

WHAT IS CLAIMED IS:

1. A variant human IL-2 protein comprising one or more amino acid changes, wherein the one or more amino acid changes is selected from the group consisting of: an amino acid substitution to methionine at position 27 (G27M), an amino acid substitution at position 28, an amino acid substitution to alanine at position 31 (Y31A), an amino acid substitution at position 32, an amino acid substitution to serine at position 35 (K35S), an amino acid substitution at position 39, an amino acid substitution at position 52, an amino acid deletion at position 82 (P82Δ), and an amino acid substitution to isoleucine at position 115 (V115I), relative to the amino acid sequence of wild-type human IL-2 protein represented by SEQ ID NO: 19.
2. A variant human IL-2 protein having greater potency for activation of IL-2 signaling pathways in cells lacking CD25 expression relative to wild-type IL-2, comprising one or more amino acid substitutions relative to wild-type human IL-2.
3. The variant human IL-2 protein of claim 2, wherein the one or more amino acid substitutions relative to wild-type human IL-2 is selected from the group consisting of: an amino acid substitution to methionine at position 27 (G27M), an amino acid substitution at position 28, an amino acid substitution to alanine at position 31 (Y31A), an amino acid substitution at position 32, an amino acid substitution to serine at position 35 (K35S), an amino acid substitution at position 39, an amino acid substitution at position 52, and an amino acid substitution to isoleucine at position 115 (V115I).
4. The variant human IL-2 protein of claim 1 or 3 further comprising an amino acid deletion at position 81 (R81Δ) or a substitution at position 81.
5. The variant human IL-2 protein of claim 1 or 3 further comprising one or more amino acid substitutions at positions selected from the group consisting of: 69, 72, a region encompassing amino acid residues 74-80, 85, 86, and 92.
6. The variant human IL-2 protein of any one of claims 1 or 3-5, wherein the amino acid substitution at position 28 comprises a substitution to leucine (I28L).

7. The variant human IL-2 protein of any one of claims 1 or 3-6, wherein the amino acid substitution at position 32 is selected from the group consisting of: a substitution to aspartic acid (K32D), a substitution to glutamic acid (K32E), and a substitution to serine (K32S).

5 8. The variant human IL-2 protein of any one of claims 1 or 3-7, wherein the amino acid substitution at position 39 is selected from the group consisting of: a substitution to leucine (M39L) and a substitution to isoleucine (M39I).

10 9. The variant human IL-2 protein of any one of claims 1 or 3-8, wherein the amino acid substitution at position 52 is selected from the group consisting of: a substitution to serine (E52S), a substitution to aspartic acid (E52D), a substitution to asparagine (E52N), and a substitution to threonine (E52T).

15 10. The variant human IL-2 protein of any one of claims 5-9, wherein the amino acid substitution at position 69 comprises a substitution to alanine (V69A).

20 11. The variant human IL-2 protein of any one of claims 5-10, wherein the amino acid substitution at position 72 is selected from the group consisting of: a substitution to alanine (L72A), a substitution to glutamine (L72Q), a substitution to aspartic acid (L72D), a substitution to histidine (L72H), and a substitution to threonine (L72T).

12. The variant human IL-2 protein of any one of claims 4-11, wherein the amino acid substitution at position 81 comprises a substitution to aspartic acid (R81D).

25 13. The variant human IL-2 protein of any one of claims 5-12, wherein the amino acid substitution at position 85 is selected from the group consisting of: a substitution to valine (L85V), a substitution to methionine (L85M), and a substitution to alanine (L85A).

30 14. The variant human IL-2 protein of any one of claims 5-13, wherein the amino acid substitution at position 86 comprises a substitution to valine (I86V).

15. The variant human IL-2 protein of any one of claims 5-14, wherein the amino acid substitution at position 92 comprises a substitution to phenylalanine (I92F).

16. The variant human IL-2 protein of any one of claims 5-15, wherein the amino acid substitution at the region encompassing amino acid residues 74-80 comprises an amino acid sequence of GDDPKTI, DSTDETV, DSTDERI, DSTDSRI, SKDQDKV, SKDQKKV, SDDQDKV, ADDKDTI, or ADDQDKI.

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17. The variant human IL-2 protein of claim 4 or 16 comprising amino acid changes G27M, I28L, K32D, M39L, E52S, substitution of the region encompassing amino acid residues 74-80 with GDDPKTI, R81Δ, P82Δ, and V115I.

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18. The variant human IL-2 protein of claim 17 wherein the amino acid changes comprise or consist of G27M, I28L, K32D, M39L, E52S, V69A, L72A, substitution of the region encompassing amino acid residues 74-80 with GDDPKTI, R81Δ, P82Δ, L85V, and V115I.

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19. The variant human IL-2 protein of claim 17 wherein the amino acid changes comprise or consist of G27M, I28L, K32D, M39L, V69A, L72A, substitution of the region encompassing amino acid residues 74-80 with GDDPKTI, R81Δ, P82Δ, and (V115I).

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20. The variant human IL-2 protein of claim 17 wherein the amino acid changes comprise or consist of G27M, I28L, K32D, M39L, E52S, V69A, L72A, substitution of the region encompassing amino acid residues 74-80 with GDDPKTI, R81Δ, P82Δ, and V115I.

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21. The variant human IL-2 protein of claim 17 wherein the amino acid changes comprise or consist of G27M, I28L, K32D, M39L, E52S, V69A, L72Q, substitution of the region encompassing amino acid residues 74-80 with GDDPKTI, R81Δ, P82Δ, and V115I.

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22. The variant human IL-2 protein of claim 17 wherein the amino acid changes comprise or consist of G27M, I28L, K32D, M39L, E52S, V69A, L72D, substitution of the region encompassing amino acid residues 74-80 with GDDPKTI, R81Δ, P82Δ, and V115I.

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23. The variant human IL-2 protein of claim 4 or 16 wherein the amino acid changes comprise or consist of G27M, I28L, K32E, E52S, V69A, L72Q, substitution of the region encompassing amino acid residues 74-80 with GDDPKTI, R81Δ, P82Δ, and V115I.

24. The variant human IL-2 protein of claim 4 or 16 wherein the amino acid changes comprise or consist of G27M, I28L, K32D, V69A, L72Q, substitution of the region encompassing amino acid residues 74-80 with DSTDETV, R81Δ, P82Δ, and V115I.

5 25. The variant human IL-2 protein of claim 4 or 16 wherein the amino acid changes comprise or consist of G27M, I28L, K32D, M39L, E52S, V69A, L72A, substitution of the region encompassing amino acid residues 74-80 with DSTDERI, R81Δ, P82Δ, L85V, I86V, I92F, and V115I.

10 26. The variant human IL-2 protein of claim 4 or 16 wherein the amino acid changes comprise or consist of G27M, I28L, K32D, M39L, E52S, V69A, L72Q, substitution of the region encompassing amino acid residues 74-80 with DSTDSRI, R81Δ, P82Δ, and V115I.

15 27. The variant human IL-2 protein of claim 4 or 16 wherein the amino acid changes comprise or consist of G27M, I28L, K32D, M39L, E52S, V69A, substitution of the region encompassing amino acid residues 74-80 with SKDQKKV, R81Δ, P82Δ, L85V, I86V, I92F, and V115I.

20 28. The variant human IL-2 protein of claim 4 or 16 wherein the amino acid changes comprise or consist of G27M, I28L, K32S, M39L, E52S, V69A, substitution of the region encompassing amino acid residues 74-80 with SDDQDKV, R81Δ, P82Δ, L85V, I86V, I92F, and V115I.

25 29. The variant human IL-2 protein of claim 4 or 16 wherein the amino acid changes comprise or consist of G27M, I28L, K32D, V69A, L72H, substitution of the region encompassing amino acid residues 74-80 with ADDKDTI, R81Δ, P82Δ, and V115I.

30 30. The variant human IL-2 protein of claim 4 or 16 wherein the amino acid changes comprise or consist of G27M, I28L, Y31A, K32S, K35S, V69A, L72T, substitution of the region encompassing amino acid residues 74-80 with ADDQDKI, R81Δ, P82Δ, and V115I.

31. The variant human IL-2 protein of claim 4 or 16 wherein the amino acid changes comprise or consist of G27M, I28L, K32D, V69A, R81D, and V115I.

32. The variant human IL-2 protein of claim 31 wherein the amino acid changes comprise or consist of G27M, I28L, K32D, V69A, L72Q, R81D, and V115I.

33. The variant human IL-2 protein of claim 4 or 16 wherein the amino acid changes
5 comprise or consist of G27M, I28L, K32D, M39L, E52S, V69A, R81D, and V115I.

34. The variant human IL-2 protein of claim 33 wherein the amino acid changes comprise or consist of G27M, I28L, K32D, M39L, E52S, V69A, L72Q, R81D, and V115I.

10 35. The variant human IL-2 protein of claim 4 wherein the amino acid changes consist of G27M, I28L, K32D, L72Q, and R81D.

36. The variant human IL-2 protein of claim 1, wherein the variant protein has a greater potency for activation of IL-2 signaling pathways in cells lacking CD25 expression.
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37. The variant human IL-2 protein of any one of the preceding claims, wherein the variant IL-2 protein has an EC₅₀ for activation of IL-2 signaling pathways in cells lacking CD25 expression at least two-fold lower than the wild-type protein.

20 38. The variant human IL-2 protein of any one of the preceding claims, wherein the variant IL-2 protein has increased binding affinity for human CD122.

39. The variant human IL-2 protein of any one of the preceding claims, wherein the variant IL-2 protein has a greater potency for activation of IL-2 signaling pathways in cells lacking CD25
25 expression, but does not have a substantially increased binding affinity relative to the wild type IL-2 protein.

40. The variant human IL-2 protein of any one of the preceding claims, wherein the variant human IL-2 protein is fused to a human IgG Fc domain.
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41. The variant human IL-2 protein of claim 40, wherein IgG Fc domain is an IgG1, IgG2, IgG3, or IgG4 Fc domain.

42. A nucleic acid encoding a variant human IL-2 protein according to any one of the preceding claims.

43. A vector comprising a nucleic acid according to claim 42.

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44. A recombinant cell comprising the nucleic acid according to claim 42 or the vector according to claim 43.

45. A pharmaceutical composition comprising a variant human IL-2 protein of any one of claims 1-41, and a pharmaceutically acceptable carrier.

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46. A method of treating disease in a subject in need thereof, the method comprising administering to the patient a variant human IL-2 protein according to any one of claims 1-41, or the pharmaceutical composition of claim 45.

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47. A method of treating a disease in need of expansion of T cells, the method comprising administering an effective amount of a variant human IL-2 protein according to any of claims 1-41 or the pharmaceutical composition of claim 45.

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48. The method of claim 46 or 47, wherein the disease is a hyperproliferative disease.

49. The method of any one of claims 46-48 wherein the disease is cancer.

50. A method for in silico design of a cytokine based on crystallographic topology of the ligand receptor interaction, the method comprising: identifying locations where packing can be improved in order to increase binding; redesigning core positions and testing in a computer model to identify positions where improvements can be introduced: generating backbone structure ensembles while allowing the entire core to redesign while monitoring the residue changes in the identified regions; visually selecting a subset of residues that improve packing; holding identities of the subset and iteratively performing the redesign until the sequences converge; generating a coding sequence for the in silico designed cytokine.

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