

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 transcriptional regulator, wherein binding of the selected ligand to the extracellular ligand-binding domain induces cleavage at a ligand-inducible proteolytic cleavage site disposed between the transcriptional regulator 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 an 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 gamma secretase cleavage site.

19. The chimeric polypeptide of any one of claims 1 to 18, wherein the transcriptional regulator 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 and a transcriptional regulator sequence selected from the group consisting of Gal4-VP16, Gal4-VP64, tetR-VP64, ZFHD1-VP64, Gal4-KRAB, and HAP1-VP16.

21. The chimeric polypeptide of any one of claims 1 to 20, 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.

22. The chimeric polypeptide of any one of claims 1 to 21, 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.

23. The chimeric polypeptide of any one of claims 1 to 22, wherein the hinge domain is derived from a CD8 α hinge domain or a functional variant thereof.

24. The chimeric polypeptide of any one of claims 1 to 22, wherein the hinge domain is derived from a CD28 hinge domain or a functional variant thereof.

25. The chimeric polypeptide of any one of claims 1 to 22, wherein the hinge domain is derived from an OX40 hinge domain or a functional variant thereof.

26. The chimeric polypeptide of any one of claims 1 to 22, wherein the hinge domain is derived from an IgG4 hinge domain or a functional variant thereof.

27. The chimeric polypeptide of any one of claims 1 to 26, wherein the hinge domain comprises an amino acid sequence having at least 80% sequence identity to any one of SEQ ID NOS: 12-16 and 39-42.

28. The chimeric polypeptide of any one of claims 1 to 27, wherein the stop-transfer-sequence comprises an amino acid sequence having at least 80% sequence identity to any one of SEQ ID NOS: 18-19, 43-63, 79, and 80.

29. The chimeric polypeptide of any one of claims 1 to 28, wherein the transmembrane domain comprises an amino acid sequence having at least 80% sequence identity to any one of SEQ ID NOS: 17, 77, and 78.

30. The chimeric polypeptide of any one of claims 1 to 29, wherein:

- a) the hinge domain comprises an amino acid sequence having at least 80% sequence identity to any one of SEQ ID NOS: 12-16 and 39-42;
- b) the transmembrane domain comprises an amino acid sequence having at least 80% sequence identity to any one of SEQ ID NOS: 17, 77, and 78; 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: 18-19, 43-63, 79, and 80.

31. The chimeric polypeptide of any one of claims 1 to 30, wherein the chimeric polypeptide comprises an amino acid sequence having at least 80% sequence identity to any one of SEQ ID NOS: 1-8, 24-35, and 73-76.

32. A recombinant nucleic acid comprising a nucleotide sequence encoding a chimeric polypeptide according to claims 1 to 31.

33. The recombinant nucleic acid of claim 32, wherein the nucleotide sequence is incorporated into an expression cassette or an expression vector.

34. The recombinant nucleic acid of claim 33, wherein the expression vector is a viral vector.

35. The recombinant nucleic acid of claim 34, wherein the viral vector is a lentiviral vector, an adeno virus vector, an adeno-associated virus vector, or a retroviral vector.
36. A recombinant cell comprising:
- a) a chimeric polypeptide according to any one of claims 1 to 31; and/or
 - b) a recombinant nucleic acid according to any one of claims 32 to 35.
37. The recombinant cell of claim 36, wherein the recombinant cell is a eukaryotic cell.
38. The recombinant cell of claim 37, wherein the eukaryotic cell is a mammalian cell.
39. The recombinant cell of claim 38, wherein the mammalian cell is an immune cell, a neuron, an epithelial cell, and endothelial cell, or a stem cell.
40. The recombinant cell of claim 39, 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.
41. The recombinant cell of any one of claims 36 to 40, comprising:
- a) a first chimeric polypeptide and a second chimeric polypeptide according to any one of claims 1 to 31; and/or
 - b) a first nucleic acid and a second nucleic acid according to any one of claims 32 to 35;
- 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.
42. The recombinant cell of claim 41, wherein the first chimeric polypeptide modulates the expression and/or activity of the second chimeric polypeptide.
43. The recombinant cell of any one of claims 36 to 42, further comprising an expression cassette encoding a protein operably linked to a promoter, wherein expression of the protein is modulated by the transcriptional regulator.
44. The recombinant cell of claim 43, wherein the protein is heterologous to the cell.
45. The recombinant cell of claim 44, wherein the promoter is a yeast GAL4 promoter.

46. The recombinant cell of any one of claims 43 to 45, wherein the protein 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.

47. A cell culture comprising at least one recombinant cell according to any one of claims 36 to 46, and a culture medium.

48. 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 32 to 35; and
- b) a recombinant cell according to any one of claims 36 to 46.

49. The pharmaceutical composition of claim 48, wherein the composition comprises a recombinant nucleic acid according to any one of claims 32 to 35, and a pharmaceutically acceptable carrier.

50. The pharmaceutical composition of claim 49, wherein the recombinant nucleic acid is encapsulated in a viral capsid or a lipid nanoparticle.

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

- a) providing a recombinant cell according to any one of claims 36 to 46; 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 regulator, wherein the released transcriptional regulator modulates an activity of the recombinant cell.

52. The method of claim 51, the contacting is carried out *in vivo*, *ex vivo*, or *in vitro*.

53. The method of any one of claims 51 to 52, 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.

54. The method of any one of claims 51 to 53, wherein the released transcriptional regulator modulates expression of a gene product of the cell.

55. The method of claim 51 to 53, wherein the released transcriptional regulator modulates expression of a heterologous gene product.

56. The method of any one of claims 54 to 55, 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.

57. The method of any one of claims 51 to 56, wherein the released transcriptional regulator modulates differentiation of the cell, and wherein the cell is an immune cell, a stem cell, a progenitor cell, or a precursor cell.

58. A method for inhibiting an activity of a target cell in an individual, the method comprising administering to the individual an effective number of the recombinant cells according to any one of claims 36 to 46, wherein the recombinant cells inhibit an activity of the target cell in the individual.

59. The method of claim 58, wherein the target cell is a pathogenic cell.

60. The method of claim 59, wherein the pathogenic cell is a cancer cell.

61. The method of claim 57, 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.

62. A method for the treatment of a health condition in an individual in need thereof, the method comprising administering to the individual a first therapy comprising an effective number of the recombinant cell according to any one of claims 36 to 46, wherein the recombinant cell treats the health condition in the individual.

63. The method of claim 62, further comprising administering to the individual a second therapy.

64. The method of claim 63, wherein the second therapy is selected from the group consisting of chemotherapy, radiotherapy, immunotherapy, hormonal therapy, and toxin therapy.

65. The method of any one of claims 63 to 64, wherein the first therapy and the second therapy are administered together in the same composition or in separate compositions.

66. The method claim 65, wherein the first therapy and the second therapy are administered at the same time.

67. The method of any one of claims 63 to 64, wherein the first therapy and the second therapy are administered sequentially.

68. The method of claim 67, wherein the first therapy is administered before the second therapy.

69. The method of claim 67, wherein the first therapy is administered after the second therapy.

70. The method of claim 67, wherein the first therapy and the second therapy are administered in rotation.

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

- a) a chimeric polypeptide according to any one of claims 1 to 31;
- b) a recombinant nucleic acid according to any one of claims 32 to 35;
- c) a recombinant cell according to any one of claims 36 to 46; and
- d) a pharmaceutical composition according to any one of claims 48 to 50.

72. A method for making the recombinant cell according to any one of claims 36 to 46, 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 32 to 35 into the provided cell.

73. The method of claim 72, wherein the cell is obtained by leukapheresis performed on a sample obtained from a subject, and the cell is contacted *ex vivo*.

74. The method of claim 72, wherein the recombinant nucleic acid is encapsulated in a viral capsid or a lipid nanoparticle.

75. 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 31;
- b) a recombinant nucleic acid according to any one of claims 32 to 35;
- c) a recombinant cell according to any one of claims 36 to 46; and
- d) a composition according to any one of claims 48 to 50.

76. The use of claim 75, wherein the health condition is cancer.

77. The use of claim 76, wherein the cancer is a solid tumor, a soft tissue tumor, or a metastatic lesion.

78. The use of the invention of any one of claims 1 to 74, for the manufacture of a medicament for the treatment of a health condition.