[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 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),

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 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 is positioned carboxy-terminally relative to the CD3-binding region,
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 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),
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),
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 (CD3ε); 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),
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 CD3ε and/or is produced by a tumor that is co-localized with cells that express CD3ε 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.

[0079] In some examples, the anti multispecific construct described herein is used in conjunction with one or more additional agents or a combination of additional agents. Suitable additional agents include current pharmaceutical and/or surgical therapies for an intended application, such as, for example, cancer. For example, the multispecific construct can be used in conjunction with an additional chemotherapeutic or anti-neoplastic agent.

[0080] In some examples, the multispecific construct and additional agent are formulated into a single therapeutic composition, and the multispecific construct and additional agent are administered simultaneously. In some examples, the multispecific construct and additional agent are separate from each other, *e.g.*, each is formulated into a separate therapeutic composition, and the multispecific construct and the additional agent are administered simultaneously, or the multispecific construct and the additional agent are administered at different times during a treatment regimen. For example, the multispecific construct is administered prior to the administration of the additional agent, the multispecific construct is administered subsequent to the administration of the additional agent, or the multispecific construct and the additional agent are administered in an alternating fashion. As described herein, the multispecific construct and additional agent are administered in single doses or in multiple doses.

[0081] In some examples, the multispecific construct naturally contains one or more disulfide bonds. In some examples, the multispecific construct can be engineered to include one or more disulfide bonds.

[0082] The disclosure also provides an isolated nucleic acid molecule or polynucleotide encoding at least a portion of a multispecific construct described herein and/or one or more nucleic acid molecules encoding a multispecific construct described herein, such as for example, at least a first nucleic acid encoding at least a portion of the first component of the multispecific construct and a second nucleic acid encoding at least a portion of the second component of the multispecific construct, as well as vectors that include these isolated nucleic acid sequences.

[0083] Among the provided examples is a polynucleotide(s) encoding any of the provided multispecific polypeptide constructs. In another aspect, the present disclosure refers to a polynucleotide(s) encoding the multispecific polypeptide construct as disclosed herein. In one example, the present disclosure refers to a polynucleotide encoding a polypeptide chain of any of the multispecific polypeptide constructs as disclosed herein. Further provided is a polynucleotide, comprising a first nucleic acid sequence encoding a first polypeptide of any of the provided multispecific constructs and a second nucleic acid sequence encoding a second polypeptide of the multispecific construct, wherein the first and second nucleic acid sequence are separated by an internal ribosome entry site (IRES), or a

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

[0088a] In another aspect, the present disclosure refers to a multispecific polypeptide construct produced by the method as disclosed herein.

[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).

[0089a] In another aspect, the present disclosure refers to an ex vivo or in vitro method of stimulating or inducing an immune response, the method comprising contacting a target cell and a T cell with the multispecific polypeptide construct as disclosed herein or the pharmaceutical composition as disclosed herein, said target cell expressing a tumor associated antigen recognized by the multispecific polypeptide construct.

[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.

[0093a] In another aspect, the present disclosure refers to a composition comprising a therapeutically effective amount of the multispecific conjugate as disclosed herein or the pharmaceutical composition as disclosed herein for use in stimulating or inducing an immune response in a subject.

[0093b] In another aspect, the present disclosure refers to use of a composition comprising the multispecific polypeptide construct as disclosed herein or the pharmaceutical composition as disclosed herein in the manufacture of a medicament for stimulating or inducing an immune response in a subject having a tumor or a cancer.

[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.

[0094a] In another aspect, the present disclosure refers to a composition comprising a therapeutically effective amount of the multispecific polypeptide construct as disclosed herein or the pharmaceutical composition as disclosed herein for use in treating a disease or a condition in a subject, wherein cells of the disease or the condition express an antigen recognized by the multispecific polypeptide construct.

[0094b] In another aspect, the present disclosure refers to use of a composition comprising the multispecific polypeptide construct as disclosed herein or the pharmaceutical composition as disclosed herein in the manufacture of a medicament for the treatment of a tumor or a cancer in a subject.

[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

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 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),

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 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 is positioned carboxy-terminally relative to the CD3-binding region,

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 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),

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 polypeptide linker.

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

6. 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),

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. 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),

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.

8. The multispecific polypeptide construct of any of claims 1 and 4-7, wherein:

(i) 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; or

(ii) the at least first antigen binding domain is positioned amino-terminally relative to the Fc region and the at least second antigen binding domain is positioned carboxy-terminally relative to the CD3-binding region.

9. The multispecific polypeptide construct of any of claims 1, 2, and 4-8, 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).

10. The multispecific polypeptide construct of any of claims 1-9, wherein the CD3-binding region binds CD3 (CD3ε).

11. The multispecific polypeptide construct of claim 3 or claim 9, 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.

12. The multispecific polypeptide construct of any of claims 1-11, wherein the Fc region is an Fc region of a human IgG1, a human IgG2, a human IgG3, or a human IgG4, or is an immunologically active fragment thereof.

13. The multispecific polypeptide construct of any of claims 2, 3, and 6, wherein the Fc region is a heterodimeric Fc region.

14. The multispecific polypeptide construct of any of claims 1, 7, and 13, 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.

15. The multispecific polypeptide construct of any of claims 1, 7, 13, and 14, 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.

16. The multispecific polypeptide construct of any of claims 1-15, wherein the Fc region comprises a polypeptide comprising at least one amino acid modification to enhance FcRn binding.

17. The multispecific polypeptide construct of any of claims 1-16, 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.

18. The multispecific polypeptide construct of any of claims 1-17, wherein the CD3-binding region is monovalent.

19. The multispecific polypeptide construct of any of claims 2, 3, and 6, wherein the Fc is a heterodimeric Fc and the VH and VL are linked to opposite polypeptides of the heterodimeric Fc.

20. The multispecific polypeptide construct of any of claims 5-7, wherein the cleavable linker is a polypeptide that functions as a substrate for a protease.

21. The multispecific polypeptide construct of claim 20, wherein the protease is selected from the group consisting of: matriptase, a matrix metalloprotease (MMP), granzyme B, and combinations thereof.

22. The multispecific polypeptide construct of any of claims 1-21, 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.

23. The multispecific polypeptide construct of any of claims 1-22, wherein the antigen binding domain, or independently each of the antigen binding domains, is a single domain antibody (sdAb).

24. The multispecific polypeptide construct of any of claims 1-23, 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 (FRα), GAL3ST1, G-CSF, G-CSFR, GD2, GITR, GLUT1, GLUT4, GM-CSF, GM-CSFR, GP IIb/IIIa receptors, Gp130, GPIIB/IIIA, GPNMB, GRP78, HER2/neu, HER3, HER4, HGF, hGH, HVEM, Hyaluronidase, ICOS, IFNalpha, IFNbeta, IFNgamma, IgE, IgE Receptor (FceRI), IGF, IGF1R, IL1B, IL1R, IL2, IL11, IL12, IL12p40, IL-12R, IL-12Rbeta1, 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.

25. The multispecific polypeptide construct of any of claims 1, 2, 6, and 7, 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.

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

27. The multispecific polypeptide construct of any of claims 1, 2, 6, and 7, 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 a different TAA.

28. The multispecific polypeptide construct of claim 3, wherein the first antigen binding domain and the second antigen binding domain bind a different TAA.

29. The multispecific polypeptide construct of any of claims 1-28, 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.

30. A polynucleotide(s) encoding the multispecific polypeptide construct of any of claims 1-29.

31. A vector, comprising a polynucleotide or polynucleotides of claim 30.

32. A cell, comprising a polynucleotide or polynucleotides of claim 30 or the vector of claim 31.

33. A method of producing a multispecific polypeptide construct, the method comprising introducing into a cell a polynucleotide or polynucleotides of claim 30 or the vector of claim 31, and culturing the cell under conditions to produce the multispecific polypeptide construct.

34. A multispecific polypeptide construct produced by the method of claim 33.

35. A pharmaceutical composition comprising the multispecific polypeptide construct of any of claims 1-29 and 34 and a pharmaceutically acceptable carrier.

36. An ex vivo or in vitro method of stimulating or inducing an immune response, the method comprising contacting a target cell and a T cell with the multispecific polypeptide construct of any of claims 1-29 and 34 or the pharmaceutical composition of claim 35, said target cell expressing a tumor associated antigen recognized by the multispecific polypeptide construct.

37. A composition comprising a therapeutically effective amount of the multispecific conjugate of any of claims 1-29 and 34 or the pharmaceutical composition of claim 35 for use in stimulating or inducing an immune response in a subject.

38. Use of a composition comprising the multispecific polypeptide construct of any of claims 1-29 and 34 or the pharmaceutical composition of claim 35 in the manufacture of a medicament for stimulating or inducing an immune response in a subject having a tumor or a cancer.

39. The composition for use of claim 37 or the use of claim 38, wherein the immune response is increased against the tumor or the cancer.

40. A composition comprising a therapeutically effective amount of the multispecific polypeptide construct of any of claims 1-29 and 34 or the pharmaceutical composition of claim 35 for use in treating a disease or a condition in a subject, wherein cells of the disease or the condition express an antigen recognized by the multispecific polypeptide construct.

41. Use of a composition comprising the multispecific polypeptide construct of any of claims 1-29 and 34 or the pharmaceutical composition of claim 35 in the manufacture of a medicament for the treatment of a tumor or a cancer in a subject, wherein cells of the tumor or the cancer express an antigen recognized by the multispecific polypeptide construct.

42. The composition for use of claim 40, wherein the disease or condition is a tumor or a cancer.