

[0006] Because direct engagement of CD3 results in T-cell activation, it is a desirable target for a variety of therapeutic and/or diagnostic indications. Accordingly, there exists a need for antibodies and therapeutics that target the CD3/TCR pathway.

SUMMARY

[0007] The present disclosure provides multispecific polypeptide constructs that exhibit constrained CD3 binding. In some examples, the multispecific polypeptide construct is composed of a first component comprising an immunoglobulin Fc region and a second component comprising a CD3-binding region, wherein the first and second components are coupled by a linker or operably linked, wherein the Fc region is positioned N-terminal to the CD3-binding region; and one or both of the first and second components comprises an antigen binding domain that binds a tumor associated antigen (TAA). In some examples, the multispecific polypeptide construct, in an inactive state, is composed of a first component and a second component, wherein the first and second components are operably linked, wherein each of the first and second components comprises an antigen binding domain that binds a tumor associated antigen (TAA), wherein the first component comprises an Fc region, wherein the second component comprises a CD3-binding region, and wherein the first and second components are coupled by a cleavable linker. In some examples, the CD3-binding region binds CD3 (CD3ε).

[0007a] In one aspect, the present disclosure refers to a multispecific polypeptide construct, the multispecific polypeptide construct comprising a first component comprising a heterodimeric immunoglobulin Fc region and a second component comprising a CD3-binding region, wherein:

the first and second components are coupled by a polypeptide linker, wherein the Fc region is positioned amino-terminally relative to the CD3-binding region;

the CD3-binding region is a disulfide stabilized anti-CD3 binding Fv fragment (dsFv) comprising a variable heavy chain region (VH) and a variable light chain region (VL), wherein the VH and VL are linked to opposite polypeptides of the heterodimeric Fc; and one or both of the first and second components comprises at least one antigen binding domain that binds a tumor associated antigen (TAA), and

wherein each of the at least one antigen binding domain is positioned amino-terminally relative to the Fc region and/or positioned carboxy-terminally relative to the CD3-binding region; and

wherein the CD3-binding region is not able to measurably bind or engage cell surface CD3 unless at least one antigen binding domain is bound to its TAA.

[0007b] In another aspect, the present disclosure refers to a multispecific polypeptide construct, the multispecific polypeptide construct comprising a first component comprising an immunoglobulin Fc region and a second component comprising a CD3-binding region, wherein:

the first and second components are coupled by a polypeptide linker, wherein the Fc region is positioned amino-terminally relative to the CD3-binding region;

the CD3-binding region is a disulfide stabilized anti-CD3 binding Fv fragment (dsFv) comprising a variable heavy chain region (VH) and a variable light chain region (VL); and

the multispecific polypeptide construct comprises at least one antigen binding domain that binds a tumor associated antigen (TAA), and wherein each of the at least one antigen binding domain is positioned amino-terminally relative to the Fc region and/or positioned carboxy-terminally relative to the CD3-binding region, wherein at least one antigen binding domain is positioned carboxy-terminally relative to the CD3-binding region; and wherein the CD3-binding region is not able to measurably bind or engage cell surface CD3 unless at least one antigen binding domain is bound to its TAA.

[0007c] In another aspect, the present disclosure refers to a multispecific polypeptide construct, the multispecific polypeptide construct comprising a first component comprising an immunoglobulin Fc region and a second component comprising a CD3-binding region, wherein: the first and second components are coupled by a polypeptide linker, wherein the Fc region is positioned amino-terminally relative to the CD3-binding region; the CD3-binding region is a disulfide stabilized anti-CD3 binding Fv fragment (dsFv) comprising a variable heavy chain region (VH) and a variable light chain region (VL); and the first component comprises at least a first antigen binding domain and the second component comprises at least a second antigen binding domain, wherein each of the antigen binding domains binds a tumor associated antigen (TAA), and wherein each of the at least one antigen binding domain is positioned amino-terminally relative to the Fc region and/or positioned carboxy-terminally relative to the CD3-binding region; and wherein the CD3-binding region is not able to measurably bind or engage cell surface CD3 unless at least one antigen binding domain is bound to its TAA.

[0007d] In another aspect, the present disclosure refers to a multispecific polypeptide construct, the multispecific polypeptide construct comprising a first component comprising an immunoglobulin Fc region and a second component comprising a CD3-binding region, wherein: the first and second components are coupled by a cleavable linker, wherein the Fc region is positioned amino-terminally relative to the CD3-binding region; the CD3-binding region is a disulfide stabilized anti-CD3 binding Fv fragment (dsFv) comprising a variable heavy chain region (VH) and a variable light chain region (VL); and the multispecific polypeptide construct further comprises at least one antigen binding domain that binds a tumor associated antigen (TAA), and wherein each of the at least one antigen binding domain is positioned amino-terminally relative to the Fc region and/or positioned carboxy-terminally relative to the CD3-binding region; and wherein the CD3-binding region is not able to measurably bind or engage cell surface CD3 unless at least one antigen binding domain is bound to its TAA.

[0008] In some examples, the antigen binding domain is positioned amino-terminally relative to the Fc region and/or carboxy-terminally relative to the CD3 binding region of the multispecific polypeptide construct. In some examples, the first component comprises a first antigen binding domain and the second component comprises a second antigen binding domain, wherein each of the antigen binding domains bind a tumor associated antigen (TAA). In some cases, the first antigen binding domain is positioned at the amino terminus of the multispecific construct and the second antigen binding domain is positioned at the carboxy terminus of the multispecific construct. In some examples, the first antigen binding domain is positioned amino-terminally relative to the Fc region and/or carboxy-terminally relative to the CD3 binding region of the multispecific polypeptide construct.

[0009] Provided herein is a multispecific polypeptide construct, wherein the multispecific construct comprises in order, from N-terminus to C-terminus: a first antigen binding domain that binds to a tumor-associated antigen (TAA); an immunoglobulin Fc region; a linker; a CD3 binding region that binds CD3 (CD3 ϵ); and a second antigen binding domain that binds a tumor-associated antigen (TAA). Also provided is a multispecific

terminal to the anti-CD3 antibody; and one or both of the first and second components comprises an antigen binding domain that binds a tumor associated antigen (TAA).

[0047a] In another aspect, the present disclosure refers to a multispecific polypeptide construct, the multispecific polypeptide construct comprising a first component comprising a heterodimeric Fc region and a second component comprising a CD3-binding region, wherein: the CD3-binding region is a disulfide stabilized anti-CD3 binding Fv fragment (dsFv) comprising a variable heavy chain region (VH) and a variable light chain region (VL); the VH and VL are linked to opposite polypeptides of the heterodimeric Fc; the first and second components are coupled by a cleavable linker, wherein the heterodimeric Fc region is positioned amino-terminally relative to the anti-CD3 antibody or antigen-binding fragment; and

one or both of the first and second components comprises at least one antigen binding domain that binds a tumor associated antigen (TAA), and wherein each of the at least one antigen binding domain is positioned amino-terminally relative to the Fc region and/or positioned carboxy-terminally relative to the CD3-binding region; and wherein the CD3-binding region is not able to measurably bind or engage cell surface CD3 unless at least one antigen binding domain is bound to its TAA.

[0048] In some examples, binding of the CD3-binding region to CD3 is substantially reduced when the multispecific polypeptide construct is in an uncleaved state compared to a cleaved state. In some examples, in a cleaved state, the first and second components are not linked.

[0049] In some examples, the cleavable linker is a polypeptide. In some examples, the cleavable linker is a polypeptide that is a substrate for a protease. In some examples, the protease is produced by an immune effector cell, by a tumor, or by cells present in the tumor microenvironment. In some examples, the protease is produced by a tumor that is in proximity to cells that express CD3 ϵ and/or is produced by a tumor that is co-localized with cells that express CD3 ϵ in a tissue, and wherein the protease cleaves the cleavable linker in the multispecific polypeptide construct when the multispecific polypeptide construct is exposed to the protease. In some examples, the protease is produced by a tumor that is in proximity to cells that express one or more tumor associated antigens (TAA) and/or is produced by a tumor that is co-localized with cells that express the target TAA(s) in a tissue, and wherein the protease cleaves the cleavable linker in the multispecific polypeptide construct when the multispecific polypeptide construct is exposed to the protease. In some examples, the protease is produced by an immune effector cell. In some examples, the protease is produced by an immune effector cell that is in proximity to cells that express the TAA. In some examples, the protease is produced by an immune effector cell and the immune effector cell is an activated T cell, a natural killer (NK) cell, or an NK T cell. In some examples, the protease cleaves the cleavable linker in the multispecific polypeptide construct when the multispecific polypeptide construct is exposed to the protease. In some examples, the protease is produced an immune effector cell that is in proximity to cells that express the TAA and wherein the protease cleaves the cleavable linker in the multispecific polypeptide construct when the multispecific polypeptide construct is exposed to the protease.

[0050] In some examples, the cleavable linker is a polypeptide of up to 50 amino acids in length. In some examples, the cleavable linker is a polypeptide of up to 25 amino acids in length. In some examples, the cleavable linker is a polypeptide of up to 15 amino acids in length.

[0088] Provided herein is a multispecific polypeptide construct produced by any of the provided methods.

[0089] Provided herein is a method of stimulating or inducing an immune response, the method comprising contacting a target cell and a T cell with the any of the provided multispecific polypeptide constructs or pharmaceutical compositions, said target cell expressing a tumor associated antigen recognized by the multispecific polypeptide construct. In some examples, the target cell is a tumor cell expressing the tumor associated antigen (TAA).

[0090] In some examples, the multispecific polypeptide construct comprises a cleavable linker that functions as a substrate for a protease and the inducing or stimulating the immune response is increased in the presence of the protease. In some cases, the protease is produced by an immune effector cell, by a tumor, or by cells present in the tumor microenvironment.

[0091] In some examples, the protease is produced by an immune effector cell and the immune effector cell is an activated T cell, a natural killer (NK) cell, or an NK T cell. In some instances, the immune effector cell is in proximity to cells that express the antigen. In some examples, the protease is produced by a tumor that is in proximity to cells that express the TAA in a tissue and/or produced by a tumor that is co-localized with TAA in a tissue, and wherein the protease cleaves the cleavable linker in the multispecific polypeptide construct when the multispecific polypeptide construct is exposed to the protease. In some examples, the protease is selected from among matriptase, a matrix metalloprotease (MMP), granzyme B, and combinations thereof. In some instances, the protease is granzyme B.

[0092] In some examples, the contacting is carried out ex vivo or in vitro. In some examples, the contacting is carried out in vivo in a subject.

[0093] Provided is a method of stimulating or inducing an immune response in a subject, the method comprising administering, to a subject in need thereof, a therapeutically effective amount of any of the provided multispecific conjugates or pharmaceutical compositions. In some cases, the method increases cell-mediated immunity. In some examples, the method increases T-cell activity. In some examples, the method increases cytolytic T-cell (CTL) activity. In some examples, the immune response is increased against a tumor or cancer. In some examples, the method treats a disease or condition in the subject.

[0094] The present disclosure also provides methods of treating, preventing, delaying the progression of or otherwise ameliorating a symptom of one or more pathologies or alleviating a symptom associated with such pathologies, by administering a multispecific polypeptide

construct of the disclosure to a subject in which such treatment or prevention is desired. Provided herein is a method of treating a disease or condition in a subject, the method comprising administering, to a subject in need thereof, a therapeutically effective amount of any of the provided multispecific conjugates or pharmaceutical compositions. In some examples, the disease or condition is a tumor or a cancer.

[0095] In some examples of any of the provided method, the subject, such as the subject to be treated is, *e.g.*, human or other mammal. In some examples of any of the provided method, the subject is a human. In some examples, the subject is a non-human mammal, such as a non-human primate, companion animal (*e.g.*, cat, dog, horse), farm animal, work animal, or zoo animal. In some examples, the subject is a rodent.

[0096] A multispecific polypeptide construct of the disclosure used in any of the examples of these methods and uses can be administered at any stage of the disease. For example, such a multispecific polypeptide construct can be administered to a patient suffering cancer of any stage, from early to metastatic. The terms subject and patient are used interchangeably herein.

[0097] A multispecific polypeptide construct of the disclosure used in any of the examples of these methods and uses can be used in a treatment regimen comprising neoadjuvant therapy.

[0098] A multispecific polypeptide construct of the disclosure used in any of the examples of these methods and uses can be administered either alone or in combination with one or more additional agents, including small molecule inhibitors, other antibody-based therapies, polypeptide or peptide-based therapies, nucleic acid-based therapies and/or other biologics. In some examples, a multispecific polypeptide construct is administered in combination with one or more additional agents such as, by way of non-limiting example, a chemotherapeutic agent, such as an alkylating agent, an anti-metabolite, an anti-microtubule agent, a topoisomerase inhibitor, a cytotoxic antibiotic, and any other nucleic acid damaging agent. In some examples, the additional agent is a taxane, such as paclitaxel (*e.g.*, Abraxane®). In some examples, the additional agent is an anti-metabolite, such as gemcitabine. In some examples, the additional agent is an alkylating agent, such as platinum-based chemotherapy, such as carboplatin or cisplatin. In some examples, the additional agent is a targeted agent, such as a kinase inhibitor, *e.g.*, sorafenib or erlotinib. In some examples, the additional agent is a targeted agent, such as another antibody, *e.g.*, a monoclonal antibody (*e.g.*, bevacizumab), a bispecific antibody, or a multispecific antibody. In some examples, the additional agent is a proteasome inhibitor, such as bortezomib or

same, *e.g.*, cleavable or non-cleavable, or the two or more linkers are different, *e.g.*, at least one cleavable and at least one non-cleavable.

[00110] The multispecific polypeptide constructs and conjugates thereof are useful in methods for treating a variety of disorders and/or diseases. Non-limiting examples of disease include: all types of cancers (breast, lung, colorectal, prostate, melanomas, head and neck, pancreatic, etc.), rheumatoid arthritis, Crohn's disease, SLE, cardiovascular damage, ischemia, etc. For example, indications would include leukemias, including T-cell acute lymphoblastic leukemia (T-ALL), lymphoblastic diseases including multiple myeloma, and solid tumors, including lung, colorectal, prostate, pancreatic, and breast, including triple negative breast cancer. For example, indications include bone disease or metastasis in cancer, regardless of primary tumor origin; breast cancer, including by way of non-limiting example, ER/PR+ breast cancer, Her2+ breast cancer, triple-negative breast cancer; colorectal cancer; endometrial cancer; gastric cancer; glioblastoma; head and neck cancer, such as esophageal cancer; lung cancer, such as by way of non-limiting example, non-small cell lung cancer; multiple myeloma ovarian cancer; pancreatic cancer; prostate cancer; sarcoma, such as osteosarcoma; renal cancer, such as by way of nonlimiting example, renal cell carcinoma; and/or skin cancer, such as by way of nonlimiting example, squamous cell cancer, basal cell carcinoma, or melanoma. In some examples, the cancer is a squamous cell cancer. In some examples, the cancer is a skin squamous cell carcinoma. In some examples, the cancer is an esophageal squamous cell carcinoma. In some examples, the cancer is a head and neck squamous cell carcinoma. In some examples, the cancer is a lung squamous cell carcinoma.

[00111] Also provided is a pharmaceutical composition comprising any of the multispecific polypeptide constructs provided herein and a pharmaceutically acceptable carrier. In some cases, the pharmaceutical composition is sterile. Pharmaceutical compositions according to the disclosure can include a multispecific polypeptide construct of the disclosure and a carrier. These pharmaceutical compositions can be included in kits, such as, for example, diagnostic kits.

[00112] One skilled in the art will appreciate that the antibodies of the disclosure have a variety of uses. For example, the proteins of the disclosure are used as therapeutic agents for a variety of disorders. The antibodies of the disclosure are also used as reagents in diagnostic kits or as diagnostic tools, or these antibodies can be used in competition assays to generate therapeutic reagents.

CLAIMS

What is claimed is:

1. A multispecific polypeptide construct, the multispecific polypeptide construct comprising a first component comprising a heterodimeric immunoglobulin Fc region and a second component comprising a CD3-binding region, wherein:

the first and second components are coupled by a polypeptide linker, wherein the Fc region is positioned amino-terminally relative to the CD3-binding region;

the CD3-binding region is a disulfide stabilized anti-CD3 binding Fv fragment (dsFv) comprising a variable heavy chain region (VH) and a variable light chain region (VL), wherein the VH and VL are linked to opposite polypeptides of the heterodimeric Fc; and

one or both of the first and second components comprises at least one antigen binding domain that binds a tumor associated antigen (TAA), and

wherein each of the at least one antigen binding domain is positioned amino-terminally relative to the Fc region and/or positioned carboxy-terminally relative to the CD3-binding region; and

wherein the CD3-binding region is not able to measurably bind or engage cell surface CD3 unless at least one antigen binding domain is bound to its TAA.

2. A multispecific polypeptide construct, the multispecific polypeptide construct comprising a first component comprising an immunoglobulin Fc region and a second component comprising a CD3-binding region, wherein:

the first and second components are coupled by a polypeptide linker, wherein the Fc region is positioned amino-terminally relative to the CD3-binding region;

the CD3-binding region is a disulfide stabilized anti-CD3 binding Fv fragment (dsFv) comprising a variable heavy chain region (VH) and a variable light chain region (VL); and

the multispecific polypeptide construct comprises at least one antigen binding domain that binds a tumor associated antigen (TAA), and

wherein each of the at least one antigen binding domain is positioned amino-terminally relative to the Fc region and/or positioned carboxy-terminally relative to the CD3-binding region, wherein at least one antigen binding domain is positioned carboxy-terminally relative to the CD3-binding region; and

wherein the CD3-binding region is not able to measurably bind or engage cell surface CD3 unless at least one antigen binding domain is bound to its TAA.

3. A multispecific polypeptide construct, the multispecific polypeptide construct comprising a first component comprising an immunoglobulin Fc region and a second component comprising a CD3-binding region, wherein:

the first and second components are coupled by a polypeptide linker, wherein the Fc region is positioned amino-terminally relative to the CD3-binding region;

the CD3-binding region is a disulfide stabilized anti-CD3 binding Fv fragment (dsFv) comprising a variable heavy chain region (VH) and a variable light chain region (VL); and

the first component comprises at least a first antigen binding domain and the second component comprises at least a second antigen binding domain, wherein each of the antigen binding domains binds a tumor associated antigen (TAA), and

wherein each of the at least one antigen binding domain is positioned amino-terminally relative to the Fc region and/or positioned carboxy-terminally relative to the CD3-binding region; and

wherein the CD3-binding region is not able to measurably bind or engage cell surface CD3 unless at least one antigen binding domain is bound to its TAA.

4. The multispecific polypeptide construct of any of claims 1-3, wherein the linker is a cleavable linker.

5. A multispecific polypeptide construct, the multispecific polypeptide construct comprising a first component comprising an immunoglobulin Fc region and a second component comprising a CD3-binding region, wherein:

the first and second components are coupled by a cleavable linker, wherein the Fc region is positioned amino-terminally relative to the CD3-binding region;

the CD3-binding region is a disulfide stabilized anti-CD3 binding Fv fragment (dsFv) comprising a variable heavy chain region (VH) and a variable light chain region (VL); and

the multispecific polypeptide construct further comprises at least one antigen binding domain that binds a tumor associated antigen (TAA), and

wherein each of the at least one antigen binding domain is positioned amino-terminally relative to the Fc region and/or positioned carboxy-terminally relative to the CD3-binding region; and

wherein the CD3-binding region is not able to measurably bind or engage cell surface CD3 unless at least one antigen binding domain is bound to its TAA.

6. A multispecific polypeptide construct, the multispecific polypeptide construct comprising a first component comprising a heterodimeric Fc region and a second component comprising a CD3-binding region, wherein:

the CD3-binding region is a disulfide stabilized anti-CD3 binding Fv fragment (dsFv) comprising a variable heavy chain region (VH) and a variable light chain region (VL);

the VH and VL are linked to opposite polypeptides of the heterodimeric Fc;

the first and second components are coupled by a cleavable linker, wherein the heterodimeric Fc region is positioned amino-terminally relative to the anti-CD3 antibody or antigen-binding fragment; and

one or both of the first and second components comprises at least one antigen binding domain that binds a tumor associated antigen (TAA), and

wherein each of the at least one antigen binding domain is positioned amino-terminally relative to the Fc region and/or positioned carboxy-terminally relative to the CD3-binding region; and

wherein the CD3-binding region is not able to measurably bind or engage cell surface CD3 unless at least one antigen binding domain is bound to its TAA.

7. The multispecific polypeptide construct of any of claims 1, 2, and 4-6, wherein the first component comprises a first antigen binding domain and the second component comprises a second antigen binding domain, wherein each of the antigen binding domains binds a tumor associated antigen (TAA).

8. The multispecific polypeptide construct of any of claims 1-7, wherein the CD3-binding region binds CD3 (CD3ε).

9. The multispecific polypeptide construct of claim 3 or claim 7, wherein the first antigen binding domain is positioned amino-terminally relative to the Fc region and the second antigen binding domain is positioned carboxy-terminally relative to the CD3-binding region.

10. The multispecific polypeptide construct of any of claims 1-9, wherein the Fc region is an Fc region of a human IgG1, a human IgG2, a human IgG3, or a human IgG4.

11. The multispecific polypeptide construct of any of claims 2, 3, and 5, wherein the Fc region is a heterodimeric Fc region.
12. The multispecific polypeptide construct of any of claims 1, 6, and 11, wherein one or both Fc polypeptide of the heterodimeric Fc region comprises at least one amino acid modification to promote heterodimerization, compared to a polypeptide of a homodimeric Fc region, optionally compared to the Fc polypeptide set forth in SEQ ID NO:1 or an immunologically active fragment thereof.
13. The multispecific polypeptide construct of any of claims 1, 6, 11, and 12, wherein each of the Fc polypeptides of the heterodimeric Fc comprises a knob-into-hole modification or comprises a charge mutation to increase electrostatic complementarity of the polypeptides.
14. The multispecific polypeptide construct of any of claims 1-13, wherein the Fc region comprises a polypeptide comprising at least one amino acid modification to enhance FcRn binding.
15. The multispecific polypeptide construct of any of claims 1-14, wherein the Fc region comprises a polypeptide comprising at least one amino acid modification that reduces effector function and/or reduces binding to Fc gamma receptor or C1q.
16. The multispecific polypeptide construct of any of claims 1-15, wherein the CD3-binding region is monovalent.
17. The multispecific polypeptide construct of any of claims 2, 3, and 5, wherein the Fc is a heterodimeric Fc and the VH and VL are linked to opposite polypeptides of the heterodimeric Fc.
18. The multispecific polypeptide construct of any of claims 4-6, wherein the cleavable linker is a polypeptide that functions as a substrate for a protease.

19. The multispecific polypeptide construct of claim 18, wherein the protease is selected from the group consisting of: matrilysin, a matrix metalloprotease (MMP), granzyme B, and combinations thereof.

20. The multispecific polypeptide construct of any of claims 1-19, wherein the antigen binding domain, or independently each of the antigen binding domains, is an antibody or antigen-binding fragment thereof selected from the group consisting of: a Fab fragment, a F(ab')₂ fragment, an Fv fragment, a scFv, a scAb, a dAb, a single domain heavy chain antibody, and a single domain light chain antibody.

21. The multispecific polypeptide construct of any of claims 1-20, wherein the antigen binding domain, or independently each of the antigen binding domains, is a single domain antibody (sdAb).

22. The multispecific polypeptide construct of any of claims 1-21, wherein the antigen binding domain, or independently each of the antigen binding domains, binds to a tumor antigen selected from the group consisting of: 1-92-LFA-3, 5T4, Alpha-4 integrin, Alpha-V integrin, alpha4beta1 integrin, alpha4beta7 integrin, AGR2, Anti-Lewis-Y, Apelin J receptor, APRIL, B7-H3, B7-H4, BAFF, BTLA, C5 complement, C-242, CA9, CA19-9, (Lewis a), Carbonic anhydrase 9, CD2, CD3, CD6, CD9, CD11a, CD19, CD20, CD22, CD24, CD25, CD27, CD28, CD30, CD33, CD38, CD40, CD40L, CD41, CD44, CD44v6, CD47, CD51, CD52, CD56, CD64, CD70, CD71, CD74, CD80, CD81, CD86, CD95, CD117, CD123, CD125, CD132 (IL-2RG), CD133, CD137, CD138, CD166, CD172A, CD248, CDH6, CEACAM5 (CEA), CEACAM6 (NCA-90), CLAUDIN-3, CLAUDIN-4, cMet, Collagen, Cripto, CSFR, CSFR-1, CTLA-4, CTGF, CXCL10, CXCL13, CXCR1, CXCR2, CXCR4, CYR61, DL44, DLK1, DLL3, DLL4, DPP-4, DSG1, EDA, EDB, EGFR, EGFRviii, Endothelin B receptor (ETBR), ENPP3, EpCAM, EPHA2, EPHB2, ERBB3, F protein of RSV, FAP, FGF-2, FGF8, FGFR1, FGFR2, FGFR3, FGFR4, FLT-3, Folate receptor alpha (FR α), GAL3ST1, G-CSF, G-CSFR, GD2, GTR, GLUT1, GLUT4, GM-CSF, GM-CSFR, GP IIb/IIIa receptors, Gp130, GPIIB/IIIA, GPNMB, GRP78, HER2/neu, HER3, HER4, HGF, hGH, HVEM, Hyaluronidase, ICOS, IFN α , IFN β , IFN γ , IgE, IgE Receptor (Fc ϵ RI), IGF, IGF1R, IL1B, IL1R, IL2, IL11, IL12, IL12p40, IL-12R, IL-12R β 1, IL13, IL13R, IL15, IL17, IL18, IL21, IL23, IL23R, IL27/IL27R (wsx1), IL29, IL-

31R, IL31/IL31R, IL2R, IL4, IL4R, IL6, IL6R, Insulin Receptor, Jagged Ligands, Jagged 1, Jagged 2, KISS1-R, LAG-3, LIF-R, Lewis X, LIGHT, LRP4, LRRC26, Ly6G6D, LyPD1, MCSP, Mesothelin, MRP4, MUC1, Mucin-16 (MUC16, CA-125), Na/K ATPase, NGF, Nicastrin, Notch Receptors, Notch 1, Notch 2, Notch 3, Notch 4, NOV, OSM-R, OX-40, PAR2, PDGF-AA, PDGF-BB, PDGFRalpha, PDGFRbeta, PD-1, PD-L1, PD-L2, Phosphatidyl-serine, P1GF, PSCA, PSMA, PSGR, RAAG12, RAGE, SLC44A4, Sphingosine 1 Phosphate, STEAP1, STEAP2, TAG-72, TAPA1, TEM-8, TGFbeta, TIGIT, TIM-3, TLR2, TLR4, TLR6, TLR7, TLR8, TLR9, TMEM31, TNFalpha, TNFR, TNFRS12A, TRAIL-R1, TRAIL-R2, Transferrin, Transferrin receptor, TRK-A, TRK-B, uPAR, VAP1, VCAM-1, VEGF, VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGFR1, VEGFR2, VEGFR3, VISTA, WISP-1, WISP-2, and WISP-3.

23. The multispecific polypeptide construct of any of claims 1, 2, 5, and 6, wherein the multispecific polypeptide construct comprises at least a first antigen binding domain and a second antigen binding domain, wherein the first antigen binding domain and second antigen binding domain bind to the same TAA.

24. The multispecific polypeptide construct of claim 3, wherein the first antigen binding domain and second antigen binding domain bind to the same TAA.

25. The multispecific polypeptide construct of any of claims 1, 2, 5, and 6, wherein the multispecific polypeptide construct comprises at least a first antigen binding domain and a second antigen binding domain, wherein the first antigen binding domain and the second antigen binding domain bind different TAAs.

26. The multispecific polypeptide construct of claim 3, wherein the first antigen binding domain and the second antigen binding domain bind different TAAs.

27. The multispecific polypeptide construct of any of claims 1-26, wherein the multispecific polypeptide construct is conjugated to an agent, optionally wherein the agent is a therapeutic agent, an antineoplastic agent, a toxin or fragment thereof, a detectable moiety, or a diagnostic agent.

28. The multispecific polypeptide construct of any of claims 1-27, wherein the anti-CD3 dsFv comprises a VH CDR1 comprising the amino acid sequence TYAMN (SEQ ID NO: 16); a VH CD2 comprising the amino acid sequence RIRSKYNNYATYYADSVKD (SEQ ID NO: 17); a VH CDR3 comprising the amino acid sequence HGNFGNSYVSWFAY (SEQ ID NO: 18), a VL CDR1 comprising the amino acid sequence RSSTGAVTTSNYAN (SEQ ID NO: 19); a VL CDR2 comprising the amino acid sequence GTNKRAP (SEQ ID NO: 20); and a VL CDR3 comprising the amino acid sequence ALWYSNLWV (SEQ ID NO: 21).

29. The multispecific polypeptide construct of any of claims 1-27, wherein the anti-CD3 dsFv comprises:

a VH having the amino acid sequence of any of SEQ ID NOS: 44 and 49-62, 197 or a sequence that exhibits at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to any of SEQ ID NOS: 44 and 49-62; and

a VL having the amino acid sequence of any of SEQ ID NOS: 64, 72, 74, 76, and 78-81, or a sequence that exhibits at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to any of SEQ ID NOS: 64, 72, 74, 76, and 78-81.

30. The multispecific polypeptide construct of any of claims 1-27, wherein the anti-CD3 dsFv comprises a VH having a sequence that exhibits at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to SEQ ID NO: 44; and a VL having a sequence that exhibits at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity SEQ ID NO: 72.

31. A polynucleotide(s) encoding the multispecific polypeptide construct of any of claims 1-30.

32. A vector, comprising a polynucleotide or polynucleotides of claim 31.

33. A cell, comprising a polynucleotide or polynucleotides of claim 31 or the vector of claim 32.

34. A method of producing a multispecific polypeptide construct, the method comprising introducing into a cell a polynucleotide or polynucleotides of claim 31 or the

vector of claim 32, and culturing the cell under conditions to produce the multispecific polypeptide construct.