

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 receptor; and which (ii) comprises a set of amino acid substitutions selected from [E15S; H16Q; L19V; D20L; M23A; and Q22K]; [E15S; H16Q; L19V; D20L; M23Q; R81D; and T51I]; [E15S; H16Q; L19V; D20L; M23Q; and R81Y] and [E15S; H16Q; L19V; D20L; and M23A] wherein numbering of amino acid positions is made relative to SEQ ID NO:4.

[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 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; pegylating the isolated recombinant IL-2; and isolating the PEGylated IL2 polypeptide.

[0009e] In one aspect, the present disclosure refers to an in vitro method for activation of a mammalian immune cell, the method comprising: (a) transfecting a mammalian immune cell with a vector comprising a nucleic acid sequence encoding an orthogonal human CD122 receptor, the orthogonal human CD122 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 immune 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 T 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 neoplastic, autoimmune or infection disease, wherein said medicament is to be administered in combination with a therapeutically amount of an engineered human IL-2 polypeptide 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 (c) in the manufacture of a medicament for use in combinatorial therapy for treating a mammalian subject suffering from a neoplastic disease, wherein said medicament is to be administered in combination with a therapeutically effective amount of an engineered human IL-2 polypeptide of the disclosure and wherein the population of T cells of step (c) is prepared by a method comprising the steps of:

WHAT IS CLAIMED IS:

1. An engineered human IL-2 polypeptide, which (i) has significantly reduced binding to native human CD122 receptor; and which (ii) comprises a set of amino acid substitutions selected from [E15S; H16Q; L19V; D20L; M23A; and Q22K]; [E15S; H16Q; L19V; D20L; M23Q; R81D; and T51I]; [E15S; H16Q; L19V; D20L; M23Q; and R81Y] and [E15S; H16Q; L19V; D20L; and M23A] wherein numbering of amino acid positions is made relative to SEQ ID NO:4.
2. The engineered human IL-2 polypeptide of claim 1 comprising a set of amino acid substitutions [E15S; H16Q; L19V; D20L; M23A; and Q22K].
3. The engineered human IL-2 polypeptide of claim 1 comprising a set of amino acid substitutions [E15S; H16Q; L19V; D20L; M23Q; R81D; and T51I].
4. The engineered human IL-2 polypeptide of claim 1 comprising a set of amino acid substitutions [E15S; H16Q; L19V; D20L; M23Q; and R81Y].
5. The engineered human IL-2 polypeptide of claim 1 comprising a set of amino acid substitutions [E15S; H16Q; L19V; D20L; and M23A].
6. The engineered human IL-2 polypeptide of any of claims 1-5, wherein the polypeptide binds to and activates an orthogonal human CD122 receptor.
7. The engineered human IL-2 polypeptide of claim 6, wherein the orthogonal human CD122 receptor is modified at one or more residues selected from R41, R42, Q70, K71, T73, T74, V75, S132, H133, Y134, F135, E136, and Q214.
8. The engineered human IL-2 polypeptide of claim 7, wherein the orthogonal human CD122 receptor is modified at H133 and Y134 wherein numbering of amino acid positions is made relative to SEQ ID NO:1.
9. The engineered human IL-2 polypeptide of claim 7, wherein the orthogonal human CD122 receptor comprises an amino acid substitution selected from H133 or Y134 wherein numbering of amino acid positions is made relative to SEQ ID NO:1.

10. The engineered human IL-2 polypeptide of claim 7, wherein the orthogonal human CD122 receptor comprises an amino acid substitution selected from H133D or Y134Y wherein numbering of amino acid positions is made relative to SEQ ID NO:1.

11. The engineered human IL-2 polypeptide of claim 8, wherein the orthogonal human CD122 receptor comprises amino acid substitutions H133D and Y134F wherein numbering of amino acid positions is made relative to SEQ ID NO:1.

12. An in vitro method for activation of a mammalian immune cell, the method comprising:
(a) transfecting a mammalian immune cell with a vector comprising a nucleic acid sequence encoding an orthogonal human CD122 receptor, the orthogonal human CD122 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 immune cell with an engineered human IL-2 polypeptide of any of claims 1-5.

13. The method of claim 12, wherein the immune cell is a T cell.

14. The method of claim 13, wherein the T cell is a CAR-T cell.

15. A pharmaceutical composition comprising an engineered human IL-2 polypeptide of any of claims 1-5; and a pharmaceutically acceptable carrier.

16. A nucleic acid encoding an engineered human IL-2 polypeptide of any of claims 1-5.

17. An expression vector comprising the nucleic acid of claim 16.

18. A cell genetically engineered to comprise the vector of claim 17.

19. Use of a therapeutically effective amount of T 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 neoplastic, autoimmune or infection disease, 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 1-5.

20. The use of claim 19, wherein the T cell is a CAR-T cell.
21. The use of any of claims 19 or 20, wherein the disease is a neoplastic disease.
22. The use of any of claims 19 or 20, wherein the disease is autoimmune disease.
23. The use of any of claims 19 or 20, wherein the disease is infection.
24. The engineered human IL-2 polypeptide of any of claims 1-5, wherein the engineered human IL-2 polypeptide is pegylated.
25. The engineered human IL-2 polypeptide of claim 24, wherein the PEG has a molecular weight of from about 5kD to about 80kD.
26. The engineered human IL-2 polypeptide of claim 24, wherein the PEG has a molecular weight of from about 5kD to about 40kD.
27. The engineered human IL-2 polypeptide of claim 24, wherein the PEG has a molecular weight of about 40kD.
28. The engineered human IL-2 polypeptide of any of claims 24-27, wherein the PEG is a branched PEG.
29. The engineered human IL-2 polypeptide of any of claims 24-27, wherein the PEG is a linear PEG.
30. The engineered human IL-2 polypeptide of any of claims 24-27, wherein the PEG is attached to the amino terminus of the human IL2 polypeptide via a linker.
31. The engineered human IL-2 polypeptide of any of claims 1-5, wherein the engineered human IL-2 polypeptide is fused to an Fc domain of IgG or albumin.
32. The method of claim 14, wherein the antigen binding domain of the chimeric antigen receptor of said CAR-T cell specifically binds to one or more antigens expressed on the surface of a target cell.

33. The method of claim 32, wherein the antigen binding domain of the chimeric antigen receptor of the CAR-T cell comprises an scFv.

34. The method of claim 32, wherein the antigen binding domain of the chimeric antigen receptor of the CAR-T cell comprises a VHH.

35. The method of claim any one of claims 32-34, wherein at least one of the one or more antigens is a tumor antigen.

36. The method of claim 35, 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.

37. The method of claim 36, wherein the tumor antigen is CD19.

38. The method of claim 37, wherein the CAR-T cell is axicabtagene, ciloleucel, or tisagenlecleucel.

39. A pre-filled syringe comprising the pharmaceutical composition of claim 15.

40. The expression vector of claim 17, wherein the vector is a viral vector.

41. The expression vector of claim 17, wherein the vector is a non-viral vector.

42. A method for the recombinant preparation of composition comprising a polypeptide of any one of claims 1-5, the method comprising:
culturing a recombinant host cell of claim 18 under conditions so as to result in expression of the recombinant human IL2 polypeptide; and
isolating the recombinant IL2 polypeptide.

43. A method for the preparation of a PEGylated IL2 polypeptide of any of claims 24-30, the method comprising:
culturing a recombinant host cell of claim 18 under conditions so as to result in expression of the recombinant human IL2 polypeptide;
isolating the recombinant IL2 polypeptide;

pegylating the isolated recombinant IL-2; and
isolating the PEGylated IL-2 polypeptide.

44. The use of claim 19, 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 T-cells.

45. The use of claim 20, wherein the antigen binding domain of the chimeric antigen receptor of said CAR-T cell specifically binds to one or more antigens expressed on the surface of a target cell.

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

47. The use of claim 45, wherein the disease is a neoplastic disease and wherein at least one of the one or more antigens is a tumor antigen.

48. The use of claim 47, 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.

49. Use of a therapeutically effective amount of the population of T cells of step (c) in the manufacture of a medicament for use in combinatorial therapy for treating a mammalian subject suffering from a neoplastic disease, 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 1-5 and wherein the population of T cells of step (c) is prepared by a method comprising the steps of:

(a) isolating a population of T cells in vitro from a quantity of blood obtained from a subject;

(b) 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

(c) culturing said isolated population of cells from step (b) under T cell activating conditions, optionally in the presence of an orthogonal IL2 polypeptide of according to any one of claims 1-5.

50. The use of claim 49, wherein the subject is one that has been lymphodepleted prior to the administration of the medicament.

51. The use of any one of claims 19-21 and 44-50, wherein one or more additional therapeutic agents is to be further administered.