CLAIMS

WHAT IS CLAIMED IS:

1. A chimeric polypeptide comprising:

i) an antigen-binding region,

ii) a transmembrane region,

and

iii) a spacer region connecting said trans-membrane region with said antigen binding region, wherein said spacer region comprises a stalk region(s) comprising at least one dimerization site, and a stalk extension region (s'-n), said stalk extension region comprising fewer dimerization sites as compared to said stalk region.

2. A chimeric polypeptide comprising:

i) an antigen-binding region,

ii) a transmembrane region,

and

iii) a spacer region connecting said trans-membrane region with said antigen binding region, wherein said spacer region comprises a stalk region which is from about 20 to about 60 amino acids in length and comprises at least one dimerization site, and a stalk extension region comprising from about 1 to about 5 times the length of the stalk region as measured by number of amino acids.

3. A chimeric polypeptide comprising:

i) an antigen-binding region,

ii) a transmembrane region, and

iii) a spacer region connecting said trans-membrane region with said antigen binding region, wherein said spacer region comprises a stalk region designated as "s" and at least one stalk extension region, designated as "s' n,"

wherein n represents the number of units of s' in the space region, and

wherein n is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20.

4. The chimeric polypeptide of any one of claims 1-3 wherein at least one stalk extension region has a sequence homologous to the stalk region except for the dimerization sites of the stalk region.

5. The chimeric polypeptide of any one of claims 1-4 wherein the spacer region is proximal to the membrane region.

6. The chimeric polypeptide of any one of claims 1-4 wherein the spacer region is distal to the membrane region.

7. The chimeric polypeptide of any one of claims 1-6 further comprising an intracellular signaling domain.

8. The chimeric polypeptide of any one of claims 1-6, said chimeric polypeptide not comprising an intracellular signaling domain.

9. The chimeric polypeptide of any one of claims 2-6, wherein said stalk extension region contains at least one fewer dimerization site as compared to said stalk region.

10. The chimeric polypeptide of any one of claims 1-9, wherein the chimeric polypeptide has improved functional activity compared to an otherwise identical antigen-binding polypeptide which lacks the stalk extension region.

11. The chimeric polypeptide of any one of claims 1-10, wherein the chimeric polypeptide has increased expression on a cell surface compared to an otherwise identical polypeptide which lacks the stalk extension region.

12. The chimeric polypeptide of any one of claims 1-11, wherein the stalk extension region lacks a dimerization site.

13. The chimeric polypeptide of any one of claims 1-11, wherein each of the stalk extension region is from about 20 to about 60 amino acids in length, wherein n is 1, 2, 3 or 4.

14. The chimeric polypeptide of claim 13, wherein each said stalk extension region has a sequence which has at least about 80% identity to the stalk region.

15. The chimeric polypeptide of claim 14, wherein at least one said stalk extension region has a sequence comprising at least one less dimerization site as compared to said stalk region.

16. The chimeric polypeptide of any one of claims 13-15, wherein each said stalk extension region has a sequence which has at least about 80% identity to the stalk region, wherein n is 2.

17. The chimeric polypeptide of any one of claims 13-15, wherein each said stalk extension region has a sequence which has at least about 80% identity to the stalk region, wherein n is 3.

18. The chimeric polypeptide of any one of claims 13-15, wherein each said stalk extension region has a sequence which has at least about 80% identity to the stalk region, wherein n is 4.

19. The chimeric polypeptide of any one of claims 13-15, wherein each said stalk extension region has a sequence which has at least about 80% identity to the stalk region, wherein n is at least 5.

20. The chimeric polypeptide of any one of claims 1-19, wherein the stalk region comprises a sequence with at least about 70%, 75%, 80%. 85%, 90%, 95% or 99% identity to at least one of a CD8alpha hinge domain, a CD28 hinge domain and a CTLA-4 hinge domain.

21. The chimeric polypeptide of claim 20, wherein the stalk region is a CD8 alpha hinge domain having a sequence as shown in SEQ ID NO: 1.

22. The chimeric polypeptide of claim 20, wherein the stalk region is a CD28 hinge domain having a sequence as shown in SEQ ID NO: 7.

23. The chimeric polypeptide of claim 20, wherein the stalk region is a CTLA-4 hinge domain having a sequence as shown in SEQ ID NO: 12.

24. The chimeric polypeptide of any one of claims 1-23, wherein interchain dimerization of said chimeric polypeptide is mediated by at least one disulfide bond between cysteine residues.

25. The chimeric polypeptide of any one of claims 1-24, wherein the antigen binding region binds an epitope on CD19.

26. The chimeric polypeptide of any one of claims 1-24, wherein the antigen binding region binds an epitope on CD33.

27. The chimeric polypeptide of any one of claims 1-24, wherein the antigen binding region binds an epitope on at least one of CD19, BCMA, CD44, α Folate receptor, CAIX, CD30, ROR1, CEA, EGP-2, EGP-40, HER2, HER3, Folate-binding Protein, GD2, GD3, IL-13R-a2, KDR, EDB-F, mesothelin, CD22, EGFR, Folate receptor α, Mucins, MUC-1, MUC-16, GPC3, CSPG4, HER1/HER3, HER2, CD44v6, CD44v7/v8, CD20, CD174, CD138, L1-CAM, FAP, c-MET, PSCA, CS1, CD38, IL-11Rα, EphA2, CLL-1, MAGE-A1, h5T4, PSMA, TAG-72, EGFR, CD20, EGFRvIII, CD123 and VEGF-R2.

28. The chimeric polypeptide of any one of claims 1-27, wherein the chimeric polypeptide comprises a chimeric antigen receptor (CAR).

29. The chimeric polypeptide of claim 28, wherein the CAR further comprises at least one costimulatory signaling domain.

30. The chimeric polypeptide of claim 29, wherein the at least one costimulatory signaling domain comprises a signaling domain from CD27, CD28, 4-1BB, ICOS, OX40, DAP10, DAP12, CD134, CD3-zeta or fragment or combination thereof.

31. The chimeric polypeptide of claim 29 or claim 30, wherein the at least one costimulatory signaling domain comprises a signaling domain from 4-1BB, CD28 or a combination thereof.

32. The chimeric polypeptide of any one of the claims 28-31, wherein the CAR further comprises a CD28 costimulatory signaling domain and CD3-zeta.

33. The chimeric polypeptide of any one of the claims 28-32, wherein the CAR further comprises a CD28 costimulatory signaling domain.

34. The chimeric polypeptide of claim 7, wherein the intracellular cell signaling domain interacts with a T cell, a Natural Killer (NK) cell, a cytotoxic T lymphocyte (CTL), or a regulatory T cell.

35. The chimeric polypeptide of any one of claims 1-24, wherein the chimeric polypeptide comprises an engineered T-cell receptor (TCR).

36. The chimeric polypeptide of claim 35, wherein the engineered TCR is an αβ TCR.

37. The chimeric polypeptide of claim 35, wherein the engineered TCR is a γδ TCR.

38. The chimeric polypeptide of any one of claims 1-24 and 35-37, wherein the antigen binding region binds an epitope on at least one of NY-ESO-1, Titin, MART-1, HPV, HBV, MAGE-A4, MAGE-A10, MAGE-A3/A6, gp100, MAGE-A1, or PRAME.

39. A polynucleotide encoding the chimeric polypeptide of any one of claims 1-38.

40. An expression vector comprising the polynucleotide of claim 39.

41. The vector of claim 40, wherein the vector is a lentivirus vector, a retroviral vector, or a non-viral vector.

42. The vector of claim 41, wherein the vector is a non-viral vector which is a Sleeping Beauty vector.

43. An engineered cell comprising the expression vector of any one of claims 38-42.

44. The engineered cell of claim 43, further comprising a Sleeping Beauty transposase.

45. The engineered cell of claim 44, wherein the Sleeping Beauty transposase is SB11, SB100X or SB110.

46. The engineered cell of any one of claims 43-45, wherein said engineered cell is an animal cell.

47. The engineered cell of claim 46, wherein said animal cell is a human cell.

48. The engineered cell of claim 47, wherein said human cell is a T cell or NK cell.

49. The engineered cell of any one of claims 43-48, further expressing a polypeptide comprising an intracellular signaling domain.

50. A chimeric antigen receptor (CAR) comprising an antigen binding region, a transmembrane region a stalk region and a stalk extension region, wherein said stalk extension region is homologous to the stalk region and comprises at least one amino acid substitution relative to said stalk region.

51. The CAR of claim 50, wherein said stalk region is capable of dimerizing with a homologous stalk region of a second CAR.

52. The CAR of any one of claims 50-51, wherein said stalk extension region is not capable of dimerizing.

53. The CAR of any one of claims 50-52, wherein said stalk region is connected to said transmembrane region.

54. The CAR of any one of claims 50-53, wherein the antigen binding region comprises a scFv.

55. The CAR of claim 54, wherein said scFv binds an epitope on CD19, BCMA, CD44, α-Folate receptor, CAIX, CD30, ROR1, CEA, EGP-2, EGP-40, HER2, HER3, Folate-binding

Protein, GD2, GD3, IL-13R-a2, KDR, EDB-F, mesothelin, CD22, EGFR, Folate receptor α, Mucins, MUC-1, GPC3, CSPG4, HER1/HER3, HER2, CD44v6, CD44v7/v8, CD20, CD174, CD138, L1-CAM, FAP, c-MET, PSCA, CS1, CD38, IL-11Rα, EphA2, CLL-1, MUC-16, MAGE-A1, h5T4, PSMA, TAG-72, EGFR, CD20, EGFRvIII, CD123 or VEGF-R2.

56. The CAR of any one of claims 50-55, wherein the stalk extension region (s'-n), wherein n comprises two or more, thereby comprising a first stalk extension region and a second stalk extension region.

57. The CAR of claim 56, wherein the first stalk extension region is homologous to the second stalk extension region.

58. The CAR of any one of claims 56-57, wherein the first stalk extension region comprises at least one amino acid residue substitution compared to the stalk region.

59. The CAR of any one of claims 56-59, wherein the first stalk extension region is not capable of dimerizing to a stalk region of a CAR.

60. The CAR of any one of claims 56-59, wherein the second stalk extension region comprises at least one amino acid residue substitution compared to the stalk region.

61. The CAR of any one of claims 56-60, wherein the second stalk extension region is not capable of dimerizing to another stalk extension region.

62. The CAR of any one of claims 56-61, further comprising a third stalk extension region.

63. The CAR of claim 62, further comprising a fourth stalk extension region.

64. The CAR of claim 63, comprising five or more stalk extension region.

65. The CAR of any one of claims 56-64, wherein at least one stalk extension region is not capable of forming a disulfide bond.

66. The CAR of any one of claims 50-65, wherein the stalk region comprises a sequence with at least about 70%, 75%, 80%. 85%, 90%, 95% or 99% identity to at least one of a CD8alpha hinge domain, a CD28 hinge domain and a CTLA-4 hinge domain.

67. The CAR of claim 66, wherein the stalk region is a CD8alpha hinge domain, a CD28 hinge domain or a CTLA-4 hinge domain.

68. A T cell or NK cell which expresses the CAR according to any of claims 50-67.

69. The CAR according to any one of claims 50-67, which comprises the sequence shown as SEQ ID No. 53-68, or a variant thereof which has at least 80% sequence identity but retains antigen binding capacity.

70. A nucleic acid sequence which encodes the CAR according to any one of claims 50-67.

71. The nucleic acid sequence according to claim 70, which comprises the sequence shown as SEQ ID No 147-162 or a variant thereof having at least 70%, 75%, 80%, 85%, 90%, 95% or 99% sequence identity.

72. A vector which comprises the nucleic acid sequence according to claim 70 or 71.

73. The vector of claim 72, wherein the vector is a lentivirus vector, a retroviral vector, a Sleeping Beauty transposon or a non-viral vector.

74. A method for making the T cell or NK cell according to claim 64, which comprises the step of introducing the nucleic acid sequence according to claim 70 or 71 into a T cell or NK cell.

75. The method of claim 74, wherein the T cell is modified at a point-of-care site and administered to a subject in need thereof, without undergoing propagation and activation.

76. The method of claim 74, wherein the T cell is stimulated for at least 0, 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30 or more days.

77. The method of claim 74, wherein the T cell is stimulated for at least 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60 or more days.

78. The method of claim 74, wherein the T cell is stimulated for at least 7, 14, 21, 28, 35, 42, 49, 56, 63 or more days.

79. A pharmaceutical composition which comprises at least one of:

-a vector according to any one of claims 72 or 73; and

-a T cell according to claim 68 or a NK cell according to claim 68; and a pharmaceutically acceptable carrier, diluent or excipient.

80. A population of cells comprising the CAR of any one of claims 50-67.

81. A method for stimulating a T cell-mediated immune response in a subject, comprising contacting said subject with an effective amount of a cell genetically modified to express the CAR, wherein the CAR comprises the CAR of any one of claims 50-67.

82. A method of increasing expression of a chimeric antigen receptor (CAR) on a cell surface comprising engineering a nucleic acid encoding the CAR to comprise a stalk extension domain, thereby generating an engineered CAR.

83. A method of increasing expansion of an engineered T cell expressing a chimeric polypeptide comprising engineering a nucleic acid encoding the chimeric polypeptide to comprise a stalk extension domain, thereby generating an engineered T cell.

84. The method of any one of claims 82-83, wherein the CAR comprises the CAR of any one of claims 50-67.

CLAIMS

WHAT IS CLAIMED IS:

1. A chimeric polypeptide comprising:

(a) an antigen-binding region;

(b) a transmembrane region; and

(c) a spacer region connecting the trans-membrane region with the antigen binding region, wherein the spacer region comprises: (i) a stalk region comprising about 20 to about 60 amino acids and at least one dimerization site; and (ii) one to five a stalk extension regions, wherein each stalk extension region comprises fewer dimerization sites than the stalk region

2. The chimeric polypeptide of claim 1, wherein at least one stalk extension region comprises an amino acid sequence having at least 80% sequence identity with the amino acid sequence of the stalk region.

3. The chimeric polypeptide of claim 1, wherein the stalk region is proximal to the transmembrane region.

4. The chimeric polypeptide of claim 1, wherein the stalk region is distal to the transmembrane region.

5. The chimeric polypeptide of claim 1, wherein the chimeric polypeptide exhibits increased expression on a cell surface compared to an otherwise identical polypeptide that lacks the stalk extension region.

6. The chimeric polypeptide of claim 1, wherein at least one stalk extension region lacks a dimerization site.

7. The chimeric polypeptide of claim 1, wherein each stalk extension region comprises about 20 to about 60 amino acids.

8. The chimeric polypeptide of claim 7, wherein each stalk extension region comprises an amino acid sequence that has at least about 80% sequence identity to the amino acid sequence of the stalk region.

9. The chimeric polypeptide of claim 1, wherein the stalk region comprises a sequence with at least about identity to a CD8alpha hinge domain, a CD28 hinge domain, and/or a CTLA-4 hinge domain.

10. The chimeric polypeptide of claim 1, wherein the antigen binding region binds an epitope on CD19, BCMA, CD44, α-Folate receptor, CAIX, CD30, ROR1, CEA, EGP-2, EGP-40, HER2, HER3, Folate-binding Protein, GD2, GD3, IL-13R-a2, KDR, EDB-F, mesothelin, CD22, EGFR, Folate receptor α, Mucins, MUC-1, MUC-16, GPC3, CSPG4, HER1/HER3, HER2, CD44v6, CD44v7/v8, CD20, CD174, CD138, L1-CAM, FAP, c-MET, PSCA, CS1, CD38, IL-11Rα, EphA2, CLL-1, MAGE-A1, h5T4, PSMA, TAG-72, EGFR, CD20, EGFRvIII, CD123, and/or VEGF-R2.

11. The chimeric polypeptide of claim 1, wherein the chimeric polypeptide comprises a chimeric antigen receptor (CAR).

12. The chimeric polypeptide of claim 1, wherein the chimeric polypeptide comprises an engineered T-cell receptor (TCR).

13. The chimeric polypeptide of claim 12, wherein the engineered TCR is an αβ TCR.

14. The chimeric polypeptide of claim 12, wherein the engineered TCR is a γδ TCR.

15. The chimeric polypeptide of claim 12, wherein the antigen binding region binds an epitope on at least one of NY-ESO-1, Titin, MART-1, HPV, HBV, MAGE-A4, MAGE-A10, MAGE A3/A6, gp100, MAGE-A1, or PRAME.

16. The chimeric polypeptide of claim 11, wherein at least one stalk extension region comprises: (a) an amino acid sequence that has at least 80% sequence identity with the amino acid sequence of stalk region; and (b) at least one amino acid substitution relative to the stalk region.

17. The chimeric polypeptide of claim 16, wherein the stalk region is capable of dimerizing with a second stalk region present in second CAR and comprises at least 80% sequence identity with the amino acid sequence of the second stalk region.

18. A method of increasing expansion of an engineered T cell expressing a chimeric polypeptide comprising engineering a nucleic acid encoding the chimeric polypeptide to comprise a stalk extension domain, thereby generating an engineered T cell.

19. The chimeric polypeptide of claim 1, wherein the antigen binding region binds an epitope on CD33, MUC-16, and/or ROR1.

20. The chimeric polypeptide of claim 1, wherein the antigen binding region binds an epitope on ROR1.

21. The chimeric polypeptide of claim 1, wherein the stalk region comprises an amino acid sequence that has at least 80% sequence identity with SEQ ID NO: 3.

22. The chimeric polypeptide of claim 1, wherein the spacer region comprises an amino acid sequence that has at least 80% sequence identity with SEQ ID NO: 5.

23. A modified effector cell that expresses the chimeric polypeptide of claim 1.

24. A modified effector cell that expresses the chimeric polypeptide of claim 20.

25. The modified effector cell of claim 24, wherein the cell is a CAR-T cell.

26. The modified effector cell of claim 23, wherein the cell expresses a cytokine.

27. The modified effector cell of claim 26, wherein the cytokine is a membrane-bound IL15.

28. The modified effector cell of claim 23, wherein the cell expresses a cell tag.

29. The modified effector cell of claim 28, wherein the cell tag comprises a truncated epidermal growth factor receptor.

30. The modified cell of claim 23, wherein the cell expresses a membrane-bound IL15 and a cell tag comprising a truncated epidermal growth factor receptor.

31. The modified cell of claim 24, wherein the cell expresses a membrane-bound IL15 and a cell tag comprising a truncated epidermal growth factor receptor.

32. The modified cell of claim 23, wherein the cell has improved cytotoxicity as compared to a cell expressing an otherwise identical antigen-binding polypeptide that lacks the stalk extension region.

33. A method for making the modified effector cell of claim 23, the method comprising transfecting an immune effector cell with an expression vector encoding the chimeric polypeptide.

34. The method of claim 33, wherein the vector is a *Sleeping Beauty* transposon.