CLAIMS

1. A polypeptide heterodimer, comprising:

(a) a first single chain polypeptide (SCP-I) comprising from one to four binding domains that specifically bind from one to four targets, a hinge (H-I), an immunoglobulin heterodimerization domain (HD-I), and an Fc region portion (FRP-I); and

(b) a second single chain polypeptide (SCP-II) comprising from zero to four binding domains that specifically bind from zero to four targets, a hinge (H-II), an immunoglobulin heterodimerization domain (HD-II), and an Fc region portion (FRP- II);

wherein

(i) the immunoglobulin heterodimerization domain of the first single chain polypeptide (HD-I) and the immunoglobulin heterodimerization domain of the second single chain polypeptide (HD-II) preferentially associate with each other to form a polypeptide heterodimer comprised of the first single chain polypeptide (SCP-I) and the second single chain polypeptide (SCP-II), and

(1) the immunoglobulin heterodimerization domain of the first single chain polypeptide (HD-I) comprises a first immunoglobulin CHI region, and the immunoglobulin heterodimerization domain of the second single chain polypeptide (HD-II) comprises a first immunoglobulin CL region, or

(2) the immunoglobulin heterodimerization domain of the first single chain polypeptide (HD-I) comprises a first immunoglobulin CL region, and the immunoglobulin heterodimerization domain of the second single chain polypeptide (HD-II) comprises a first immunoglobulin CHI region; and

(ii) the Fc region portion of the first single chain polypeptide (FCP-I) and the Fc region portion of the second single chain polypeptide (FCP-II) each comprise an immunoglobulin CH2 and CH3 domain of IgGl, IgG2, IgG3, IgG4, IgAl, IgA2, IgD, or any combination thereof; an immunoglobulin CH3 domain of IgGl, IgG2, IgG3, IgG4, IgAl, IgA2, IgD, IgE, IgM, or any combination thereof; or an immunoglobulin CH3 and CH4 domain of IgE, IgM, or a combination thereof,

provided that the polypeptide heterodimer comprises at least two binding domains that specifically bind at least two different targets.

2. The polypeptide heterodimer of claim 1, wherein the binding domains are single chain Fv (scFv) polypeptides.

3. The polypeptide heterodimer of claim 1, wherein the polypeptide heterodimer comprises two binding domains (BDl and BD2), three binding domains (BD1, BD2 and BD3), four binding domains (BD1, BD2, BD3 and BD4), or five to eight binding domains.

4. The polypeptide heterodimer of any one of claims 1 to 3, wherein at least one of the binding domains is an agonist of IL-10, HLA-G, HGF, IL-35, PD-1, BTLA, TNFR1, TNFR2, DR4, DR5, TWEAKR, or FAS.

5. The polypeptide heterodimer of any one of claims 1 to 3, wherein at least one binding domain specifically binds a TCR complex or a component thereof, and at least another binding domain specifically binds to PSMA, CD79b, CD19, HLA-DR, CD20, RON, c-Met, or CEACAM-6.

6. The polypeptide heterodimer of any one of claims 1 to 3, wherein at least one binding domain specifically binds to CD28, and at least another binding domain specifically binds to, or is an antagonist of, CD79b, hyperIL-6, PDL2, monoIL-10, CD86, LIGHT, GITRL, CD40, PDL1, HVEM, or LTBR.

7. The polypeptide heterodimer of any one of claim 1 to 3, wherein at least one binding domain specifically binds to CD28, and at least another binding domain is an agonist of IL-10, HLA-G, HGF, IL-35, PD-1, or BTLA.

8. The polypeptide heterodimer of any one of claims 1 to 7 wherein the immunoglobulin heterodimerization domain of the first single chain polypeptide (HD-I) comprises the first immunoglobulin CHl region and the immunoglobulin heterodimerization domain of the second single chain polypeptide (HD-II) comprises the first immunoglobulin CL region.

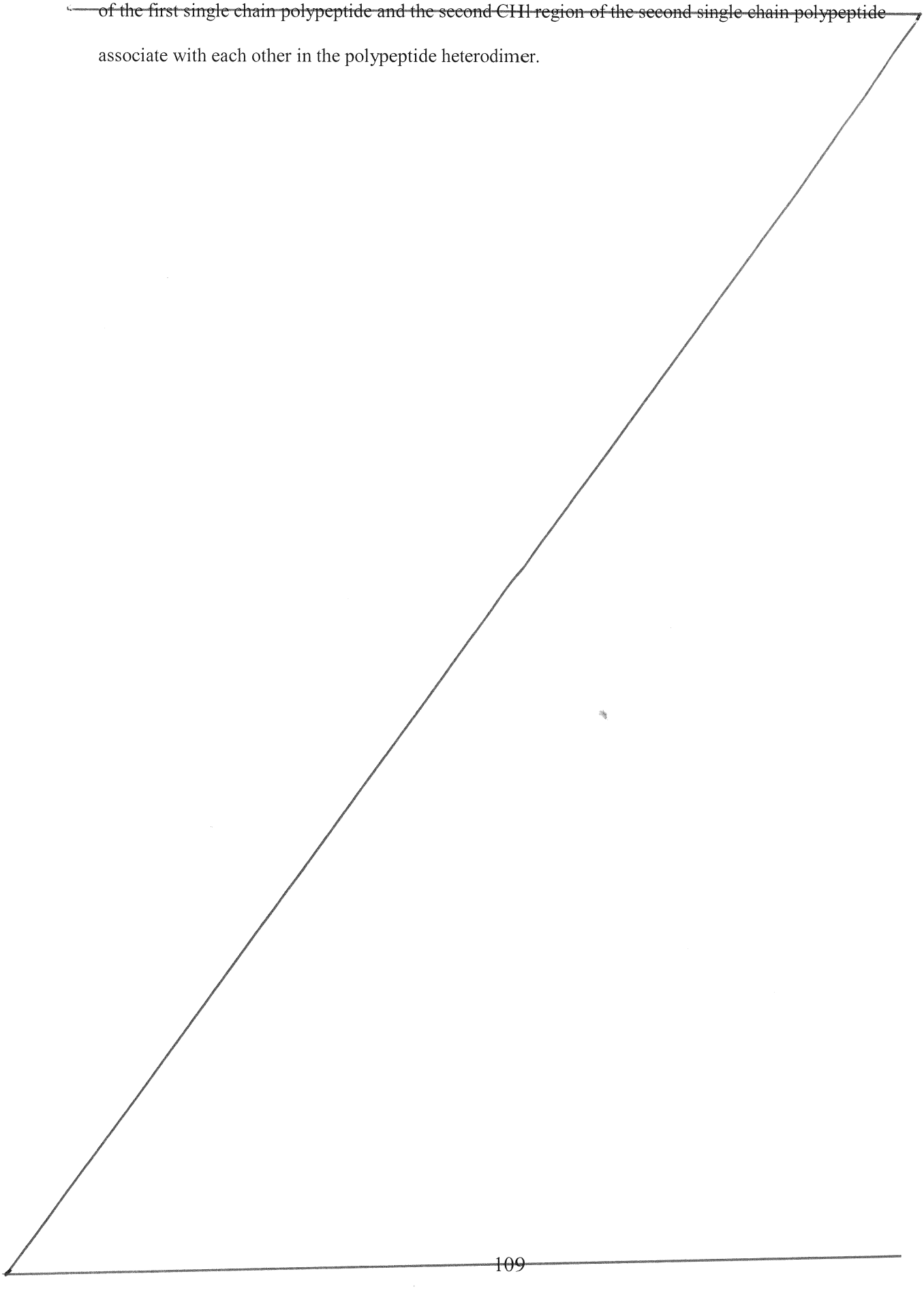
9. The polypeptide heterodimer of claim 8 wherein the first single chain polypeptide further comprises a second CHl region and the second single chain polypeptide further comprises a second CL region, and wherein the second CHl region

~~of the first single chain polypeptide and the second CL region of the second single chain polypeptide associate with~~ each other in the polypeptide heterodimer.

10. The polypeptide heterodimer of any one of claims 1 to 7 wherein the immunoglobulin heterodimerization domain of the first single chain polypeptide (HD-I) comprises a first immunoglobulin CL region, and the immunoglobulin heterodimerization domain of the second single chain polypeptide (HD-II) comprises a first immunoglobulin CHl region.

11. The polypeptide heterodimer of claim 10 wherein the first single chain polypeptide further comprises a second CL region and the second single chain polypeptide further comprises a second CHl region, and wherein the second CL region

of the first single chain polypeptide and the second CH1 region of the second single chain polypeptide associate with each other in the polypeptide heterodimer.



12. The polypeptide heterodimer of any one of claims 1 to 11 wherein the first CL region is a Cκ region or a Cλ region.

13. The polypeptide heterodimer of claim 9 or claim 11, wherein the second CL region is a Cκ region or a Cλ region.

14. The polypeptide heterodimer of claim 12 or claim 13, wherein the Cκ region is a wild type human immunoglobulin Cκ region.

15. The polypeptide heterodimer of claim 12 or claim 13, wherein the Cκ region is an altered human immunoglobulin Cκ region with one or more amino acids of a wild type human Cκ region substituted at N29, N30, Q52, V55, T56, S68, or T70.

16. The polypeptide heterodimer of claim 15, wherein the one or more amino acid substitutions are selected from Ala (A), Arg (R), Trp (W), Tyr (Y), Glu (E), Gln (Q), Lys (K), Asp (D), Met (M), Ser (S), and Phe (F).

17. The polypeptide heterodimer of claim 12 or claim 13, wherein the CHl region is an altered human immunoglobulin CHl region comprising an amino acid substitution by which Val (V) at position 68 is substituted by Lys K), Arg (R) or His (H), and wherein the Cκ region is an altered human immunoglobulin Cκ region comprising an amino acid substitution by which Leu (L) at position 27 is substituted by Asp (D) or Glu (E).

18. The polypeptide heterodimer of claim 12 or claim 13, wherein the CHl region is an altered human immunoglobulin CHl region comprising an amino acid substitution by which Val (V) at position 68 is changed to Asp (D) or Glu (E), and wherein the Cκ region is an altered human immunoglobulin Cκ region comprising an amino acid substitution by which Leu (L) at position 27 is changed to Lys (K), Arg (R) or His (H).

19. The polypeptide heterodimer of any one of claims 1 to 18, wherein the first CHl region is an altered human immunoglobulin CHl region with the cysteine of a wild type human immunoglobulin CHl region that is involved in forming a disulfide bond with a wild type human immunoglobulin CL region deleted or substituted.

20. The polypeptide heterodimer of claim 12 or 13, wherein the Cκ region is an altered human immunoglobulin Ck region with the cysteine residue of a wild type human Cκ region that is involved in forming a disulfide bond with a wild type human immunoglobulin CH1 region deleted or substituted.

21. The polypeptide heterodimer of claim 12 or claim 13, wherein the Cλ region is an altered human immunoglobulin Cλ region with the cysteine residue of a wild type human Cλ region that is involved in forming a disulfide bond with a wild type human immunoglobulin CH1 region deleted or substituted.

22. The polypeptide heterodimer of claim 19, wherein the first CHl region is a polypeptide comprising SEQ ID NO: 114, 844 or 845.

23. The polypeptide heterodimer of claim 12 or 20, wherein the Cκ region is selected from any one of the polypeptides comprising SEQ ID NOS: 141-178, 202, and 838-843.

24. The polypeptide heterodimer of claim 12 or 21, wherein the Cλ region is a polypeptide comprising SEQ ID NO: 140.

25. The polypeptide heterodimer of any one of claims 1 to 24, wherein the Fc region portion of the first single chain polypeptide (FRP-I) and the Fc region portion of the second single chain polypeptide (FRP-II) each comprise an immunoglobulin CH2 domain or an immunoglobulin CH3 domain.

26. The polypeptide heterodimer of any one of claims 1 to 25, wherein the Fc region portion of the first single chain polypeptide (FRP-I) and the Fc region portion of the second single chain polypeptide (FRP-II) each comprise an immunoglobulin CH2 domain and an immunoglobulin CH3 domain.

27. The polypeptide heterodimer of any one of claims 1 to 26 wherein the hinge of both the first and second single chain polypeptides is an immunoglobulin hinge region.

28. The polypeptide heterodimer of claim 27 wherein the hinge region is

(a) amino terminal to the Fc region portion,

(b) disposed between the binding domain and the immunoglobulin heterodimerization domain,

(c) disposed between the immunoglobulin heterodimerization domain and the Fc region portion, or

(d) at the amino terminus of the first or second single chain polypeptide.

29. The polypeptide heterodimer of any one of claims 1 to 28 wherein the hinges of the first and second single chain polypeptides are different.

30. The heterodimer of any one of claims 1-29, wherein the first single chain polypeptide comprises a binding domain that specifically binds a

TCR complex or a component thereof, and the second single chain polypeptide comprises a binding domain that specifically binds a tumor-specific antigen.

31. The heterodimer of claim 1, wherein the first and second single chain polypeptides comprise SEQ ID NOS: SEQ ID NOS:2 and 4, SEQ ID NOS:6 and 8, SEQ ID NOS: 10 and 12, SEQ ID NOS: 14 and 16, SEQ ID NOS: 18 and 20, SEQ ID NOS:20 and 22, SEQ ID NOS:20 and 24, SEQ ID NOS:30 and 32, SEQ ID NOS:29 and 31, SEQ ID NOS:29 and 32, SEQ ID NOS:30 and 72, SEQ ID NOS:53 and 72, SEQ ID NOS:54 and 72, SEQ ID NOS:55 and 72, SEQ ID NOS:70 and 72, SEQ ID NOS:71 and 72, SEQ ID NOS:63 and 56, SEQ ID NOS:64 and 57, SEQ ID NOS:65 and 60, SEQ ID NOS:66 and 58, SEQ ID NOS:67 and 59, SEQ ID NOS:68 and 61, SEQ ID NOS:69 and 62, SEQ ID NOS:54 and 811, SEQ ID NOS:54 and 812, SEQ ID NOS:54 and 813, SEQ ID NOS:814 and 818, SEQ ID NOS:815 and 818, SEQ ID NOS:816 and 818, SEQ ID NOS:817 and 818, SEQ ID NOS:814 and 820, SEQ ID NOS:814 and 821, SEQ ID NOS:54 and 819, SEQ ID NOS:814 and 826, SEQ ID NOS:814 and 822, SEQ ID NOS: 814 and 823, SEQ ID NOS:814 and 824, SEQ ID NOS:859 and 862, SEQ ID NOS:860 and 863, SEQ ID NOS:861 and 864, SEQ ID NOS:874 and 825, SEQ ID NOS:875 and 879, SEQ ID NOS:876 and 880, SEQ ID NOS:877 and 881, or SEQ ID NOS:878 and 882.

32. A composition comprising a polypeptide heterodimer of any one of claims 1 to 31 and a pharmaceutically acceptable excipient.

33. An expression vector capable of expressing the polypeptide heterodimer of any one of claims 1 to 32, comprising a first polynucleotide encoding

the first single chain polypeptide and a second polynucleotide encoding the second single chain polypeptide.

34. A host cell comprising the expression vector of claim 33.

35. A host cell comprising first and second expression vectors capable of expressing the first and second single chain polypeptides, respectively, of the polypeptide heterodimer of any one of claims 1 to 31.

36. A method for making a polypeptide heterodimer, comprising

(a) culturing the host cell of claim 34 or claim 35 under conditions suitable to express first and second single chain polypeptides, and

(b) optionally isolating or purifying the heterodimers formed from the first and second single chain polypeptides from the culture.

37. Use of a polypeptide heterodimer according to any one of claims 1 to 31 in the manufacture of a medicament for directing T cell activation, wherein the polypeptide heterodimer comprises a binding domain that specifically binds TCRα, TCRβ, CD3γ, CD3δ, CD3ε or a combination thereof, and a second binding domain that specifically binds a different target.

38. Use of a polypeptide heterodimer according to any one of claims 1 to 31 in the manufacture of a medicament for inhibiting growth, metastasis or metastatic growth of a malignancy, wherein the polypeptide heterodimer comprises a binding domain that specifically binds TCRα, TCRβ, CD3γ, CD3δ, CD3ε, c-Met, or Ron.

39. Use of a polypeptide heterodimer according to any one of claims 1 to 31 in the manufacture of a medicament for treating an autoimmune or inflammatory condition, wherein the polypeptide heterodimer comprises a binding domain that specifically binds TCRα, TCRβ, CD3γ, CD3δ, CD3ε, or CD28.

40. Use of a polypeptide heterodimer according to any one of claims 1 to 31 in the manufacture of a medicament for treating a B-cell associated disorder or disease, wherein the

polypeptide heterodimer comprises a binding domain that specifically binds TCRα, TCRβ, CD3γ, CD3δ, CD3ε, and a second binding domain that specifically binds to CD19, CD20, CD79b or HLA-DR.

41. The polypeptide heterodimer of claim 15, wherein the altered human immunoglobulin Cκ region comprises the following substitutions: (i) N30Y, V55A, and T70E; or (ii) N30M, V55A, and T70E.

CLAIMS

1. A polypeptide heterodimer, comprising:

(a) a first single chain polypeptide (SCP-I) comprising from one to four binding domains that specifically bind from one to four targets, a hinge (H-I), an immunoglobulin heterodimerization domain (HD-I), and an immunoglobulin CH2 and CH3 domain of IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgD, or any combination thereof (CH2CH3-I); and

(b) a second single chain polypeptide (SCP-II) comprising from zero to four binding domains that specifically bind from zero to four targets, a hinge (H-II), an immunoglobulin heterodimerization domain (HD-II), and an immunoglobulin CH2 and CH3 domain of IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgD, or any combination thereof (CH2CH3-II);

wherein

(i) the immunoglobulin heterodimerization domain of the first single chain polypeptide (HD-I) and the immunoglobulin heterodimerization domain of the second single chain polypeptide (HD-II) preferentially associate with each other to form a polypeptide heterodimer comprised of the first single chain polypeptide (SCP-I) and the second single chain polypeptide (SCP-II), and

(1) the immunoglobulin heterodimerization domain of the first single chain polypeptide (HD-I) comprises a first immunoglobulin CHI region, and the immunoglobulin heterodimerization domain of the second single chain polypeptide (HD-II) comprises a first immunoglobulin CL region, or

(2) the immunoglobulin heterodimerization domain of the first single chain polypeptide (HD-I) comprises a first immunoglobulin CL region, and the immunoglobulin heterodimerization domain of the second single chain polypeptide (HD-II) comprises a first immunoglobulin CHI region;

(ii) wherein the first immunoglobulin CL region is an altered human immunoglobulin Cκ region with one or more amino acids of a wild type human Cκ region substituted at N29, N30, Q52, V55, T56, S68, or T70,

provided that the polypeptide heterodimer comprises at least two binding domains that specifically bind at least two different targets.

2. The polypeptide heterodimer of claim 1, wherein the binding domains are single chain Fv (scFv) polypeptides.

3. The polypeptide heterodimer of claim 1, wherein the polypeptide heterodimer comprises two binding domains (BDl and BD2), wherein preferably the first binding domain (BD1) is on the first single chain polypeptide (SCP-I) and the second binding domain (BD2) is on the second single chain polypeptide (SCP-II).

4. The polypeptide heterodimer of claim 3, wherein the first binding domain (BD1) is amino terminal to the CH2CH3-I of the first single chain polypeptide, and the second binding domain (BD2) is amino terminal to the CH2CH3-II of the second single chain polypeptide.

5. The polypeptide heterodimer of claim 1 or 2, wherein the polypeptide heterodimer comprises three binding domains (BD1, BD2 and BD3).

6. The polypeptide heterodimer of claim 1, wherein the polypeptide heterodimer comprises four binding domains (BD1, BD2, BD3, and BD4), wherein preferably the HD-I and CH2CH3-I are disposed between BD1 and BD2, and the HD-II and CH2CH3-II are disposed between BD3 and BD4.

7. The polypeptide heterodimer of claim 1 or 2, wherein the polypeptide heterodimer comprises five to eight binding domains.

8. The polypeptide heterodimer of any one of claims 1 to 7, wherein at least one binding domain specifically binds to, or is an antagonist of, a TCR complex, TCRα, TCRβ, CD3γ, CD3δ, CD3ε, CD28, CD79b, hyperIL-6, monoIL-10, CD86, CD20, PSMA, CD19, HLA-DR, Ron, c-Met, CEACAM-6, LIGHT, GITRL, CD40, PDL1, PDL2, HVEM, LTBR, EGFR, EGFRvIII, ErbB2, ErbB3, ErbB4, IGF1R, EphA2, PDGFR, VEGFR1-4, Angiopoietin 2, CD64, CD32A, CD16, CD71, TNFR1, TNFR2,

TWEAKR, TACI, BAFF-R, BCMA, FAS, CD32B, CD21, CD22, CD30, CD33, CD37, CD38, CD70, TNFα, IL-6, hyperIL-6, IL-2, IL-1, IL-7, IL-8, IL-17A/C, IP-10, IFNγ, IFNα, RANKL, FASL, TGFβ, IL10, IL17A/F, CSF2, IGF1, IGF2, BLyS/APRIL, HGF, MSP, EGF (including epiregulin, herregulin, β-regulin, neuregulin), HIF-1α, VEGFA, VEGFB, VEGFC, VEGFD, TNFα, Wnt, sHH, TGFβ, PDGF, TWEAK, EpCAM, CEA, PCTA-1, STEAP-1, PSCA, ALCAM (CD166), EphA2, CD151, CA-125, MUC-1, MAGE-1, TROP2, CCR5, HER-3, HER-4, EGFR, CEA, MUC2, MUC3, MUC4, $MUC5_{AC}$, $MUC5_b$, MUC7, βhCG, Lewis-Y, ganglioside GD3, 9-O-Acetyl-GD3, GM2, Globo H, fucosyl GM1, Poly SA, GD2, Carboanhydrase IX (MN/CA IX), CD44v6, Sonic Hedgehog (Shh), Wue-1, Plasma Cell Antigen, (membrane-bound) IgE, Melanoma Chondroitin Sulfate Proteoglycan (MCSP), CCR8, TNF-alpha precursor, STEAP, mesothelin, A33 Antigen, Prostate Stem Cell Antigen (PSCA), Ly-6; desmoglein 4, E-cadherin neoepitope, Fetal Acetylcholine Receptor, CD25, CA19-9 marker, CA-125 marker and Muellerian Inhibitory Substance (MIS) Receptor type II, sTn (sialylated Tn antigen; TAG-72), FAP (fibroblast activation antigen), endosialin, EGFRvIII, LG, SAS, CD63, IGF1R, CD151, TGFBR2, GHRHR, GHR, IL-6R, gp130, TNFR2, OSMRβ, Patched-1, Frizzled, Robo1, CD80, CD81, CD86, OX40, CD40, CD137, LIFRβ, TLR7 or TLR9.

9. The polypeptide heterodimer of any one of the preceding claims, wherein the one or more amino acids of a wild type human Cκ region are substituted with one or more amino acid substitutions selected from Ala (A), Arg (R), Trp (W), Tyr (Y), Glu (E), Gln (Q), Lys (K), Asp (D), Met (M), Ser (S), and Phe (F).

10. The polypeptide heterodimer of any one of the preceding claims, wherein the altered human immunoglobulin Cκ region comprises the following substitutions: (i) N30Y V55A T70E; or (ii) N30M, V55A T70E.

11. The polypeptide heterodimer of any one of the preceding claims, wherein the CH1 region is an altered human immunoglobulin CH1 region comprising an amino acid substitution by which Val (V) at position 68 is substituted by Lys (K), Arg (R) or His (H), and wherein the Cκ region is an altered human immunoglobulin Cκ region comprising an amino acid substitution by which Leu (L) at position 27 is

substituted by Asp (D) or Glu (E) or wherein the CH1 region is an altered human immunoglobulin CH1region comprising an amino acid substitution by which Val (V) at position 68 is substituted by Asp (D) or Glu (E), and wherein the Cκ region is an altered human immunoglobulin Cκ region comprising an amino acid substitution by which Leu (L) at position 27 is changed to Lys (K), Arg (R) or His (H).

12. The polypeptide heterodimer of any one of claims 1 to 11, wherein the first CH1 region is an altered human immunoglobulin CH1 region with the cysteine of a wild type human immunoglobulin CH1 region that is involved in forming a disulfide bond with a wild type human immunoglobulin CL region deleted or substituted.

13. The polypeptide heterodimer of any one of the preceding claims, wherein the Cκ region is an altered human immunoglobulin Cκ region with the cysteine residue of a wild type human Cκ region that is involved in forming a disulfide bond with a wild type human immunoglobulin CH1 region deleted or substituted.

14. The polypeptide heterodimer of any one of claims 1 to 13 wherein the hinge of both the first and second single chain polypeptides is an immunoglobulin hinge region.

15. The polypeptide heterodimer of any one of claims 1 to 14 wherein the hinge region is

(a) at the amino terminal to CH2CH3-I or CH2CH3-II,

(b) disposed between the binding domain and the first immunoglobulin heterodimerization domain,

(c) disposed between a immunoglobulin heterodimerization domain and CH2CH3-I or CH2CH3-II,

(d) at the amino terminus of the first or second single chain polypeptide,

(e) a C-type lectin hinge region,

(f) an NKg2A or NKg2D peptide, or a derivative thereof,

(g) disposed between CH2CH3-I and a binding domain, or

(h) at the carboxyl terminus of the first or second single chain polypeptide.

16. The heterodimer of any one of the preceding claims, wherein the first single chain polypeptide comprises a binding domain that specifically binds a TCR complex or a component thereof, and the second single chain polypeptide comprises a binding domain that specifically binds a tumor-specific antigen.

17. A composition comprising a polypeptide heterodimer of any one of claims 1 to 16 and a pharmaceutically acceptable excipient.

18. The polypeptide heterodimer according to any one of claims 1 to 17 for use in:

a) a method for directing T cell activation, wherein the polypeptide heterodimer comprises a binding domain that specifically binds TCRα, TCRβ, CD3γ, CD3δ, CD3ε or a combination thereof, and a second binding domain that specifically binds a different target; or

b) inhibiting growth, metastasis or metastatic growth of a malignancy, wherein the polypeptide heterodimer comprises a binding domain that specifically binds TCRα, TCRβ, CD3γ, CD3δ, CD3ε, c-Met or Ron;

c) treating an autoimmune or inflammatory condition, wherein the polypeptide heterodimer comprises a binding domain that specifically binids TCRα, TCRβ, CD3γ, CD3δ, CD3ε, or CD28.

d) treating a B-cell associated disorder or disease, wherein the polypeptide heterodimer comprises a binding domain that specifically binds TCRα, TCRβ, CD3γ, CD3δ, CD3ε, and a second binding domain that specifically binds to CD19, CD20, CD79b or HLA-DR.

19. An expression vector capable of expressing the polypeptide heterodimer of any one of claims 1 to 16, comprising a first polynucleotide encoding the first single chain polypeptide and a second polynucleotide encoding the second single chain polypeptide.

20. A host cell comprising (i) the expression vector of claim 19 or (ii) first and second expression vectors capable of expressing the first and second single chain polypeptides, respectively, of the polypeptide heterodimer of any one of claims 1 to 16.

21. A method for making a polypeptide heterodimer, comprising

(a) culturing the host cell of claim 20 under conditions suitable to express first and second single chain polypeptides, and

(b) optionally isolating or purifying the heterodimers formed from the first and second single chain polypeptides from the culture.