of any of claims 34-37, wherein the PEG is attached to the amino terminus of the human IL2 polypeptide via a linker.

41. The engineered human IL-2 polypeptide of any of claims 1-10 or 30-33, wherein the engineered human IL-2 polypeptide is fused to an Fc domain of IgG or albumin.

42. The method of claim 19, wherein the antigen binding domain of the chimeric antigen receptor of said CAR-T cell is specifically binds to one or more antigens expressed on the surface of a target cell.

43. The method of claim 42, wherein the antigen binding domain of the chimeric antigen receptor of the CAR-T cell comprises an scFv.

44. The method of claim 42, wherein the antigen binding domain of the chimeric antigen receptor of the CAR-T cell comprises a VHH.

45. The method of claim any one of claims 42-44, wherein at least one of the one or more antigens is a tumor antigen.

46. The method of claim 45, wherein the tumor antigen is selected from the group consisting of CD19, CD20, HER2, 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), and Wnt1 antigens.

47. The method of claim 46, wherein the tumor antigen is CD19.

48. The method of claim 47, wherein the CAR-T cell is axicabtagene ciloleucel or tisagenlecleucel.

49. A pre-filled syringe comprising the pharmaceutical composition of claim 20.

50. The expression vector of claim 22, wherein the vector is a viral vector.

51. The expression vector of claim 22, wherein the vector is a non-viral vector.

52. A method for the recombinant preparation of composition comprising a substantially pure polypeptide of any one of claims 1-10 or 30-33, the method comprising:
culturing a recombinant host cell of claim 23 under conditions so as to result in expression of the recombinant human IL2 polypeptide; and
isolating the recombinant IL2 polypeptide.

53. A method for the preparation of a PEGylated IL2 polypeptide of any of claims 34-40, the method comprising:
culturing a recombinant host cell of claim 23 under conditions so as to result in expression of the recombinant human IL2 polypeptide;
isolating the recombinant IL2 polypeptide;
reacting the isolated recombinant IL2 polypeptide in the presence of a molar excess of a PEG molecule under conditions so as to chemically conjugate the PEG molecule the IL2 polypeptide; and
isolating the PEGylated IL2 polypeptide.

54. The use of claim 25, wherein the T cell is selected from the group consisting of naïve CD8⁺ T cells, cytotoxic CD8⁺ T cells, naïve CD4⁺ T cells, helper T cells; regulatory T cells; memory T cells, NK T cells, $\gamma\delta$ T cells, CAR-T cells, TILs, and TCR $\alpha\beta$ receptor knock-out cells.

55. The use of claim 26, wherein the antigen binding domain of the chimeric antigen receptor of said CAR-T cell is specifically binds to one or more antigens expressed on the surface of a target cell.

56. The use of claim 57, wherein the tumor antigen is CD19.

57. The use of claim 27, wherein the disease, disorder or condition is a neoplastic disease and wherein at least one of the one or more antigens is a tumor antigen.

58. The use of claim 56, wherein the tumor antigen is selected from the group consisting of CD19, CD20, HER2, 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), and Wnt1 antigens.

59. Use of a therapeutically effective amount of the population of T cells of step (d) in the manufacture of a medicament for use in combinatorial therapy for treating a mammalian subject suffering from a neoplastic disease, disorder or condition, wherein said medicament is to be administered in combination with a therapeutically effective amount of an engineered human IL-2 polypeptide according to any of claims 1-10 or 30-41 or a recombinant vector of any of claims 22, 50 or 51, and wherein the population of T cells of step (d) is prepared by a method comprising the steps of:

- (a) obtaining a quantity of blood from said subject;
- (b) isolating a population of T cells from said quantity of blood;
- (c) contacting said population of isolated T cells with a recombinant viral vector comprising:

- a nucleic acid sequence encoding an orthogonal human CD122 receptor comprising amino acid substitutions at H133 and Y134 and a nucleic acid sequence encoding a chimeric antigen receptor the extracellular domain of said chimeric antigen receptor exhibits specific binding to a tumor antigen, such that a fraction of the population of isolated T cells are transduced by said viral vector and expresses both the chimeric antigen receptor and the orthogonal human CD122 receptor; and

- (d) culturing said isolated population of cells from step (c) under T cell activating conditions, optionally in the presence of an orthogonal IL2 polypeptide of according to any one of claims 1-10 or 31-42.

60. The use of claim 59, wherein the subject is one that has been lymphodepleted prior to the administration of the medicament.

61. The use of any one of claims 24-27 and 54-60, wherein one or more additional therapeutic agents is to be further administered.