

Claims

1. A modified T lymphocyte comprising:

a. a first polypeptide comprising a first extracellular antigen binding domain that binds a first antigen, and a first intracellular signaling domain, wherein said first polypeptide does not comprise a co-stimulatory domain; and

b. a second polypeptide comprising a second extracellular antigen binding domain binding a second antigen, or a receptor that binds said second antigen; a second intracellular signaling domain; and one or more co-stimulatory domains;

wherein said second antigen is an antigen on an antibody that binds to an antigen presented by a tumor cell, vascular endothelial growth factor (VEGF), or a damage associated molecular pattern molecule (DAMP),

wherein said DAMP is a heat shock protein, chromatin-associated protein high mobility group box 1 (HMGB1), S100A8 (MRP8, calgranulin A), S100A9 (MRP14, calgranulin B), serum amyloid A (SAA), deoxyribonucleic acid, adenosine triphosphate, uric acid, or heparin sulfate; and

wherein said modified lymphocyte becomes maximally cytotoxic only when said first signaling domain and said second signaling domain are both activated by said first antigen and said second antigen, respectively.

2. The modified T lymphocyte of claim 1, wherein binding of said first antigen to said first antigen binding domain without binding of said second antigen to said second binding domain, or binding of said second antigen to said second antigen binding domain without binding of said second antigen to said first binding domain induces anergy of said modified T lymphocyte.

3. The modified T lymphocyte of claim 1, wherein said first antigen binding domain and said second antigen binding domain are independently an antigen-binding portion of a receptor or an antigen-binding portion of an antibody.

4. The modified T lymphocyte of claim 3, wherein either or both of said first antigen binding domain or said second antigen binding domain are scFv antibody fragments.

5. The modified T lymphocyte of claim 1, wherein said first polypeptide additionally comprises a transmembrane domain.
6. The modified T lymphocyte of claim 1, wherein said second polypeptide additionally comprises a transmembrane domain.
7. The modified T lymphocyte of any of claims 1-6, wherein said first polypeptide or said second polypeptide comprises a T cell survival motif.
8. The modified T lymphocyte of claim 1, wherein said first antigen is an antigen on a tumor cell.
9. The modified T lymphocyte of claim 8, wherein said tumor cell is a cell in a solid tumor.
10. The modified T lymphocyte of claim 8, wherein said antigen is a tumor-associated antigen or a tumor-specific antigen.
11. The modified T lymphocyte of claim 10, wherein said tumor-associated antigen or tumor-specific antigen is Her2, prostate stem cell antigen (PSCA), PSMA, BCMA, alpha-fetoprotein (AFP), carcino embryonic antigen (CEA), cancer antigen-125 (CA-125), CA19-9, calretinin, MUC-1, epithelial membrane protein (EMA), epithelial tumor antigen (ETA), tyrosinase, melanoma-associated antigen (MAGE), CD34, CD45, CD99, CD117, chromogranin, cytokeratin, desmin, glial fibrillary acidic protein (GFAP), gross cystic disease fluid protein (GCDFP-15), HMB-45 antigen, protein melan-A (melanoma antigen recognized by T lymphocytes; MART-1), myo-Dl, muscle-specific actin (MSA), neurofilament, neuron-specific enolase (NSE), placental alkaline phosphatase, synaptophysin, thyroglobulin, thyroid transcription factor-1, the dimeric form of the pyruvate kinase isoenzyme type M2 (tumor M2-PK), CD 19, CD22, CD27, CD30, CD70, GD2 (ganglioside G2), EGFRvIII (epidermal growth factor variant III), sperm protein 17 (Sp17), mesothelin, PAP (prostatic acid phosphatase), prostatein, TARP (T cell receptor gamma alternate reading frame protein), Trp-p8, STEAP1 (six-transmembrane epithelial antigen of the prostate 1), an abnormal ras protein, or an abnormal p53 protein.
12. The modified T lymphocyte of claim 1, wherein said first antigen is integrin $\alpha v \beta 3$ (CD61), galactin, K-Ras (V-Ki-ras2 Kirsten rat sarcoma viral oncogene) or Ral-B.

13. The modified T lymphocyte of claim 1, wherein said first intracellular signaling domain comprises a polypeptide sequence comprising an immunoreceptor tyrosine-based activation motif (ITAM).
14. The modified T lymphocyte of claim 13, wherein said polypeptide sequence is a CD3 ζ signaling domain.
15. The modified T lymphocyte of claim 1-14, wherein said one or more co-stimulatory domains comprises one or more of a co-stimulatory CD27 polypeptide sequence, a co-stimulatory CD28 polypeptide sequence, a co-stimulatory OX40 (CD 134) polypeptide sequence, a co-stimulatory 4-1BB (CD 137) polypeptide sequence, and a co-stimulatory inducible T-cell costimulatory (ICOS) polypeptide sequence.
16. The modified T lymphocyte of claim 1, wherein said first polypeptide comprises an extracellular tumor antigen-binding domain and a CD3 ζ signaling domain.
17. The modified T lymphocyte of claim 16, wherein said second antigen is VEGF.
18. The modified T lymphocyte of claim 16, wherein said one or more co-stimulatory domains comprise co-stimulatory domains from one or more of CD27, CD28, OX40, ICOS, and 4-1BB.
19. The modified T lymphocyte of claim 18, wherein said first polypeptide comprises an extracellular tumor antigen-binding domain and a CD3 ζ signaling domain, and wherein said second polypeptide comprises an antigen-binding domain wherein said antigen is VEGF, and co-stimulatory domains from one or more of CD27, CD28, OX40, ICOS, and 4-1BB.
20. The modified T lymphocyte of claim 1, wherein said first polypeptide or said second polypeptide comprises a T cell survival motif.
21. The modified T lymphocyte of claim 19, wherein said T cell survival motif is, or is derived from, an intracellular signaling domain of IL-7 receptor (IL-7R), an intracellular signaling domain of IL-12 receptor, an intracellular signaling domain of IL-15 receptor, an intracellular signaling domain of IL-21 receptor, or an intracellular signaling domain of transforming growth factor β (TGF β) receptor.

22. The modified T lymphocyte of claim 16, wherein said second polypeptide comprises-an IL-7 receptor intracellular T cell survival motif, and wherein the one or more co-stimulatory domains comprise one or more of CD27, CD28, OX40, ICOS, and 4-1BB.
23. The modified T lymphocyte of claim 1, wherein said first antigen is a tumor-specific antigen or a tumor-associated antigen, and said first intracellular signaling domain comprises a CD3 ζ signaling domain; and wherein the one or more co-stimulatory domains comprise one or more of CD27, CD28, OX40, ICOS, and 4-1BB.
24. The modified T lymphocyte of claim 23, wherein said second polypeptide further comprises an intracellular T cell survival motif.
25. The modified T lymphocyte of claim 24, wherein said T cell survival motif is, or is derived from, an intracellular signaling domain of IL-7 receptor (IL-7R), an intracellular signaling domain of IL-12 receptor, an intracellular signaling domain of IL-15 receptor, an intracellular signaling domain of IL-21 receptor, or an intracellular signaling domain of transforming growth factor β (TGFB) receptor.
26. The modified T lymphocyte of claim 25, wherein said T cell survival motif is, or is derived from, an intracellular signaling domain of IL-7R.
27. The modified T lymphocyte of claim 23, wherein said second antigen is VEGF.
28. The modified T lymphocyte of claim 25, wherein said second antigen is VEGF.
29. A modified T lymphocyte comprising:
- a. a first polypeptide comprising a first extracellular antigen binding domain that binds a first antigen, a first intracellular signaling domain, and one or more co-stimulatory domains; and
 - b. a second polypeptide comprising a second extracellular antigen binding domain binding a second antigen, or a receptor that binds said second antigen; and a second intracellular signaling domain, wherein said second polypeptide does not comprise a co-stimulatory domain;
- wherein said second antigen is an antigen on an antibody that binds to an antigen presented by a tumor cell, vascular endothelial growth factor (VEGF), or a damage-associated molecular pattern molecule (DAMP),

wherein said DAMP is a heat shock protein, chromatin-associated protein high mobility group box 1 (HMGB1), S100A8 (MRP8, calgranulin A), S100A9 (MRP14, calgranulin B), serum amyloid A (SAA), deoxyribonucleic acid, adenosine triphosphate, uric acid, or heparin sulfate; and

wherein said modified lymphocyte becomes maximally cytotoxic only when said first signaling domain and said second signaling domain are both activated by said first antigen and said second antigen, respectively.

30. The modified T lymphocyte of claim 29, wherein binding of said first antigen to said first antigen binding domain without binding of said second antigen to said second binding domain, or binding of said second antigen to said second antigen binding domain without binding of said second antigen to said first binding domain induces anergy of said modified T lymphocyte.

31. The modified T lymphocyte of claim 29, wherein said first antigen-binding domain and said antigen-binding domain are independently an antigen-binding portion of a receptor or an antigen-binding portion of an antibody.

32. The modified T lymphocyte of claim 31, wherein either or both of said first antigen binding domain or said second antigen binding domain are scFv antibody fragments.

33. The modified T lymphocyte of claim 29, wherein said first polypeptide additionally comprises a transmembrane domain.

34. The modified T lymphocyte of claim 29, wherein said second polypeptide additionally comprises a transmembrane domain.

35. The modified T lymphocyte of any of claims 29-34, wherein said first polypeptide or said second polypeptide comprises a T cell survival motif.

36. The modified T lymphocyte of claim 29, wherein said first antigen is an antigen on a tumor cell.

37. The modified T lymphocyte of claim 36, wherein said tumor cell is a cell in a solid tumor.

38. The modified T lymphocyte of claim 36, wherein said first antigen is a tumor-associated antigen or a tumor-specific antigen.

39. The modified T lymphocyte of claim 38, wherein said tumor-associated antigen or tumor-specific antigen is Her2, prostate stem cell antigen (PSCA), PSMA, BCMA, alpha-fetoprotein (AFP), carcinoembryonic antigen (CEA), cancer antigen-125 (CA-125), CA19-9, calretinin, MUC-1, epithelial membrane protein (EMA), epithelial tumor antigen (ETA), tyrosinase, melanoma-associated antigen (MAGE), CD34, CD45, CD99, CD117, chromogranin, cytokeratin, desmin, glial fibrillary acidic protein (GFAP), gross cystic disease fluid protein (GCDFP-15), HMB-45 antigen, protein melan-A (melanoma antigen recognized by T lymphocytes; MART-1), myo-D1, muscle-specific actin (MSA), neurofilament, neuron-specific enolase (NSE), placental alkaline phosphatase, synaptophysin, thyroglobulin, thyroid transcription factor-1, the dimeric form of the pyruvate kinase isoenzyme type M2 (tumor M2-PK), CD 19, CD22, CD27, CD30, CD70, GD2 (ganglioside G2), EGFRvIII (epidermal growth factor variant III), sperm protein 17 (Sp17), mesothelin, PAP (prostatic acid phosphatase), prostatein, TARP (T cell receptor gamma alternate reading frame protein), Trp-p8, STEAP1 (six-transmembrane epithelial antigen of the prostate 1), an abnormal ras protein, or an abnormal p53 protein.

40. The modified T lymphocyte of claim 29, wherein said first antigen is integrin $\alpha\beta3$ (CD61), galactin, K-Ras (V-Ki-ras2 Kirsten rat sarcoma viral oncogene) or Ral-B.

41. The modified T lymphocyte of claim 29, wherein said second intracellular signaling domain comprises a polypeptide sequence comprising an immunoreceptor tyrosine-based activation motif (ITAM).

42. The modified T lymphocyte of claim 41, wherein said polypeptide sequence is a CD3 ζ signaling domain.

43. The modified T lymphocyte of claim 29, wherein said one or more co-stimulatory domains comprises one or more of a co-stimulatory CD27 polypeptide sequence, a co-stimulatory CD28 polypeptide sequence, a co-stimulatory OX40 (CD 134) polypeptide sequence, a co-stimulatory 4-1BB (CD137) polypeptide sequence, or a co-stimulatory inducible T-cell costimulatory (ICOS) polypeptide sequence.

44. The modified T lymphocyte of claim 29, wherein said first polypeptide or said second polypeptide comprises a T cell survival motif.

45. The modified T lymphocyte of claim 44, wherein said T cell survival motif is, or is derived from, an intracellular signaling domain of IL-7 receptor (IL-7R), an intracellular signaling domain of IL-12 receptor, an intracellular signaling domain of IL-15 receptor, an intracellular signaling domain of IL-21 receptor, or an intracellular signaling domain of transforming growth factor β (TGFB) receptor.

46. A modified T lymphocyte comprising:

a. a first polypeptide comprising a first extracellular antigen binding domain that binds a first antigen, an intracellular signaling domain, and one or more co-stimulatory domains; and

b. a second polypeptide comprising a second extracellular antigen binding domain that binds a second antigen, and a T cell survival motif, wherein said second polypeptide does not comprise an intracellular signaling domain or a co-stimulatory domain;

wherein said second antigen is an antigen on an antibody that binds to an antigen presented by a tumor cell, vascular endothelial growth factor (VEGF), or a damage associated molecular pattern molecule (DAMP),

wherein said DAMP is a heat shock protein, chromatin-associated protein high mobility group box 1 (HMGB1), S100A8 (MRP8, calgranulin A), S100A9 (MRP14, calgranulin B), serum amyloid A (SAA), deoxyribonucleic acid, adenosine triphosphate, uric acid, or heparin sulfate; and

wherein said modified lymphocyte survives only when said intracellular signaling domain and said T cell survival motif are both activated by said first antigen and said second antigen, respectively.

47. The modified T lymphocyte of claim 46, wherein said T cell survival motif is derived from, an intracellular signaling domain of IL-7 receptor (IL-7R), an intracellular signaling domain of IL-12 receptor, an intracellular signaling domain of IL-15 receptor, an intracellular signaling domain of IL-21 receptor, or an intracellular signaling domain of transforming growth factor β (TGFB) receptor.

48. The modified T lymphocyte of claim 46, wherein said intracellular signaling domain is or comprises a CD3 ζ signaling domain.

49. The modified T lymphocyte of claim 46, wherein said one or more co-stimulatory domains comprise one or more of a co-stimulatory CD27 polypeptide sequence, a co-stimulatory CD28 polypeptide sequence, a co-stimulatory OX40 (CD 134) polypeptide sequence, a co-stimulatory 4- IBB (CD 137) polypeptide sequence, and a co-stimulatory inducible T-cell costimulatory (ICOS) polypeptide sequence.
50. The modified T lymphocyte of claim 46, wherein said first antigen is an antigen on a tumor cell.
51. The modified T lymphocyte of claim 50, wherein said tumor cell is a cell in a solid tumor.
52. The modified T lymphocyte of claim 50, wherein said antigen is a tumor- associated antigen or a tumor-specific antigen.
53. The modified T lymphocyte of claim 52, wherein said tumor-associated antigen or tumor-specific antigen is Her2, prostate stem cell antigen (PSCA), alpha-fetoprotein (AFP), carcinoembryonic antigen (CEA), cancer antigen-125 (CA-125), CA19-9, calretinin, MUC-1, epithelial membrane protein (EMA), epithelial tumor antigen (ETA), tyrosinase, melanoma-associated antigen (MAGE), CD34, CD45, CD99, CD 117, chromogranin, cytokeratin, desmin, glial fibrillary acidic protein (GFAP), gross cystic disease fluid protein (GCDFF-15), HMB-45 antigen, protein melan-A (melanoma antigen recognized by T lymphocytes; MART-1), myo-DI, muscle-specific actin (MSA), neurofilament, neuron-specific enolase (NSE), placental alkaline phosphatase, synaptophysin, thyroglobulin, thyroid transcription factor- 1, the dimeric form of the pyruvate kinase isoenzyme type M2 (tumor M2-PK), CD 19, CD22, CD27, CD30, CD70, GD2 (ganglioside G2), EGFRvIII (epidermal growth factor variant III), sperm protein 17 (Sp17), mesothelin, PAP (prostatic acid phosphatase), prostein, TARP (T cell receptor gamma alternate reading frame protein), Trp-p8, STEAP1(six-transmembrane epithelial antigen of the prostate 1), an abnormal ras protein, or an abnormal p53 protein.
54. The modified T lymphocyte of claim 46, wherein said first antigen is integrin $\alpha\beta3$ (CD61), galactin, K-Ras (V-Ki-ras2 Kirsten rat sarcoma viral oncogene) or Ral-B.
55. A modified T lymphocyte comprising:

a. a first polypeptide comprising a first extracellular antigen binding domain that binds a first antigen, and a T cell survival motif, wherein said first polypeptide does not comprise an intracellular signaling domain or a co-stimulatory domain; and

b. a second polypeptide comprising a second extracellular antigen binding domain that binds a second antigen, an intracellular signaling domain and one or more co-stimulatory domains;

wherein said second antigen is an antigen on an antibody that binds to an antigen presented by a tumor cell, vascular endothelial growth factor (VEGF), or a damage associated molecular pattern molecule (DAMP),

wherein said DAMP is a heat shock protein, chromatin-associated protein high mobility group box 1 (HMGB1), S100A8 (MRP8, calgranulin A), S100A9 (MRP14, calgranulin B), serum amyloid A (SAA), deoxyribonucleic acid, adenosine triphosphate, uric acid, or heparin sulfate; and

wherein said modified lymphocyte survives only when said T cell survival motif and said intracellular signaling domain are both activated by said first antigen and said second antigen, respectively.

56. The modified T lymphocyte of claim 55, wherein said T cell survival motif is derived from, an intracellular signaling domain of IL-7 receptor (IL-7R), an intracellular signaling domain of IL-12 receptor, an intracellular signaling domain of IL-15 receptor, an intracellular signaling domain of IL-21 receptor, or an intracellular signaling domain of transforming growth factor β (TGFB) receptor.

57. The modified T lymphocyte of claim 55, wherein said intracellular signaling domain is a CD3 ζ signaling domain.

58. The modified T lymphocyte of claim 55, wherein said one or more co-stimulatory domains comprise one or more of a co-stimulatory CD27 polypeptide sequence, a co-stimulatory CD28 polypeptide sequence, a co-stimulatory OX40 (CD 134) polypeptide sequence, a co-stimulatory 4-1BB (CD 137) polypeptide sequence, or a co-stimulatory inducible T-cell costimulatory (ICOS) polypeptide sequence.

59. The modified T lymphocyte of claim 55, wherein said first antigen is an antigen on a tumor cell.
60. The modified T lymphocyte of claim 59, wherein said tumor cell is a cell in a solid tumor.
61. The modified T lymphocyte of claim 59, wherein said antigen is a tumor-associated antigen or a tumor-specific antigen.
62. The modified T lymphocyte of claim 61, wherein said tumor-associated antigen or tumor-specific antigen is Her2, prostate stem cell antigen (PSCA), alpha-fetoprotein (AFP), carcinoembryonic antigen (CEA), cancer antigen-125 (CA-125), CA19-9, calretinin, MUC-1, epithelial membrane protein (EMA), epithelial tumor antigen (ETA), tyrosinase, melanoma-associated antigen (MAGE), CD34, CD45, CD99, CD 117, chromogranin, cytokeratin, desmin, glial fibrillary acidic protein (GFAP), gross cystic disease fluid protein (GCDFP-15), HMB-45 antigen, protein melan-A (melanoma antigen recognized by T lymphocytes; MART-1), myo-D1, muscle-specific actin (MSA), neurofilament, neuron-specific enolase (NSE), placental alkaline phosphatase, synaptophysin, thyroglobulin, thyroid transcription factor- 1, the dimeric form of the pyruvate kinase isoenzyme type M2 (tumor M2-PK), CD 19, CD22, CD27, CD30, CD70, GD2 (ganglioside G2), EGFRvIII (epidermal growth factor variant III), sperm protein 17 (Sp17), mesothelin, PAP (prostatic acid phosphatase), prostein, TARP (T cell receptor gamma alternate reading frame protein), Trp-p8, STEAP1(six-transmembrane epithelial antigen of the prostate 1), an abnormal ras protein, or an abnormal p53 protein.
63. The modified T lymphocyte of claim 55, wherein said first antigen is integrin $\alpha\beta3$ (CD61), galactin, K-Ras (V-Ki-ras2 Kirsten rat sarcoma viral oncogene) or Ral-B.
64. The modified T lymphocyte of claim 1, wherein said second antigen is VEGF.
65. The modified T lymphocyte of claim 29, wherein said second antigen is VEGF.
66. The modified T lymphocyte of claim 46, wherein said second antigen is VEGF.
67. The modified T lymphocyte of claim 55, wherein said second antigen is VEGF.