

CLAIMS

WHAT IS CLAIMED IS:

1. A chimeric polypeptide comprising, from N-terminus to C-terminus:
 - a) an extracellular ligand-binding domain having a binding affinity for a selected ligand;
 - b) a hinge domain capable of promoting oligomer formation of the chimeric polypeptide via intermolecular disulfide bonding;
 - c) a transmembrane domain comprising one or more ligand-inducible proteolytic cleavage sites; and
 - d) an intracellular domain comprising a human or humanized transcriptional effector,wherein binding of the selected ligand to the extracellular ligand-binding domain results in cleavage at a ligand-inducible proteolytic cleavage site between the transcriptional effector and the hinge domain,
and wherein the chimeric polypeptide does not comprise a LIN-12-Notch repeat (LNR) and/or a heterodimerization domain (HD) of a Notch receptor.
2. The chimeric polypeptide of claim 1, wherein the transmembrane domain further comprises a stop-transfer-sequence.
3. The chimeric polypeptide of any one of claims 1 to 2, wherein the extracellular domain comprises an antigen-binding moiety capable of binding to a ligand on the surface of a cell.
4. The chimeric polypeptide of claim 3, wherein the cell is a pathogen.
5. The chimeric polypeptide of claim 3, wherein the cell is a human cell.
6. The chimeric polypeptide of claim 5, wherein the human cell is a tumor cell.
7. The chimeric polypeptide of claim 5, wherein the human cell is a terminally-differentiated cell.

8. The chimeric polypeptide of any one of claims 1 to 7, wherein the ligand comprises a protein or a carbohydrate.

9. The chimeric polypeptide of any one of claims 1 to 8, wherein the ligand is selected from the group consisting of CD1, CD1a, CD1b, CD1c, CD1d, CD1e, CD2, CD3d, CD3e, CD3g, CD4, CD5, CD7, CD8a, CD8b, CD19, CD20, CD21, CD22, CD23, CD25, CD27, CD28, CD33, CD34, CD40, CD45, CD48, CD52, CD59, CD66, CD70, CD71, CD72, CD73, CD79A, CD79B, CD80 (B7.1), CD86 (B7.2), CD94, CD95, CD134, CD140 (PDGFR4), CD152, CD154, CD158, CD178, CD181 (CXCR1), CD182 (CXCR2), CD183 (CXCR3), CD210, CD246, CD252, CD253, CD261, CD262, CD273 (PD-L2), CD274 (PD-L1), CD276 (B7H3), CD279, CD295, CD339 (JAG1), CD340 (HER2), EGFR, FGFR2, CEA, AFP, CA125, MUC-1, MAGE, alkaline phosphatase, placental-like 2 (ALPPL2), B-cell maturation antigen (BCMA), green fluorescent protein (GFP), enhanced green fluorescent protein (EGFP), and signal regulatory protein α (SIRP α).

10. The chimeric polypeptide of any one of claims 1 to 9, wherein the ligand is selected from cell surface receptors, adhesion proteins, integrins, mucins, lectins, tumor associated antigens, and tumor-specific antigens.

11. The chimeric polypeptide of any one of claims 1 to 10, wherein the ligand is a tumor-associated antigen or a tumor-specific antigen.

12. The chimeric polypeptide of any one of claims 1 to 11, wherein the extracellular ligand-binding domain comprises the ligand-binding portion of a receptor.

13. The chimeric polypeptide of any one of claims 3 to 12, wherein the antigen-binding moiety is selected from the group consisting of an antibody, a nanobody, a diabody, a triabody, a minibody, an F(ab')₂ fragment, an F(ab)_v fragment, a single chain variable fragment (scFv), a single domain antibody (sdAb), and a functional fragment thereof.

14. The chimeric polypeptide of claim 13, wherein the antigen-binding moiety comprises a scFv.

15. The chimeric polypeptide of any one of claims 3 to 14, wherein the antigen-binding moiety specifically binds to a tumor-associated antigen selected from the group consisting of CD19, B7H3 (CD276), BCMA (CD269), ALPPL2, CD123, CD171, CD179a, CD20, CD213A2, CD22, CD24, CD246, CD272, CD30, CD33, CD38, CD44v6, CD46, CD71, CD97, CEA, CLDN6, CLECL1, CS-1, EGFR, EGFRvIII, ELF2M, EpCAM, EphA2, Ephrin B2, FAP, FLT3, GD2, GD3, GM3, GPRC5D, HER2 (ERBB2/neu), IGLL1, IL-11R α , KIT (CD117), MUC1, NCAM, PAP, PDGFR- β , PRSS21, PSCA, PSMA, ROR1, SIRP α , SSEA-4, TAG72, TEM1/CD248, TEM7R, TSHR, VEGFR2, ALPI, citrullinated vimentin, cMet, and Axl.

16. The chimeric polypeptide of claim 15, wherein the tumor-associated antigen is CD19, BCMA, CEA, HER2, MUC1, CD20, ALPPL2, SIRP α , or EGFR.

17. The chimeric polypeptide of claim 16, wherein the tumor-associated antigen is CD19, BCMA, HER2, or ALPPL2.

18. The chimeric polypeptide of any one of claims 1 to 17, wherein the one or more ligand-inducible proteolytic cleavage sites comprises a γ -secretase cleavage site.

19. The chimeric polypeptide of any one of claims 1 to 18, wherein the transcriptional effector comprises a transcriptional activator, or a transcriptional repressor.

20. The chimeric polypeptide of any one of claims 1 to 19, wherein the intracellular domain comprises a nuclear localization sequence.

21. The chimeric polypeptide of any one of claims 1 to 20, wherein the transcriptional effector comprises: (i) a DNA-binding domain and (ii) an effector domain through which the transcriptional effector exerts its effect.

22. The chimeric polypeptide of claim 21, wherein the DNA-binding domain is N-terminally linked to the effector domain.

23. The chimeric polypeptide of claim 21, wherein the DNA-binding domain is C-terminally linked to the effector domain.

24. The chimeric polypeptide of any one of claims 21 to 23, wherein the DNA-binding domain is derived from a human hepatocyte nuclear factor 1 homeobox A (HNF1 α) protein, a human hepatocyte nuclear factor 1 homeobox B (HNF1 β) protein, a human paired box protein (Pax-6), a human paired box protein (Pax-1) protein, a human paired box protein (Pax-2) protein, a human paired box protein (Pax-3) protein, a human paired box protein (Pax-4), or a human paired box protein (Pax-7).

25. The chimeric polypeptide of claim 24, wherein the DNA-binding domain is derived from a human hepatocyte nuclear factor 1 homeobox A (HNF1 α) protein.

26. The chimeric polypeptide of claim 25, wherein the HNF1 α DNA-binding domain comprises the amino acid sequence of SEQ ID NO: 52 or a functional variant thereof.

27. The chimeric polypeptide of claim 24, wherein the DNA-binding domain is derived from a human paired box protein (Pax6).

28. The chimeric polypeptide of claim 27, wherein the Pax6 DNA-binding domain comprises the amino acid sequence of SEQ ID NO: 53 or a functional variant thereof.

29. The chimeric polypeptide of any one of claims 21 to 28, wherein the effector domain is selected from the group consisting of a transcriptional activation domain, a transcriptional repressor domain, and an epigenetic effector domain.

30. The chimeric polypeptide of claim 29, wherein the transcriptional activation domain is derived from human nuclear factor NF κ B p65 subunit (p65).

31. The chimeric polypeptide of claim 30, wherein the transcriptional activation domain is derived from human nuclear factor NF κ B p65 subunit (p65).

32. The chimeric polypeptide of claim 31, wherein the p65 effector domain comprises the amino acid sequence of any one of SEQ ID NOS: 54-55 or a functional variant thereof.

33. The chimeric polypeptide of any one of claims 1 to 32, further comprising an additional proteolytic cleavage site, a signal sequence, a detectable label, a tumor-specific cleavage site, a disease-specific cleavage site, and combinations thereof.

34. The chimeric polypeptide of any one of claims 1 to 33, wherein the hinge domain is derived from a CD8 α hinge domain, a CD28 hinge domain, a CD152 hinge domain, a PD-1 hinge domain, a CTLA4 hinge domain, an OX40 hinge domain, an IgG1 hinge domain, an IgG2 hinge domain, an IgG3 hinge domain, and an IgG4 hinge domain, or a functional variant of any thereof.

35. The chimeric polypeptide of any one of claims 1 to 34, wherein the hinge domain is derived from a CD8 α hinge domain or a functional variant thereof.

36. The chimeric polypeptide of any one of claims 1 to 34, wherein the hinge domain is derived from a CD28 hinge domain or a functional variant thereof.

37. The chimeric polypeptide of any one of claims 1 to 34, wherein the hinge domain is derived from an OX40 hinge domain or a functional variant thereof.

38. The chimeric polypeptide of any one of claims 1 to 34, wherein the hinge domain is derived from an IgG4 hinge domain or a functional variant thereof.

39. The chimeric polypeptide of any one of claims 1 to 38, wherein the hinge domain comprises an amino acid sequence having at least 80% sequence identity to any one of SEQ ID NOS: 15-19.

40. The chimeric polypeptide of any one of claims 1 to 39, wherein the stop-transfer-sequence comprises an amino acid sequence having at least 80% sequence identity to any one of SEQ ID NOS: 31-51.

41. The chimeric polypeptide of any one of claims 1 to 40, wherein the transmembrane domain comprises an amino acid sequence having at least 80% sequence identity to any one of SEQ ID NOS: 21-30.

42. The chimeric polypeptide of any one of claims 1 to 41, wherein:

- a) the hinge domain comprises an amino acid sequence having at least 80% sequence identity to any one of SEQ ID NOS: 15-19;
- b) the transmembrane domain comprises an amino acid sequence having at least 80% sequence identity to SEQ ID NOS: 21-30; and
- c) the stop-transfer-sequence domain comprises an amino acid sequence having at least 80% sequence identity to any one of SEQ ID NO: 31-51.

43. The chimeric polypeptide of any one of claims 1 to 42, wherein the chimeric polypeptide comprises an amino acid sequence of a length shorter than 500, shorter than 600, shorter than 700, shorter than 800, or shorter than 900 amino acid residues.

44. The chimeric polypeptide of claim 43, wherein the chimeric polypeptide comprises an amino acid sequence is of a length shorter than 600 amino acid residues.

45. The chimeric polypeptide of any one of claims 1 to 44, wherein the chimeric polypeptide comprises an amino acid sequence having at least 80% sequence identity to any one of SEQ ID NOS: 2, 4-12, and 65-66.

46. The chimeric polypeptide of any one of claims 1 to 45, wherein the domains recited in (a) through (d) are derived from human polypeptides.

47. The chimeric polypeptide of any one of claims 1 to 46, wherein the domains recited in (a) through (d) are substantially non-immunogenic in a human subject.

48. The chimeric polypeptide of claim 47, wherein the intracellular domain is substantially non-immunogenic in a human subject.

49. A recombinant nucleic acid comprising a nucleotide sequence encoding a chimeric polypeptide according to claims 1 to 48.

50. The recombinant nucleic acid of claim 49, wherein the nucleotide sequence is incorporated into an expression cassette or an expression vector.

51. The recombinant nucleic acid of claim 50, wherein the expression vector is a viral vector.
52. The recombinant nucleic acid of claim 51, wherein the viral vector is a lentiviral vector, an adeno virus vector, an adeno-associated virus vector, or a retroviral vector.
53. The recombinant nucleic acid of any one of claims 49 to 52, wherein the recombinant nucleic acid further comprises a nucleic acid sequence encoding a protein of interest operably linked to a promoter, wherein expression of the protein of interest is modulated by the transcriptional effector.
54. A recombinant cell comprising:
- a) a chimeric polypeptide according to any one of claims 1 to 48; and/or
 - b) a recombinant nucleic acid according to any one of claims 49 to 52.
55. The recombinant cell of claim 54, wherein the recombinant cell is a eukaryotic cell.
56. The recombinant cell of claim 55, wherein the eukaryotic cell is a mammalian cell.
57. The recombinant cell of claim 56, wherein the mammalian cell is an immune cell, a neuron, an epithelial cell, and endothelial cell, or a stem cell.
58. The recombinant cell of claim 57, wherein the immune cell is a B cell, a monocyte, a natural killer cell, a basophil, an eosinophil, a neutrophil, a dendritic cell, a macrophage, a regulatory T cell, a helper T cell, a cytotoxic T cell, or other T cell.
59. The recombinant cell of any one of claims 54 to 58, comprising:
- a) a first chimeric polypeptide and a second chimeric polypeptide according to any one of claims 1 to 48; and/or
 - b) a first nucleic acid and a second nucleic acid according to any one of claims 49 to 52;
- wherein the first chimeric polypeptide and the second chimeric polypeptide do not have the same sequence, and/or the first nucleic acid or the second nucleic acid do not have the same sequence.

60. The recombinant cell of claim 59, wherein the first chimeric polypeptide modulates the expression and/or activity of the second chimeric polypeptide.

61. The recombinant cell of any one of claims 54 to 60, further comprising an expression cassette encoding a protein of interest operably linked to a promoter, wherein expression of the protein of interest is modulated by the transcriptional effector.

62. The recombinant cell of claim 61, wherein the protein of interest is heterologous to the cell.

63. The recombinant cell of claim 62, wherein the promoter is a phosphoglycerate kinase (PGK) promoter or a yeast GAL4 promoter.

64. The recombinant cell of any one of claims 61 to 63, wherein the protein of interest is a cytokine, a cytotoxin, a chemokine, an immunomodulator, a pro-apoptotic factor, an anti-apoptotic factor, a hormone, a differentiation factor, a de-differentiation factor, an immune cell receptor (e.g., a TCR or CAR), or a reporter.

65. The recombinant cell of any one of claims 61 to 64, wherein the expression cassette is incorporated into a second recombinant nucleic acid molecule that is separate from the recombinant nucleic acid molecule encoding the chimeric polypeptide.

66. The recombinant cell of any one of claims 61 to 64, wherein the expression cassette is incorporated into the same recombinant nucleic acid molecule that encodes the chimeric polypeptide.

67. A cell culture comprising at least one recombinant cell according to any one of claims 54 to 66, and a culture medium.

68. A pharmaceutical composition comprising a pharmaceutically acceptable carrier, and one or more of the following:

- a) a recombinant nucleic acid according to any one of claims 49 to 52; and
- b) a recombinant cell according to any one of claims 54 to 66.

69. The pharmaceutical composition of claim 68, wherein the composition comprises a recombinant nucleic acid according to any one of claims 49 to 53, and a pharmaceutically acceptable carrier.

70. The pharmaceutical composition of claim 69, wherein the recombinant nucleic acid is encapsulated in a viral capsid or a lipid nanoparticle.

71. A method for modulating an activity of a cell, the method comprising:

- a) providing a recombinant cell according to any one of claims 54 to 66; and
- b) contacting the recombinant cell with the selected ligand, wherein binding of the selected ligand to the extracellular ligand-binding domain induces cleavage of a ligand-inducible proteolytic cleavage site and releases the transcriptional effector, wherein the released transcriptional effector modulates an activity of the recombinant cell.

72. The method of claim 71, the contacting is carried out *in vivo*, *ex vivo*, or *in vitro*.

73. The method of any one of claims 71 to 72, wherein the activity of the cell to be modulated is selected from the group consisting of: expression of a selected gene, proliferation, apoptosis, non-apoptotic death, differentiation, dedifferentiation, migration, secretion of a molecule, cellular adhesion, and cytolytic activity.

74. The method of any one of claims 71 to 73, wherein the released transcriptional effector modulates expression of a gene product of the cell.

75. The method of claim 71 to 73, wherein the released transcriptional effector modulates expression of a heterologous gene product.

76. The method of any one of claims 74 to 75, wherein the gene product of the cell is selected from the group consisting of chemokine, a chemokine receptor, a chimeric antigen receptor, a cytokine, a cytokine receptor, a differentiation factor, a growth factor, a growth factor receptor, a hormone, a metabolic enzyme, a pathogen-derived protein, a proliferation inducer, a receptor, an RNA guided nuclease, a site-specific nuclease, a T cell receptor, a toxin, a toxin derived protein, a transcriptional regulator, a transcriptional activator, a transcriptional repressor,

a translational regulator, a translational activator, a translational repressor, an activating immuno-receptor, an antibody, an apoptosis inhibitor, an apoptosis inducer, an engineered T cell receptor, an immuno-activator, an immuno-inhibitor, and an inhibiting immuno-receptor.

77. The method of any one of claims 71 to 76, wherein the released transcriptional effector modulates differentiation of the cell, and wherein the cell is an immune cell, a stem cell, a progenitor cell, or a precursor cell.

78. A method for inhibiting an activity of a target cell in a subject, the method comprising administering to the subject an effective number of the recombinant cells according to any one of claims 54 to 66, wherein the recombinant cells inhibit an activity of the target cell in the subject.

79. The method of claim 78, wherein the target cell is a pathogenic cell.

80. The method of claim 79, wherein the pathogenic cell is a cancer cell.

81. The method of claim 80, wherein the target cancer cell is an acute myeloma leukemia cell, an anaplastic lymphoma cell, an astrocytoma cell, a B-cell cancer cell, a breast cancer cell, a colon cancer cell, an ependymoma cell, an esophageal cancer cell, a glioblastoma cell, a glioma cell, a leiomyosarcoma cell, a liposarcoma cell, a liver cancer cell, a lung cancer cell, a mantle cell lymphoma cell, a melanoma cell, a neuroblastoma cell, a non-small cell lung cancer cell, an oligodendroglioma cell, an ovarian cancer cell, a pancreatic cancer cell, a peripheral T-Cell lymphoma cell, a renal cancer cell, a sarcoma cell, a stomach cancer cell, a carcinoma cell, a mesothelioma cell, or a sarcoma cell.

82. A method for the treatment of a health condition in a subject in need thereof, the method comprising administering to the subject a first therapy comprising an effective number of the recombinant cell according to any one of claims 54 to 66, wherein the recombinant cell treats the health condition in the subject.

83. The method of claim 82, further comprising administering to the subject a second therapy.

84. The method of claim 83, wherein the second therapy is selected from the group consisting of chemotherapy, radiotherapy, immunotherapy, hormonal therapy, toxin therapy, and surgery.

85. The method of any one of claims 83 to 84, wherein the first therapy and the second therapy are administered together in the same composition or in separate compositions.

86. The method claim 85, wherein the first therapy and the second therapy are administered at the same time.

87. The method of any one of claims 83 to 84, wherein the first therapy and the second therapy are administered sequentially.

88. The method of claim 88, wherein the first therapy is administered before the second therapy.

89. The method of claim 88, wherein the first therapy is administered after the second therapy.

90. The method of claim 88, wherein the first therapy and the second therapy are administered in rotation.

91. A system for modulating an activity of a cell, inhibiting a target cancer cell, or treating a health condition in a subject in need thereof, the system comprising one or more of the following:

- a) a chimeric polypeptide according to any one of claims 1 to 48;
- b) a recombinant nucleic acid according to any one of claims 49 to 53;
- c) a recombinant cell according to any one of claims 54 to 66; and
- d) a pharmaceutical composition according to any one of claims 68 to 70.

92. A method for making the recombinant cell according to any one of claims 54 to 64, comprising:

- a) providing a cell capable of protein expression;
- b) contacting the provided cell with a recombinant nucleic acid according to any

one of claims 49 to 53.

93. The method of claim 92, wherein the cell is obtained by leukapheresis performed on a sample obtained from a subject, and the cell is contacted *ex vivo*.

94. The method of claim 92, wherein the recombinant nucleic acid is encapsulated in a viral capsid or a lipid nanoparticle.

95. The use of one or more of the following for the treatment of a health condition:

- a) a chimeric polypeptide according to any one of claims 1 to 48;
- b) a recombinant nucleic acid according to any one of claims 49 to 53;
- c) a recombinant cell according to any one of claims 54 to 66; and
- d) a composition according to any one of claims 68 to 70.

96. The use of claim 95, wherein the health condition is cancer.

97. The use of claim 96, wherein the cancer is a solid tumor, a soft tissue tumor, or a metastatic lesion.

98. The use of the invention of any one of claims 1 to 94, for the manufacture of a medicament for the treatment of a health condition.