CLAIMS

What is claimed is:

1. A polypeptide construct useful to inhibit an effect of a Transforming Growth Factor Beta (TGFβ) isoform, the construct comprising:

a TGFβ-binding region, and

a multimerization domain;

wherein the N-terminus of the multimerization domain is joined to the C-terminus of the TGFβ-binding region;

wherein the TGFβ-binding region comprises, in an N- to C- terminal orientation, an N-terminal region, a first TGFβ receptor ligand-binding domain (TGFβR-LBD), a first linker, a second TGFβR ligand-binding domain, and a second linker;

wherein the inhibitory potency of the polypeptide construct for both TGFβ1 isoform activity and TGFβ3 isoform activity is greater than for TGFβ2 isoform activity; and

wherein the first linker and the second linker are selected so that the relative inhibitory potency of the polypeptide construct for TGFβ3 isoform activity compared to TGFβ1 isoform activity (IC_{50} ratio for TGFβ3:TGFβ1) is about 2.5:1 or less, about 2.3:1 or less, about 2:1 or less, about 1.8:1 or less, about 1.5:1 or less, about 1.3:1 or less, about 1:1 or less, about 1:1 or less, about 0.8:1 or less, or about 0.5:1 or less;

optionally wherein the polypeptide construct inhibits both TGFβ1 isoform activity and TGFβ3 isoform activity with at least 20-fold, 100-fold, 200-fold, 300-fold, 400-fold, 500-fold, 600-fold, 700-fold, 800-fold, 900-fold, or 1000-fold greater potency than TGFβ2 isoform activity.

2. The polypeptide construct of claim 1, wherein:

a. the first linker is:

i. 33 amino acids or shorter; and/or

ii. 10 amino acids or longer, 15 amino acids or longer, or 18 amino acids or longer; and/or

iii. from about 15 to 33 amino acids long, or from about 18 to about 30 amino acids long; and/or

b. the second linker is:

i. 10 amino acids or longer; and/or

ii. 35 amino acids or shorter, or from 10 to 34 amino acids long; and/or

iii. from about 15 to about 35 amino acids long;

preferably wherein the first linker is about 16, about 18, about 30, or about 32 amino acids long and/or the second linker is about 16, about 30, about 32, or about 34 amino acids long; and

optionally wherein the first linker and/or the second linker comprises or consists of an IDR linker, an IDR linker variant, a non-IDR linker, a hybrid linker, a hybrid linker variant, a truncated linker, a truncated linker variant or an elongated linker.

3. The polypeptide construct of claim 1 or claim 2, wherein:

a. one or more of the first linker and the second linker comprises or consists of the amino acid sequence set forth in any one of SEQ ID NOs: 4 and 8-26, or a sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical thereto; and/or

b. the first linker comprises or consists of the amino acid sequence set forth in any one of SEQ ID NOs: 8, 9, 10, 11, 12, 13, 14, 16, 21, 22, 23, and 26, or a sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical thereto; and/or

c. the second linker comprises or consists of the amino acid sequence set forth in any one of SEQ ID NOs: 4, 9, 11, 15, 17, 18, 19, 20, 22, 23, 24, 25, and 26, or a sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical thereto;

preferably wherein the first linker comprises or consists of the amino acid sequence set forth in SEQ ID NO: 12 or SEQ ID NO: 8; and/or the second linker comprises or consists of the amino acid sequence set forth in SEQ ID NO: 11 or SEQ ID NO: 9.

4. The polypeptide construct of any one of claims 1 to 3, wherein:

a. the first linker comprises or consists of an amino acid sequence having:

i. a deletion of at least one N-terminal amino acid residue in comparison with SEQ ID NO: 3, SEQ ID NO: 12 or SEQ ID NO: 8;

ii. a deletion of at least one C-terminal amino acid residue in comparison with SEQ ID NO: 3, SEQ ID NO: 12 or SEQ ID NO: 8;

iii. a deletion of at least one internal amino acid residue in comparison with SEQ ID NO: 3, SEQ ID NO: 12 or SEQ ID NO: 8; or

iv. one or more substitution in the amino acid sequence in comparison with SEQ ID NO: 3, SEQ ID NO: 12, SEQ ID NO: 8, or of any one of (a) to (c); and/or

b. the second linker comprises or consists of an amino acid sequence having:

i. a deletion of at least one N-terminal amino acid residue in comparison with SEQ ID NO: 9 or SEQ ID NO: 11;

ii. a deletion of at least one C-terminal amino acid residue in comparison with SEQ ID NO: 9 or SEQ ID NO: 11;

iii. a deletion of at least one internal amino acid residue in comparison with SEQ ID NO: 9 or SEQ ID NO: 11; or

iv. one or more substitution in the amino acid sequence in comparison with SEQ ID NO: 4, 9 or 11, or of any one of (a) to (c)

optionally wherein the amino acid deletion of the first linker is a deletion of 16 amino acids of SEQ ID NO: 3.

5. The polypeptide construct of any one of claims 1 to 4, wherein the N-terminal region comprises or consists of:

a. an IDR linker, an IDR linker variant, a hybrid linker, a hybrid linker variant, a truncated linker, a truncated linker variant or an elongated linker; and/or

b. the amino acid sequence set forth in SEQ ID NO: 3, or a sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical thereto.

6. The polypeptide construct of any one of claims 1 to 5, wherein:

a. one or more of the first TGFβR-LBD and the second TGFβR-LBD comprises or consists of the amino acid sequence set forth in SEQ ID NO: 2, or a sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical thereto, optionally wherein the first TGFβR-LBD and the second TGFβR-LBD are the same or substantially the same; and/or

b. both the first TGFβR-LBD and the second TGFβR-LBD comprise or consist of the amino acid sequence set forth in SEQ ID NO: 2, or a sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical thereto.

7. The polypeptide construct of any one of claims 1 to 6, wherein

a. the multimerization domain allows dimerization of the polypeptide construct with a second polypeptide construct as defined in any one of claims 1 to 6, in a covalent or non-covalent manner; and/or

b. the multimerization domain comprises one or more constant region of an antibody; and/or

c. the multimerization domain comprises the second constant domain (C_H2) and/or the third constant domain (C_H3) of an antibody heavy chain; and/or

d. the multimerization domain comprises an Fc region of an antibody heavy chain.

8. The polypeptide construct of claim 7, wherein

a. the antibody is an IgG antibody; and/or

b. the antibody is an IgG1, IgG2, IgG3 or IgG4 antibody, optionally a human antibody; and/or

c. the multimerization domain has at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity with a human IgG1, IgG2, IgG3 or IgG4 constant region.

9. The polypeptide construct of any one of claims 1 to 8, wherein

a. the multimerization domain comprises cysteine residues for crosslinking of the polypeptide construct with a second polypeptide construct as defined in any one of claims 1 to 8; and/or

b. the multimerization domain comprises at least two cysteine residues for forming a disulfide bridge with the second polypeptide construct; and/or

c. the multimerization domain is engineered to reduce aggregation or to modulate stability of a dimer or multimer of the polypeptide construct; and/or

d. the multimerization domain comprises or consists of the amino acid sequence set forth in any one of SEQ ID NOs: 49-80 or a sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical thereto;

preferably wherein the multimerization domain comprises or consists of the amino acid sequence set forth in SEQ ID NO:49 or SEQ ID NO:50.

10. The polypeptide construct of any one of claims 1 to 9, wherein the TGFβ-binding region comprises or consists of the amino acid sequence set forth in SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 32, SEQ ID NO: 40, or SEQ ID NO: 41.

11. The polypeptide construct of any one of claims 1 to 10, wherein the polypeptide construct comprises or consists of the amino acid sequence set forth in any one of SEQ ID NOs: 81 to 103 and 105, or a sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical thereto;

preferably wherein the polypeptide construct comprises or consists of the amino acid sequence set forth in SEQ ID NO: 81, SEQ ID NO: 84, SEQ ID NO: 87, SEQ ID NO: 95, or SEQ ID NO: 96, or a sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO: 81, SEQ ID NO: 84, SEQ ID NO: 87, SEQ ID NO: 95, or SEQ ID NO: 96; and

optionally wherein the polypeptide construct further comprises a signal peptide having the sequence set forth in SEQ ID NO: 104, or a sequence substantially identical thereto.

12. The polypeptide construct of any one of claims 1 to 11, wherein the polypeptide construct is a dimeric polypeptide comprising a first and a second polypeptide construct as defined in any

one of claims 1 to 11 linked between respective multimerization domains by at least one disulfide bridge; and

wherein the first and the second polypeptide construct comprise the same or substantially the same amino acid sequence, optionally wherein the first and the second polypeptide construct comprise from N-terminus to C-terminus: (i) an amino acid sequence consisting of the amino acid sequence of SEQ ID NO:40; and (ii) an Fc region of human IgG1; or

the first and the second polypeptide construct comprise different amino acid sequences, optionally wherein the first and the second polypeptide construct comprise the same or substantially the same multimerization domains, and different TGFβ-binding regions.

13. The polypeptide construct of claim 12, wherein the first polypeptide and/or the second polypeptide construct further comprises a site for conjugation;

optionally wherein the first polypeptide and/or the second polypeptide construct is conjugated with a targeting agent, a therapeutic moiety, a detectable moiety, or a diagnostic moiety; and

optionally wherein the targeting agent, the therapeutic moiety, the detectable moiety, or the diagnostic moiety comprises an antibody or antigen binding fragment thereof, a binding agent having affinity for another member of the TGFβ family or for another therapeutic target, a radiotherapy agent, an imaging agent, a fluorescent moiety, a cytotoxic agent, an anti-mitotic drug, a nanoparticle-based carrier, a polymer-conjugated drug, a nanocarrier, an imaging agent, a stabilizing agent, a drug, a nanocarrier, or a dendrimer.

14. A nucleic acid molecule encoding the polypeptide construct of any one of claims 1 to 13, optionally wherein the nucleic acid molecule encodes the polypeptide construct in a form that is secretable by a selected expression host.

15. A vector comprising the nucleic acid molecule of claim 14.

16. A cellular host comprising the nucleic acid molecule of claim 14 or the vector of claim 15.

17. A TGFβ binding agent comprising:

a first polypeptide construct as defined in any one of claims 1 to 13, and

a second polypeptide construct as defined in any one of claims 1 to 13;

wherein the first polypeptide construct and the second polypeptide construct are associated together through their respective multimerization domains

wherein the inhibitory potency of the TGFβ binding agent for both TGFβ1 isoform activity and TGFβ3 isoform activity is greater than for TGFβ2 isoform activity; and

wherein the first linker and the second linker are selected so that the relative inhibitory potency of the TGFβ binding agent for TGFβ3 isoform activity compared to TGFβ1 isoform activity (IC_{50} ratio for TGFβ3 TGFβ1) is about 2.5:1 or less, about 2.3:1 or less, about 2:1 or less, about 1.8:1 or less, about 1.5:1 or less, about 1.3:1 or less, about 1:1 or less, about 1:1 or less, about 0.8:1 or less, or about 0.5:1 or less;

optionally wherein the first polypeptide construct comprises from N-terminus to C-terminus: (i) an amino acid sequence consisting of the amino acid sequence of SEQ ID NO:40; and (ii) a first Fc region of human IgG1, and the second polypeptide construct comprises from N-terminus to C-terminus: (i) an amino acid sequence consisting of the amino acid sequence of SEQ ID NO:40; and (ii) a second Fc region of human IgG1; and wherein the first polypeptide construct and the second polypeptide construct are linked together through the first and second Fc region of human IgG1.

18. A pharmaceutical composition comprising the polypeptide construct of any one of claims 1 to 13 or the TGFβ binding agent of claim 17 and a pharmaceutically acceptable carrier, diluent or excipient, optionally wherein the composition is formulated for administration by injection or infusion, and optionally wherein the composition is formulated for intravenous, subcutaneous, intraperitoneal, or intramuscular administration.

19. Use of the polypeptide construct of any one of claims 1 to 13, the TGFβ binding agent of claim 17, or the pharmaceutical composition of claim 18, in the manufacture of a medicament for treating or preventing a TGFβ-associated disease or condition in a subject in need thereof, optionally wherein the subject is human.

20. The polypeptide construct of any one of claims 1 to 13, the TGFβ binding agent of claim 17, or the pharmaceutical composition of claim 18 for use in therapy.