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Inventor(s)	Zwaagstra; John C. et al.

TGF-β-receptor ectodomain fusion molecules and uses thereof

Abstract

The present invention relates, in general, to polypeptides capable of binding and neutralizing transforming growth factor beta (TGF-beta) ligands, and uses of these polypeptides for treating disorders related to TGF-beta expression or activation (e.g. cancer and fibrotic diseases), and methods of making such molecules.

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Background/Summary

RELATED APPLICATIONS (1) This application is a continuation of U.S. application Ser. No. 15/755,595, filed Feb. 27, 2018, which is a national stage filing under 35 U.S.C. § 371 of International Application No. PCT/IB2016/055204, filed Aug. 31, 2016, and claims the benefit under 35 U.S.C. § 119(e) of U.S. provisional application Ser. No. 62/212,058, filed Aug. 31, 2015, the entire contents of each of which is incorporated by reference herein in its entirety.

FIELD OF THE INVENTION
(1) The present invention relates to TGF-β superfamily receptor ectodomain fusion molecules and uses thereof. More specifically, the present invention relates to TGF-β superfamily receptor ectodomain fusion molecules and their use in TGF-β superfamily ligand neutralization.
BACKGROUND OF THE INVENTION
(2) TGF-β is part of a superfamily of over 30 ligands that regulate several physiological processes, including cell proliferation, migration and differentiation. Perturbation of their levels and/or signaling gives rise to significant pathological effects. For instance, TGF-β and activin ligands play critical pathogenic roles in many diseases including cancer (Hawinkels & Ten Dijke, 2011; Massague et al, 2000; Rodgarkia-Dara et al, 2006). TGF-β, in particular, is considered as a critical regulator of tumor progression and is overexpressed by most tumor types. It favors tumorigenesis in part by inducing an epithelial-mesenchymal transition (EMT) in the epithelial tumor cells, leading to aggressive metastasis (Thiery et al, 2009). TGF-β also promotes tumorigenesis by acting as a powerful suppressor of the immune response in the tumor microenvironment (Li et al, 2006). In fact, TGF-β is recognized as one of the most potent immunosuppressive factors present in the tumor microenvironment. TGF-β interferes with the differentiation, proliferation and survival of many immune cell types, including dendritic cells, macrophages, NK cells, neutrophils, B-cells and T-cells; thus, it modulates both innate and adaptive immunity (Santarpia et al, 2015; Yang et al, 2010). The importance of TGF-beta in the tumor microenvironment is highlighted by evidence showing that, in several tumor types (including melanoma, lung, pancreatic, colorectal, hepatic and breast), elevated levels of TGF-β ligand are correlated with disease progression and recurrence, metastasis, and mortality. Hence, significant effort has been invested in devising anti-tumor therapeutic approaches that involve TGF-β inhibition (Arteaga, 2006; Mourskaia et al, 2007; Wojtowicz-Praga, 2003).
(3) One approach to developing therapeutic agents that inhibit TGF-β function has been to use antibodies or soluble decoy receptors (also termed receptor ectodomain (ED)-based ligand traps) to bind and sequester ligand, thereby blocking access of ligand to its normal cell surface receptors (Zwaagstra et al, 2012). In general, receptor ED-based traps are a class of therapeutic agents that are able to sequester a wide range of ligands and that can be optimized using protein engineering approaches (Economides et al, 2003; Holash et al, 2002; Jin et al, 2009).
(4) Previously, a novel protein engineering design strategy was used to generate single-chain,

bivalent traps that are able to potently neutralize members of the TGF-β superfamily of ligands due to avidity effects (Zwaagstra et al, 2012) [WO 2008/113185; WO 2010/031168]. In this case, bivalency was achieved via covalent linkage of two TβRII ectodomains using portions of the intrinsically disordered regions (IDR) that flank the structured, ligand-binding domain of TβRII-ED. One example of these single-chain bivalent traps, T22d35, exhibited TGF-β neutralization potencies ˜100-fold higher than the monovalent non-engineered TβRII ectodomain, though it did not neutralize the TGF-β2 isoform and had a relatively short circulating half-life.

(5) While research to date indicates that single-chain TGF-β traps have promising therapeutic potential, their circulating half-lives and manufacturability present challenges to the commercial application.

SUMMARY OF THE INVENTION

(6) The present invention relates to TGF-β superfamily receptor ectodomain fusion molecules and uses thereof. More specifically, the present invention relates to TGF-β superfamily receptor ectodomain fusion molecules and their use in TGF-β superfamily ligand neutralization.

(7) In some aspects, the invention relates to TGF-β superfamily receptor ectodomain-based polypeptides that are similar to typical Fc fusions in design, in that the ectodomain is fused to a dimeric antibody constant domain. In particular, with respect to the present polypeptides, the Fc portion occupies the N-terminal position. Fc fusions in the prior art typically provide the Fc portion at the C-terminal end of the fusion. As will be evident from the results presented herein, this difference in orientation provides a number of significant advantages.

(8) In other aspects, the present polypeptides incorporate at least two TGF-β superfamily receptor ectodomains that are linked in tandem to the C-terminus of an antibody constant domain.

(9) Thus, there is provided a polypeptide construct comprising: a first portion comprising the second constant domain (C.sub.H2) and/or third constant domain (C.sub.H3) of an antibody heavy chain, and a second portion comprising at least two TGF-β superfamily receptor ectodomains (TβSR-ED) linked in tandem; wherein the N-terminus of the second portion is linked to the C-terminus of the first portion.

(10) There is also provided a polypeptide construct comprising: a first portion comprising the second constant domain (CH2) and/or third constant domain (CH3) of an antibody heavy chain, and a second portion comprising at least one TGF-β superfamily receptor ectodomains (TβSR-ED), wherein the N-terminus of the second portion is linked to the C-terminus of the first portion, and further wherein the first portion does not further comprise an antibody that binds to an antigen that is PD-L1, EGFR1, Her-2, CD4, CD6, CD20, CD25, MUC-1, IL-2, IL-6, or CTLA-4.

(11) There is provided a polypeptide construct comprising: a first portion comprising the second constant domain (C.sub.H2) and/or third constant domain (C.sub.H3) of an antibody heavy chain, and a second portion comprising at least one TGF-β superfamily receptor ectodomain (TβSR-ED), wherein the N-terminus of the second portion is directly fused to the C-terminus of the first portion.

(12) In an embodiment, there is provided a polypeptide construct comprising a first portion comprising the second constant domain (C.sub.H2) and/or third constant domain (C.sub.H3) of an antibody heavy chain, and a second portion comprising at least one TGF-β superfamily receptor ectodomain (TβSR-ED), wherein the N-terminus of the second portion is linked to the C-terminus of the first portion, and wherein the polypeptide construct neutralizes TGF-β with at least 100-fold more potency than the TβSR-ED alone.

(13) In a preferred embodiment, the second portion comprises one, two, or multiple TGF-β superfamily receptor ectodomain (TβSR-ED). In a preferred embodiment, the TβSR-ED is a TGF-β receptor type II ectodomain (TβR-II-ED). In a preferred embodiment, the TβSR-ED comprises a sequence selected from the group consisting of SEQ ID NO:35, SEQ ID NO:69, SEQ ID NO:75, SEQ ID NO:81, and a sequence substantially identical thereto.

(14) The second portion may comprise a sequence selected from the group consisting of SEQ ID NO:43-SEQ ID NO:51, SEQ ID NO:61-SEQ ID NO:68, SEQ ID NO:73, SEQ ID NO:74, SEQ ID

NO:79, SEQ ID NO:80, SEQ ID NO:85, SEQ ID NO:86, SEQ ID NO:88, and a sequence substantially identical thereto.
(15) In a preferred embodiment, the first portion of a polypeptide construct of the present invention further comprises a C.sub.H1, a C.sub.H1 and V.sub.H, or C.sub.H1 and scFv.
(16) There is provided a polypeptide construct of the present invention wherein the antibody heavy chain is of human origin. In a preferred embodiment, the antibody heavy chain is selected from the group consisting of a human IgG1, IgG2, IgG3, or IgG4 heavy chain. More preferably, the antibody heavy chain is a human IgG1.
(17) In accordance with the present invention, the polypeptide construct shows longer in vivo half-life compared to the half-life of the second portion alone.
(18) There is provided a polypeptide construct of the present invention, wherein the polypeptide construct is a single chain polypeptide.
(19) In an embodiment, the polypeptide construct forms a dimeric polypeptide. In another embodiment, the polypeptide construct is heterodimeric.
(20) There is provided a polypeptide construct selected from the group consisting of any one of SEQ ID NO:91 to SEQ ID NO:120, and a sequence substantially identical thereto.
(21) There is provided a polypeptide construct according to the present invention, wherein the construct comprises an antibody, antigen binding fragment thereof, or a targeting moiety. In a preferred embodiment, the antibody, the antigen binding fragment, or the targeting moiety is at the N-terminus of the first portion.
(22) In a preferred embodiment, the antigen binding fragment may be selected from the group consisting of a Fv, scFv, Fab, or sdAb. In a preferred embodiment, the antigen binding fragment binds to any antigen, provided that it is not PD-L1, EGFR1, Her-2, CD4, CD6, CD20, CD25, MUC-1, IL-2, IL-6, or CTLA-4.
(23) In a preferred embodiment, a polypeptide construct of the present invention comprises an antibody selected from the group consisting of Cetuximab, Avastin, Herceptin, Synagis, and FC5. In a preferred embodiment, the antibody is Cetuximab.
(24) In a preferred embodiment, a polypeptide construct of the present invention comprises a targeting moiety, wherein the targeting moiety comprises a poly-aspartate sequence motif for bone targeting. In a preferred embodiment, the targeting moiety comprises D10.
(25) There is provided a polypeptide construct according to the present invention wherein the construct is a dimeric polypeptide; wherein the dimeric polypeptide comprises: a first single chain polypeptide comprising a first portion comprising the second constant domain (C.sub.H2) and third constant domain (C.sub.H3) of an antibody heavy chain, and a heavy chain variable region of a given antibody; a second portion comprising one or more TGF-β superfamily receptor ectodomains (TβSR-ED), wherein the N-terminus of the second portion is linked to the C-terminus of the first portion, and a second single chain polypeptide comprising a first portion comprising the second constant domain (C.sub.H2) and third constant domain (C.sub.H3) of an antibody heavy chain, and a light chain variable region of said given antibody; a second portion comprising one or more TGF-β superfamily receptor ectodomain (TβSR-ED) which is the same or different from the ectodomain(s) in the first polypeptide, wherein the N-terminus of the second portion is linked to the C-terminus of the first portion.
(26) There is also provided a nucleic acid molecule encoding the polypeptide construct of the present invention. There is also provided a vector comprising the nucleic acid molecule of claim the present invention.
(27) There is also provided a composition comprising one or more than one independently selected polypeptide construct of the present invention and a pharmaceutically-acceptable carrier, diluent, or excipient.
(28) There is also provided a transgenic cellular host comprising the nucleic acid molecule or a vector of the present invention. The transgenic cellular host further comprising a second nucleic

acid molecule or a second vector encoding a second polypeptide construct different from the first polypeptide construct.
(29) There is also provided the use of a polypeptide construct according to the present invention for treatment of a medical condition, disease or disorder; wherein the medical condition, disease or disorder comprises, but is not limited to, cancer, ocular diseases, fibrotic diseases, or genetic disorders of connective tissue.
(30) In a preferred embodiment, there therefore provided a polypeptide construct comprising: a first portion comprising the second constant domain (CH2) and/or third constant domain (CH3) of an antibody heavy chain, and a second portion comprising at least two TGF-β superfamily receptor ectodomains (TβSR-ED), wherein the N-terminus of the second portion is linked to the C-terminus of the first portion.
(31) The antibody constant domain can further comprise, either linked thereto or formed integrally therewith, a binding agent such as a full size antibody, a ligand or any other protein of interest. In the alternative, the antibody constant domain comprises only the CH2 and/or CH3 regions, and not a full size antibody. In these and other types of constructs, the CH2 and/or CH3 region can be altered by deleting or substituting amino acids including one or more of the cysteines that provide cross-linking when the present constructs are provided as dimeric constructs.
(32) In other aspects of the present invention, there is provided a polypeptide construct that incorporates one or more such ectodomains. When the constructs comprise only one ectodomain linked to the antibody constant domain, then the construct is further characterized by at least one of the following: (1) when the constant domain further comprises a full sized antibody, that antibody does not bind effectively to PD-L1 or to an immunoregulatory antigen selected, (2) the constant domain comprises only the CH2 and/or CH3 regions, (3) the constant domain comprises an amino acid alteration relative to a wild type counterpart, such as a cysteine residue alteration; and (4) the first portion is linked to the second portion directly and without intervening amino acids.
(33) In another of its aspects, the present invention provides a polypeptide construct comprising a first portion comprising the second constant domain (C.sub.H2) and/or third constant domain (C.sub.H3) of an antibody heavy chain, and a second portion comprising at least one TGF-β superfamily receptor ectodomain (TβSR-ED), wherein
(34) the N-terminus of the second portion is linked to the C-terminus of the first portion. These polypeptide constructs can neutralize TGF-β, and with at least 100-fold more potency than the TβSR-ED alone.
(35) The second portion of the polypeptide construct of the present invention may comprise one or two or more TβSR-ED. In a preferred embodiment the construct comprises two or more independently selected ectodomains linked in tandem and to the C-terminus of the constant domain. The TβSR-ED may be selected from the group consisting of a TGF-β receptor type II ectodomain (TβRII-ED), a bone morphogenetic protein receptor type Ia ectodomain (BMPR-ED), an activin receptor type Ila ectodomain (ActRIIa-ED), and an activin receptor type IIb ectodomain (ActRII-ED). In another preferred embodiment, the ectodomain is a TβR-II ectodomain.
(36) In the polypeptide construct described herein, the first portion further may comprise a C.sub.H1, a C.sub.H1 and V.sub.H, or a C.sub.H1 and scFv. It may constitute an Fc region, an antibody, or any ligand binding agent or moiety.
(37) The polypeptide construct of the present invention may comprise a C.sub.H2 and C.sub.H3 from an antibody heavy chain that is of human or mouse origin. For example, and without wishing to be limiting, the antibody heavy chain may be selected from the group consisting of a human IgG1, IgG2, IgG3, or IgG4 heavy chain. In embodiments, the constant domain in the constructs is CH2 per se, or CH3 per se or CH2-CH3.
(38) The polypeptide construct described herein may show longer in vivo half-life compared to the half-life of TβSR-ED alone.
(39) In one example, the polypeptide construct of the present invention may be a single chain

polypeptide. The polypeptide construct as described herein may also form a dimeric polypeptide. This dimeric polypeptide may be heterodimeric.

(40) The present invention further provides a polypeptide construct comprising a first portion comprising the second constant domain (C.sub.H2) and/or third constant domain (C.sub.H3) of an antibody heavy chain, and a second portion comprising at least one TGF-β superfamily receptor ectodomain (TβSR-ED),

(41) wherein the N-terminus of the second portion is linked to the C-terminus of the first portion; additionally, in the construct as just described, the first portion is not derived from certain antibodies discussed infra.

(42) The present invention also provides a polypeptide construct, comprising: a first single chain polypeptide comprising a first portion comprising the second constant domain (C.sub.H2) and/or third constant domain (C.sub.H3) of an antibody heavy chain, and a heavy chain variable region of a given antibody; and a second portion comprising one or more TGF-β superfamily receptor ectodomains (TβSR-ED),

(43) wherein the N-terminus of the second portion is linked to the C-terminus of the first portion, and a second single chain polypeptide comprising a first portion comprising the second constant domain (C.sub.H2) and/or third constant domain (C.sub.H3) of an antibody heavy chain, and a light chain variable region of said given antibody; and a second portion comprising one or more TGF-β superfamily receptor ectodomain (TβSR-ED) which is the same or different from the ectodomain(s) in the first polypeptide, wherein the N-terminus of the second portion is linked to the C-terminus of the first portion.

(44) In alternative constructs of the present invention, the polypeptide construct comprises an antibody Fc fragment linked at the C-terminus of each heavy chain to at least one TGF-β superfamily receptor ectodomain (TβSR-ED), as described above. In embodiments the receptor ectodomain portion comprises two independently selected ectodomains that are linked in tandem, i.e., in a linear manner. In some embodiments, the ectodomains are the same in sequence, or least the same with respect to their target ligand. The construct may further comprise a binding fragment or moiety at the N-terminus of the Fc; the binding fragment may be selected from the group consisting of a Fv, scFv, Fab, or sdAb, or any other binding moiety such as a motif for bone targeting, also as described above. In the polypeptide constructs as described above, the TGF-β receptor ectodomain does not interfere in the native function or specificity of the binding fragment.

(45) The present invention also provides a nucleic acid molecule encoding the polypeptide constructs as described herein. A vector comprising the nucleic acid molecule just described is also encompassed by the invention. The invention also includes a transgenic cellular host comprising the nucleic acid molecule or a vector as described herein; the cellular host may further include a second nucleic acid molecule or a second vector encoding a second polypeptide construct different from the first polypeptide construct. Systems used to produce the present polypeptides can be secretion systems, particularly in the case where dimerization through disulfide bridges is required, and the expression polynucleotides thus encode secretion signals that are cleaved by the host upon secretion into the culturing medium.

(46) Compositions comprising one or more than one independently selected polypeptide construct described herein and a pharmaceutically-acceptable carrier, diluent, or excipient are also encompassed by the present invention.

(47) Additional aspects and advantages of the present invention will be apparent in view of the following description. The detailed descriptions and examples, while indicating preferred embodiments of the invention, are given by way of illustration only, as various changes and modifications within the scope of the invention will become apparent to those skilled in the art in light of the teachings of this invention.

Description

BRIEF DESCRIPTION OF THE DRAWINGS

(1) These and other features of the invention will now be described by way of example, with reference to the appended drawings, wherein:

(2) FIG. **1**A is a schematic diagram showing TGF-β Type II receptor ectodomain (TβRII-ED)-based molecules T2m and T22d35 along with their sequences (SEQ ID NO:43 and 46, respectively). Natural linker sequences (SEQ ID NO:36, 39 and 40) are underlined and depicted as dark grey lines; the sequence of the TβR-II-ED structured domain (SEQ ID NO:35) is shown in bold, and the domain labeled and depicted in dark grey; the site of the fusion of natural linkers is depicted by a slash. FIGS. **1**B-**1**D are schematic diagrams of IgG Fc-based scaffolds: an IgG Fc region (FIG. **1**B), a V.sub.HH-IgG Fc (comprising a V.sub.HH single domain antibody fused to the N-terminus of an Fc region; FIG. **1**C), and a full-size antibody (FIG. **1**D).

(3) FIGS. **2**A-**2**G are schematic representations of TGF-β superfamily receptor-ectodomain-based fusion constructs of the present invention. (FIG. **2**A) represents constructs in which T22d35 (dark grey) is fused to the C-terminus of IgG Fc regions (IgG isoforms 1, 2, 3 or 4) with no Fab or other functional binding moiety at the N-terminus (Fc-T22d35, FIG. **2**A), (FIG. **2**D) represents constructs in which T22d35 (dark grey) is fused to the C-terminus of the IgG Fc region of full-size antibodies with heavy and light chain Fabs (FSA-T22d35, FIG. **2**D), (FIG. **2**E) represents constructs in which T22d35 (dark grey) is fused to the C-terminus of IgG Fc regions that have a non-Fab binding/localization moiety at the N-terminus, such as the variable region of a camelid V.sub.HH antibody (V.sub.HH-Fc-T22d35) or a deca-aspartate motif for bone targeting (D10-Fc-T22d35). Similarly, (FIG. **2**B), (FIG. **2**C) and (FIG. **2**F) and (FIG. **2**G) represent constructs in which T2m (the TGF-β Type II receptor ectodomain, TβRII-ED—dark grey) is fused at the N-terminus of an IgG Fc (T2m-Fc, FIG. **2**B) or the C-terminus of IgG Fc regions with no Fab or other functional binding moiety at the N-terminus (Fc-T2m, FIG. **2**C), or the C-terminus of full-size antibodies with heavy and light chain Fabs (FSA-T2m, FIG. **2**F), or the C-terminus of IgG Fc regions that have a non-Fab binding/localization moiety at the N-terminus, such as the variable region of a camelid V.sub.HH antibody (V.sub.HH-Fc-T2m, FIG. **2**G) or a deca-aspartate motif for bone targeting (D10-Fc-T2m).

(4) FIGS. **3**A-**3**L presents (ProtA)-affinity column elution profiles, size exclusion (SEC) purification profiles, SDS-PAGE gels, and UPLC-SEC profiles of representatives of constructs type C (FIG. **2**C) and D (FIG. **2**D) in FIGS. **2**A-**2**G. FIG. **3**A is a (ProtA)-affinity column elution profile for T22d35 fused to the Cetuximab FSA (Cet-T22d35—a representative of construct D (FIG. **2**D) in FIGS. **2**A-**2**G). FIG. **3**B is the size exclusion (SEC) purification profile of the Cet-T22d35. FIG. **3**C show 4-15% SDS-PAGE gels of ProtA-purified Cet-T22d35 under reducing (left panel) and non-reducing (right panel) conditions (CetHC-T22d35, Cetuximab heavy chain fused to T22d35; CetLC, Cetuximab light chain). Lanes 1 are the pooled Prot-A eluted fractions, while lanes 2 are the pooled SEC fractions. FIG. **3**D shows the UPLC-SEC profile of ProtA-purified Cet-T22d35. FIG. **3**E shows the (ProtA)-affinity column elution profile for hIgG1 FcΔK(C)-T2m (a construct with T2m fused to an Fc region with no functional binding moiety at the N-terminus; a representative of construct D (FIG. **2**D) in FIGS. **2**A-**2**G). FIGS. **3**F, **3**G, and **3**H show the UPLC-SEC profile before SEC (FIG. **3**F), the UPLC-SEC profile after SEC (FIG. **3**G) and the SDS-PAGE (NR & R) (FIG. **3**H) of hIgG1FcΔK(C)-T2m. FIGS. **3**I, **3**K and **3**L show the SEC profile before purification and UPLC-SEC profile after SEC purification for Fc-T2m, Fc-T22d35 and hIgG1FcΔK(C)-T22d35, respectively. FIG. **3**J shows the UPLC-SEC profile for hIgG2FcΔK(CC)-Tm.

(5) FIG. **4**A shows graphs depicting the efficient inhibition of TGF-β1 (top panel), TGF-β3 (middle panel) and TGF-β2 (bottom panel) signaling in Mv1Lu luciferase reporter cells by Cet-T2m (a

representative of construct F (FIG. **2**F) in FIGS. **2**A-**2**G) and Cet-T22d35 (a representative of construct D (FIG. **2**D) in FIGS. **2**A-**2**G), compared to the significantly lower inhibition potency of non-Fc-fused T22d35.

(6) FIG. **4**B shows graphs and a summary table depicting the efficient inhibition of TGF-β1 signaling in an A549/IL-11 cell-based assay by several representatives of FSA-T22d35 constructs (Type D construct (FIG. **2**D) from FIGS. **2**A-**2**G), compared to the lower inhibition potency of Fc-T2m (Type C construct (FIG. **2**C)) and non-Fc-fused T22d35.

(7) FIG. **4**C shows graphs and a summary table depicting the efficient inhibition of TGF-β1 signaling in an A549/IL-11 release cell-based assay by several representatives of "headless"-T2m constructs (Type C construct (FIG. **2**C) from FIGS. **2**A-**2**G), compared to the lower inhibition potency of non-Fc-fused T22d35.

(8) FIG. **4**D is a graph showing competitive SPR analysis of binding of Cetuximab-fusion constructs to TGF-β isoforms in solution, compared to T22d35.

(9) FIG. **5**A is a SDS-PAGE gel showing the inhibition of EGFR phosphorylation in A549 cells by Cetuximab-fusion constructs. FIG. **5**B is a graph showing Cet-T22d35 (triangles) cytotoxicity in MDA-MB-468 and HaCat cells compared to Cetuximab (circles) and T22d35 (squares).

(10) FIG. **6** is a bar graph showing the apparent permeability coefficient (P.sub.app) values, as a measure of transport of FC5-Fc, FCS-Fc-fusion constructs, T22d35, and T2m across a human brain endothelial cell barrier in vitro, relative to a non-transporting V.sub.HH control (A20.1).

(11) FIGS. **7**A-**7**D demonstrate the Cet-T22d35 inhibition of EGF+TGF-β1 induced EMT in A549 cells. FIG. **7**A shows pictures of cultured A549 cells showing their morphologies before treatment (left panel A) and after treatment with EGF+TGF-β1 (right panel B). FIG. **7**B shows a western blot of whole cell lysates of A549 cells treated with EGF+TGF-β1 in the presence or absence of various concentrations of Cetuximab (Cetux), Cet-T22d35 or T22d35, probed for the epithelial marker E-Cadherin, while FIG. **7**C is the densitometer quantification of the E-cadherin bands in the Western blot. Results show that Cet-T22d35 is much more potent than T22d35 alone or Cetuximab alone in upregulating E-cadherin, i.e. blocking EMT. FIG. **7**D shows the inhibition of EGF+TGF-β-induced EMT by Cetuximab (Cetux), Cet-T22d35, T22d35 or Cet-T22d35 plus T22d35 as measured by flow cytometry detection of the epithelial E-Cadherin (top panel) and mesenchymal N-Cadherin (bottom panel) markers.

(12) FIG. **8**A represents the pharmacokinetic (PK) profile of Cet-T22d35 in the serum collected from BALB/C mice that were injected with a single dose of Cet-T22d35. The fusion construct appears to be cleaved in vivo; the terminal half-life of the T22d35 portion of the construct was determined to be 45.8 hours, while the terminal half-life of the Cetuximab portion of the construct was determined to be 262.5 hours. FIG. **8**B represents the PK profiles and data table (serum half-lives in bold) for constructs in which the lysine at the C-terminus of the Fc region was removed, i.e. is not present at the fusion joint between the Fc region and T2m (CetΔK-T2m, hIgG1 FcΔK(SS)-T2m, hIgG1FcΔK(AC)-T2m, and hIg2GFcΔK(SS)-T2m). The data demonstrate that the removal of the lysine prevents cleavage of the constructs in vivo.

(13) FIGS. **9**A, **9**B and **9**C present graphs showing the effect of "headless" Fc-T2m constructs (representatives of construct C (FIG. **2**C) in FIGS. **2**A-**2**G) and a FSA-T2m construct (a representative of construct F (FIG. **2**F) in FIGS. **2**A-**2**G) on tumor growth and T-cell function in an immune-competent syngeneic triple negative breast cancer (4T1) model (for comparison, the effects of the 1D11 antibody and non-Fc-fused T22d35 are also shown). The results demonstrate the improved efficacy on T-cell function of two headless-T2m constructs relative to the FSA-T2m construct, and relative to 1D11 and non-Fc-fused T22d35.

(14) FIGS. **10**A-**10**D show data illustrating enhanced bone localization of two constructs containing a deca-aspartate motif for bone targeting at the N-terminus of the Fc region (D10-hIgG1FcΔK(CC)-T2m (SEQ ID NO:136) and D10-GSL-hIgG1FcΔK(CC)-T2m (SEQ ID NO:137) —representatives of construct G (FIG. **2**G) in FIGS. **2**A-**2**G). FIGS. **10**A and **10**B show results

from an A549/IL-11 release cell-based assay demonstrating that the addition of D10 at the N-terminus did not affect TGF-β neutralization potency. FIGS. **10**C and **10**D show images demonstrating significant enhancement of the accumulation of the D10-containing constructs in bones relative to a construct without D10.

DETAILED DESCRIPTION OF THE INVENTION

(15) The present invention relates to TGF-β superfamily receptor ectodomain fusion molecules and uses thereof. More specifically, the present invention relates to TGF-β superfamily receptor ectodomain fusion molecules and their use in TGF-β superfamily ligand neutralization.

(16) The present invention provides polypeptide constructs, comprising a first portion comprising the second constant domain (C.sub.H2) and/or third constant domain (C.sub.H3) of an antibody heavy chain, and a second portion comprising at least one TGF-β superfamily receptor ectodomain (TβSR-ED),

(17) wherein the N-terminus of the second portion (ectodomain) is linked to the C-terminus of the first portion (Fc region), and wherein the polypeptide construct neutralizes TGF-β with at least 100-fold more potency than the TβSR-ED alone. The polypeptide construct referred to herein is a synthetic polypeptide produced via protein engineering. It comprises two protein "portions" (or "parts") that are linked to form the chimeric polypeptide construct. When the polypeptide construct is expressed, two polypeptide chains dimerize, such that the C.sub.H2 and C.sub.HH domains form an antibody Fc region.

(18) In specific embodiments of the present invention, descriptions of which are elaborated further herein, there are provided polypeptide constructs in which TGF-β superfamily receptor-ectodomains were fused to IgG Fc regions. Specifically, the T2m (single ectodomain) or T22d35 (double ectodomain) moieties were linked (fused) to the C-terminal end of the Fc region. It was observed that fusion constructs of this type have advantages relative to several other versions of receptor-ectodomain based molecules, including non-Fc fused monovalent or multivalent TGF-β receptor ectodomain constructs (such as T2m and T22d35) and constructs in which a receptor ectodomain is fused to the N-terminus of an Fc region. In particular, the constructs have improved manufacturability due to the presence of the Fc region (for example, purification can be accomplished using protein A chromatography). The Fc region also allows for improved circulating half-lives. Importantly, the present constructs have substantially higher TGF-β neutralization potencies compared to T2m and T22d35 alone or to constructs where a receptor ectodomain is fused to the N-terminus of an Fc region. Thus, an advantage of the present invention is unexpected high potency TGF-β superfamily ligand neutralization, including some degree of neutralization of TGF-β2, which was not observed for the non-Fc fused constructs T2m and T22d35. Finally, constructs in accordance with embodiments of the present invention, that is where the ectodomain(s) is/are fused to the C-terminus of an Fc region of an antibody, allows for preservation of the structure and function of the natural N-terminal regions/domains of an antibody; as such, antigen binding to the antibody CDR regions is not perturbed. This leads to the generation of a bifunctional construct able to interact with the target of the antibody while interacting with, and neutralizing, members of the TGF-β superfamily of ligands.

(19) The invention relates not only to bifunctional constructs, but also to constructs that are monofunctional, and comprise an Fc region that consists only of the CH2 and/or CH3 regions of an antibody constant region. Preferably, the G1, G2 or G4 subclasses are used, and particularly G1 as well as G2. These constructs are monofunctional in the sense that the constant region itself has no particular activity, other than to act as a structure through which dimers of the polypeptide constructs can form. These minimal constant regions can also be altered to provide some benefit, by incorporating the corresponding hinge regions (SEQ ID NO:5-8) and optionally changing the cysteine residue composition. Thus, some or all of the cysteine residues involved in bridging the two Fc fragments or naturally used to bridge between the heavy and light chains of a full-length antibody can be replaced or deleted. These cysteine residues are seen in hinge sequences listed in

SEQ ID NO:5-8. First, not all of the naturally-occurring inter-hinge disulfide bonds need to be formed for the Fc homodimerization to occur, while noting that the stability of the Fc homodimer may depend on the number of intermolecular disulphide bridges. Secondly and perhaps more importantly, the presence of hinge-region cysteine residues may become problematic when the Fc region lacks its N-terminal Fab fragment (i.e., is a headless Fc) as in the case of some polypeptide constructs of the present invention. This leads to untethering and exposure of these hinge-region cysteines, and in turn that may result in complex mixtures of high-order polymeric chains, which creates manufacturability issues in addition to potentially diminishing the intended biological activity and efficacy. Because it is practically impossible to predict the outcome of varying the number of inter-hinge disulphide bridges for the “headless” polypeptide constructs of the present invention, we generated a systematic array of N-terminal Fc variants for all four human IgG isotypes either by a deletion approach (in which hinge-region cysteine residues are progressively eliminated by N-terminal truncations) or by a mutagenesis approach (in which hinge-region cysteine residues are progressively mutated to serine from the N-terminus of the hinge region). Non-limiting examples of such N-terminal variants of headless Fc regions are listed in SEQ ID NO:9-34.

(20) In the present disclosure, an “antibody”, also referred to in the art as “immunoglobulin” (Ig), refers to a protein constructed from paired heavy and light polypeptide chains; various Ig isotypes exist, including IgA, IgD, IgE, IgG, and IgM. The structure of an antibody and of each of the domains is well-established and familiar to those of skill in the art, though is summarized herein. When an antibody is correctly folded, each chain folds into a number of distinct globular domains joined by more linear polypeptide sequences; the immunoglobulin light chain folds into a variable (V.sub.L) and a constant (C.sub.L) domain, while the heavy chain folds into a variable (V.sub.H) and three constant (C.sub.H, C.sub.H2, C.sub.H3) domains. Once paired, interaction of the heavy and light chain variable domains (V.sub.H and V.sub.L) and first constant domain (C.sub.L and C.sub.H1) results in the formation of a Fab (Fragment, antigen-binding) containing the binding region (Fv); interaction of two heavy chains results in pairing of C.sub.H2 and C.sub.H3 domains, leading to the formation of a Fc (Fragment, crystallisable). Characteristics described herein for the C.sub.H2 and C.sub.H3 domains also apply to the Fc.

(21) While the light and heavy chain variable regions show significant sequence diversity between antibodies, the constant regions show less sequence diversity and are responsible for binding a number of natural proteins to elicit important biochemical events. Specifically, and without wishing to be limiting, the Fc fragment binds to endogenous Fc receptors on the surface of lymphocytes.

(22) The C.sub.H2 and C.sub.H3 domains of the first portion may be of any isotype, including one selected from the group consisting of IgA, IgD, IgE, and IgG. The C.sub.H2 and C.sub.H3 domains may also be from any suitable source. For example and without wishing to be limiting, the C.sub.H2 and C.sub.H3 domains may originate from a human, mouse and other rodents like rats and degu, rabbit, monkey, or other mammalian source. In one example, the C.sub.H2 and C.sub.H3 domains may be of the IgG isotype; in another example, the C.sub.H2 and C.sub.H3 domains are from human.

(23) In a specific, non-limiting example, the C.sub.H2 and C.sub.H3 domains of the first portion may be of an isotype or comprise a sequence selected from the group consisting of:

(24) TABLE-US-00001 a human IgG1, for example but not limited to SEQ ID NO: 1 (APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA LPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSD IAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSC SVMHEALHNHYTQKSLSLSPGK) as comprised in P01857 of the UniProtKB/Swiss-Prot database; a human IgG2, for example but not limited to SEQ ID NO: 2 (APPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYV

DGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGL
PAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDI
SVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCS
VMHEALHNHYTQKSLSLSPGK), as comprised in P01859 of the UniProtKB/Swiss-Prot database; a human IgG3, for example but not limited to SEQ ID NO: 3 (APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFKWY
VDGVEVHNAKTKPREEQYNSTFRVVSVLTVLHQDWLNGKEYKCKVSNKA
LPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSD
IAVEWESSGQPENNYNTTPPMLDSDGSFFLYSKLTVDKSRWQQGNIFSC
SVMHEALHNRFTQKSLSLSPGK), as comprised in P01860 of the UniProtKB/Swiss-Prot database; a human IgG4, for example but not limited to SEQ ID NO: 4 (APEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWY
VDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKG
LPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSD
IAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSC
SVMHEALHNHYTQKSLSLSLGK), as comprised in P01861 of the UniProtKB/Swiss-Prot database;
and a sequence substantially identical to any of the sequences listed above.

(25) In the protein constructs of the present invention, the first portion may further comprise a sequence corresponding to the hinge region at the N-terminus of the C.sub.H2 domain. For example, the first portion may further comprise a sequence selected from the group consisting of:

(26) TABLE-US-00002 (SEQ ID NO: 5) EPKSCDKTHTCPPCP for human IgG1; (SEQ ID NO: 6) ERKCCVECPPCP for human IgG2; (SEQ ID NO: 7) ELKTPLGDTTHTCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCPRCPEP KSCDTPPPCPRCP for human IgG3; (SEQ ID NO: 8) ESKYGPPCPSCP for human IgG4;
and a sequence substantially identical to any of the sequences listed above.

(27) Thus, the first portion of the polypeptide construct of the present invention consists of naturally fused Fc and hinge regions for the various IgG isoforms and in embodiments is selected from the group consisting of SEQ ID NO:1-4 for the Fc region and SEQ ID NO:5-8 for the hinge region.

(28) In specific embodiments, the first portion of the polypeptide construct of the present invention is selected from a group of sequences displaying variation in the N-terminal sequence as exemplified by SEQ ID NO:9-34. These differ in the number of cysteine residues retained from the hinge region as a means to modulating the degree of Fc-region dimerization and hence impacting on both efficacy and manufacturability. Thus, in embodiments, the polypeptide construct comprises a variation in the constant domain, wherein at least one cysteine residue involved in cross-linking is deleted or substituted. Suitable substitutions include serine or alanine, and preferably by serine. A substantially identical sequence may comprise one or more conservative amino acid mutations that still provide for proper folding upon secretion into the culturing medium. It is known in the art that one or more conservative amino acid mutations to a reference sequence may yield a mutant peptide with no substantial change in physiological, chemical, physico-chemical or functional properties compared to the reference sequence; in such a case, the reference and mutant sequences would be considered “substantially identical” polypeptides. A conservative amino acid substitution is defined herein as the substitution of an amino acid residue for another amino acid residue with similar chemical properties (e.g. size, charge, or polarity). These conservative amino acid mutations may be made to the framework regions while maintaining the overall structure of the constant domains; thus the function of the Fc is maintained.

(29) In a non-limiting example, a conservative mutation may be an amino acid substitution. Such a conservative amino acid substitution may substitute a basic, neutral, hydrophobic, or acidic amino acid for another of the same group. By the term “basic amino acid” it is meant hydrophilic amino

acids having a side chain pK value of greater than 7, which are typically positively charged at physiological pH. Basic amino acids include histidine (His or H), arginine (Arg or R), and lysine (Lys or K). By the term “neutral amino acid” (also “polar amino acid”), it is meant hydrophilic amino acids having a side chain that is uncharged at physiological pH, but which has at least one bond in which the pair of electrons shared in common by two atoms is held more closely by one of the atoms. Polar amino acids include serine (Ser or S), threonine (Thr or T), cysteine (Cys or C), tyrosine (Tyr or Y), asparagine (Asn or N), and glutamine (Gln or Q). The term “hydrophobic amino acid” (also “non-polar amino acid”) is meant to include amino acids exhibiting a hydrophobicity of greater than zero according to the normalized consensus hydrophobicity scale of (Eisenberg et al, 1984). Hydrophobic amino acids include proline (Pro or P), isoleucine (Ile or I), phenylalanine (Phe or F), valine (Val or V), leucine (Leu or L), tryptophan (Trp or W), methionine (Met or M), alanine (Ala or A), and glycine (Gly or G).

(30) “Acidic amino acid” refers to hydrophilic amino acids having a side chain pK value of less than 7, which are typically negatively charged at physiological pH. Acidic amino acids include glutamate (Glu or E), and aspartate (Asp or D).

(31) In another non-limiting example, a conservative mutation in the C.sub.H2 and/or C.sub.H3 domain may be a substitution that enhances a property selected from the group consisting of the stability, half-life, or Fc properties of C.sub.H2 and/or C.sub.H3 domains or alter glycosylation of the C.sub.H2 and/or C.sub.H3 domain. For example, and without wishing to be limiting in any manner, the mutation may be an alteration at position 228 (EU numbering, 241 according to Kabat) where the serine is substituted by a proline (S228P), which stabilizes the disulfide linkage within the Fc dimer. Another alteration is the mutation at position 409 (EU numbering, 440 according to Kabat) where an arginine is substituted to a lysine for further stabilization of the Fc homodimer at the C.sub.H3-domain level (Yang & Ambrogelly, 2014). Yet another alteration within the C.sub.H2 and/or C.sub.H3 domain may be a substitution of Asn297 (EU numbering, 314 according to Kabat) by glycine or alanine to alter glycosylation of the constant domain. In yet another example, the C.sub.H2 and/or C.sub.H3 domain may be altered by substitution of one or more threonine (T252L, T253S, and/or T256F; see [U.S. Pat. No. 62,777,375]) to increase half-life. Particularly useful are those alterations that enhance Fc properties while remaining silent with respect to conformation, e.g., retaining Fc receptor binding.

(32) In yet another non-limiting example, the conservative mutations in the C.sub.H2 and/or C.sub.H3 domain may be a substitution that is naturally-occurring. Such mutations may occur in nature as minor sequence differences between species or race.

(33) Sequence identity is used to evaluate the similarity of two sequences; it is determined by calculating the percent of residues that are the same when the two sequences are aligned for maximum correspondence between residue positions. Any known method may be used to calculate sequence identity; for example, computer software is available to calculate sequence identity. Without wishing to be limiting, sequence identity can be calculated by software such as NCBI BLAST2 service maintained by the Swiss Institute of Bioinformatics (and as found at ca.expasy.org/tools/blast/), or any other appropriate software that is known in the art.

(34) The substantially identical sequences of the present invention may be at least 90% identical; in another example, the substantially identical sequences may have an identity selected from the group consisting of at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% identical, or any percentage there between, at the amino acid level to sequences described herein. Importantly, the substantially identical sequences retain the activity, specificity, and functionality of the reference sequence. In a non-limiting embodiment, the difference in sequence identity may be due to conservative amino acid mutation(s). In a non-limiting example, the first portion of the polypeptide construct of the present invention may comprise a Fc comprising a sequence selected from the group consisting of a sequence at least 95%, 98% or 99% identical to that of the Fc described herein.

(35) Accordingly, it will be appreciated that the first portion of a construct will include at least an antibody region that preferably provides for cross-linking of the polypeptide constructs, thereby to provide a dimeric protein. This first portion comprises at least the minimal CH2 and/or CH3 domain. That portion can be altered (i) by substituting or deleting cysteine residues from the hinge regions (SEQ ID NO:5-8) involved in crosslinking between the antibody heavy chains or between the heavy and light chains in order to potentially improve preparation homogeneity and efficacy, and/or (ii) by deleting or suitably replacing (e.g., by mutation to alanine) the terminal lysine residue 447 (EU numbering, 478 according to Kabat) of an IgG heavy chain in order to improve chemical stability of C-terminal fusions to enzymatic proteolysis (e.g., by several serine proteases and typically by trypsin). These changes have a positive impact on potency and/or manufacturability, as revealed herein. The first portion can also be extended to become a full Fc region, by including the CH1 domain. As a full Fc, this portion will provide normal Fc effector functions that include involvement in immune cell recruitment, ADCC, CDC and other antibody functions. Moreover, and in embodiments of the present invention, the first portion can include a complete antibody or any equivalent thereof. In certain embodiments, such as when a construct comprises just one ectodomain that is a TGF-β receptor II ectodomain, there is the proviso that the second portion is not an antibody that binds to an immune checkpoint protein such as PD-L1 (programmed death ligand 1) and is not an antibody that binds to an immunomodulating agent that counteracts immune tolerance of cancer cells, the nature and identity of which is as described in [U.S. Pat. No. 8,815,247], and is not an antibody that binds one of EGFR1, her-2, CD4, CD6, CD20, CD25, MUC-1, IL-2, IL-6, and CTLA-4.

(36) The second portion of the polypeptide construct of the present invention comprises at least one and preferably two TGF-β superfamily receptor ectodomain/s (TβSR-ED); for example, the second portion may comprise one or two TβSR-ED. The ectodomain of the Transforming Growth Factor-β superfamily receptor (TβSR) is the N-terminal extracellular, ligand-binding portion of the receptor. Without wishing to be limiting in any manner, the TβSR ectodomain may bind a molecule selected from the group consisting of TGF-β, bone morphogenetic protein (BMP) including BMP2, BMP3, BMP4, BMPS, BMP, BMP6, BMP7, BMP8, BMP9, BMP10, BMP11, BMP12, BMP13, BMP14, an BMP15, activin including activins βA, βB and βC, growth differentiation factor (GDF-1) including GDF-3, GDF-8, GDF-9, and GDF-15, nodal, inhibin-α, anti-Mullerian hormone, Lefty-1, Lefty-2, arteman, persephin, neurturin, myostatin, or other known TGF-β superfamily ligands. For example, the TβR ectodomain may be selected from the group consisting of the human TGF-β receptor type II ectodomain (TβR-II-ED), the human TGF-β receptor type IIb (TβR-IIb) ectodomain, the human activin receptor type IIa (ActR-IIa) ectodomain, the human activin receptor type IIb (ActR-IIb) ectodomain, or the BMP type Ia (BMPR-Ia) ectodomain.

(37) In a preferred embodiment the ectodomain binds TGF-β1 and/or TGF-β3. In another preferred embodiment, the ectodomain itself is a human TGF-β receptor type II ectodomain including particularly the TGF-β receptor type IIa (TβRIIa). In one specific, non-limiting example, the TβSR-ED is the TGF-β receptor type II ectodomain (TβRII-ED; SEQ ID NO:35).

(38) In the second portion as described above, the TβSR ectodomain-based portion may further comprise natural linkers. Appropriate, naturally-derived linkers that can be used to fuse two ectodomains head-to-tail are known to those of skill in the art; for example, and without wishing to be limiting, suitable natural linkers are described in [WO2008/113185].

(39) In this embodiment, the natural linker, if present, may be selected from the group consisting of

(40) TABLE-US-00003 (SEQ ID NO: 36) IPPHVQKSVNNDMIVTDNNGAVKFP; (SEQ ID NO: 37) IPPHVQKSDVEMEAQKDEIICPSCNRTAHPLRHINNDMIVTDNNGAVKF P; (SEQ ID NO: 39) SEEYNTSNPD; (SEQ ID NO: 40) SEEYNTSNPDIPPHVQKSVNNDMIVTDNNGAVKFP; (SEQ ID NO: 41) SEEYNTSNPDIPPHVQKSDVEMEAQKDEIICPSCNRTAHPLRHINNDMI VTDNNGAVKFP; and a combination thereof.

(41) In a specific, non-limiting example, the second portion of the polypeptide construct of the present invention may comprise the sequence selected from the group consisting of: A single TGF-β Type II receptor ectodomain, such as:

(42) TABLE-US-00004

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCS
ITSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKC
IMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPD (SEQ ID NO: 43, also referred to herein as T2m); (SEQ ID NO: 44)
IPPHVQKSDVEMEAQKDEIICPSCNRTAHPLRHINNDMIVTDNNGAVKF
PQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRKNDENIT
LETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECND
NIIFSEEYNTSNPD; A TGF-β Type II receptor ectodomain "doublet", in which a TGF-β Type II receptor ectodomain is linked with another TGF-β Type II receptor ectodomain, which ectodomains can be the same or different TGF-β superfamily receptor ectodomains, such as:

(43) TABLE-US-00005

IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCS
ITSICEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKC
IMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPDIPPHVQKSVNN
DMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEV
CVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGET
FFMCSCSSDECNDNIIFSEEYNTSNPD (SEQ ID NO: 46, also referred to herein as T22d35); (SEQ ID NO: 47)
IPPHVQKSDVEMEAQKDEIICPSCNRTAHPLRHINNDMIVTDNNGAVKF
PQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRKNDENIT
LETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECND
NIIFSEEYNTSNPDIPPHVQKSDVEMEAQKDEIICPSCNRTAHPLRHIN
NDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQE
VCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGE
TFFMCSCSSDECNDNIIFSEEYNTSNPD;

and a sequence substantially identical thereto. "Substantially identical" is as defined above.

(44) In another specific, non-limiting example, the TGF-β receptor ectodomain is the bone morphogenetic protein receptor Ia (BMPRIa; SEQ ID NO:69). In this embodiment, the natural linker, if present, may be selected from the group consisting of

(45) TABLE-US-00006 (SEQ ID NO: 70)
QNLDSMLHGTGMKSDSDQKKSENGVTLAPED; (SEQ ID NO: 71) PVVIGPFFDGSIR; (SEQ ID NO: 72) PVVIGPFFDGSIRQNLDSMLHGTGMKSDSDQKKSENGVTLAPED; and a combination thereof.

(46) Thus, in a specific, non-limiting example, the second portion of the polypeptide construct of the present invention may comprise the sequence selected from the group consisting of:

(47) TABLE-US-00007 (SEQ ID NO: 74)
QNLDSMLHGTGMKSDSDQKKSENGVTLAPEDTLPFLKCYCSGHCPDDAI
NNTCITNGHCFAIIEEDDQGETTLASGCMKYEGSDFQCKDSPKAQLRRT
IECCRTNLCNQYLQPTLPPVVIGPFFDGSIRQNLDSMLHGTGMKSDSDQ
KKSENGVTLAPEDTLPFLKCYCSGHCPDDAINNTCITNGHCFAIIEEDD
QGETTLASGCMKYEGSDFQCKDSPKAQLRRTIECCRTNLCNQYLQPTLP
PVVIGPFFDGSIR;

and a sequence substantially identical thereto. "Substantially identical" is as defined above.

(48) In another specific, non-limiting example, the TβSR ectodomain is the activin receptor Ila (ActRIIa; SEQ ID NO:75). In this embodiment, the natural linker, if present, may be selected from the group consisting of

(49) TABLE-US-00008 (SEQ ID NO: 76) AILGRSE; (SEQ ID NO: 77) EMEVTQPTSNPVTPKPPYYNI; (SEQ ID NO: 78) EMEVTQPTSNPVTPKPPYYNIAILGRSE;
and a combination thereof.
(50) Thus, another specific non-limiting example of the second portion of the polypeptide construct of the present invention comprises the sequence selected from the group consisting of:
(51) TABLE-US-00009 (SEQ ID NO: 80)
AILGRSETQECLFFNANWEKDRTNQTGVEPCYGDKDKRRHCFATWKNIS
GSIEIVKQGCWLDDINCYDRTDCVEKKDSPEVYFCCCEGNMCNEKFSYF
PEMEVTQPTSNPVTPKPPYYNIAILGRSETQECLFFNANWEKDRTNQTG
VEPCYGDKDKRRHCFATWKNISGSIEIVKQGCWLDDINCYDRTDCVEKK
DSPEVYFCCCEGNMCNEKFSYFPEMEVTQPTSNPVTPKPPYYNI;
and a sequence substantially identical thereto. “Substantially identical” is as defined above.
(52) In another specific, non-limiting example, the TGF-β receptor ectodomain is the activin receptor IIb (ActRIIb; SEQ ID NO:81). In this embodiment, the natural linker, if present, may be selected from the group consisting of
(53) TABLE-US-00010 (SEQ ID NO: 82) SGRGEAET; (SEQ ID NO: 83) EAGGPEVTYEPPPTAPT; (SEQ ID NO: 84) EAGGPEVTYEPPPTAPTSGRGEAET;
and a combination thereof.
(54) Thus, another specific non-limiting example of the second portion of the polypeptide construct of the present invention comprises the sequence selected from the group consisting of:
(55) TABLE-US-00011 (SEQ ID NO: 86)
SGRGEAETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSS
GTIELVKKGCWLDDFNCYDRQECVATEENPQVYFCCCEGNFCNERFTHL
PEAGGPEVTYEPPPTAPTSGRGEAETRECIYYNANWELERTNQSGLERC
EGEQDKRLHCYASWRNSSGTIELVKKGCWLDDFNCYDRQECVATEENPQ
VYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT;
and a sequence substantially identical thereto. “Substantially identical” is as defined above.
(56) Thus, in various embodiments of the present invention, the present constructs have an ectodomain comprising an amino acid sequence selected from the group consisting of SEQ ID NO:35, SEQ ID NO:69, SEQ ID NO:75, SEQ ID NO:81, and a sequence substantially identical thereto. In other embodiments, the second portion comprises the entire extracellular portion of a TβSR-ED consisting of a sequence selected from the group consisting of SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:73, SEQ ID NO:79, SEQ ID NO:85, and a sequence substantially identical thereto.
(57) The at least two ectodomain portion can have the same or different ectodomains, all belonging to the superfamily. In embodiments, the ectodomains bind the same target. In other embodiments, the ectodomains originate from the same receptor species. In other embodiments, the ectodomains are identical and thus are homomeric. In other embodiments the ectodomains are different and thus are heteromeric. In these embodiments, the ectodomain can be for instance a TβRII-ED that is type a, and another ectodomain can be a TβRII-ED that is type b. A third ectodomain could be the same as either one of these, or different still. For example, when there is more than one ectodomain in the second portion of the polypeptide construct of the present invention, the ectodomains may be all the same (homomers) or all different (heteromers), or any combination of superfamily ectodomains.
(58) Thus, in embodiments, the second portion of the polypeptide construct of the present invention comprises a repeat of a given TβSR-ED selected from the group consisting of SEQ ID NO:46, 47, 48, 74, 80, 86, and a sequence substantially identical thereto.
(59) In specific embodiments, the second portion of the polypeptide construct of the present invention comprises heteromeric repeats of two distinct TβSR-EDs genetically fused and selected

from the group consisting of SEQ ID NO:61, 62, 63, 88, and a sequence substantially identical thereto.

(60) In yet other embodiments, the second portion of the polypeptide construct of the present invention comprises homo-multimeric and hetero-multimeric repeats of one or more TβSR-EDs selected for instance from the group consisting of SEQ ID NO:49, 50, 51, 64, 65, 66, 67, 68, and a sequence substantially identical thereto.

(61) In the protein construct of the present invention, the first and second portions of the polypeptide construct of the present invention are linked. By the term “linked”, it is meant that the two portions are covalently bonded. The chemical bond may be achieved by chemical reaction, or may be the product of recombinant expression of the two portions in a single polypeptide chain. In one specific, non-limiting example, the C-terminus of the first portion is linked directly to the N-terminus of the second portion, that is, no additional “linker” amino acids are present between the two portions. In the case where no linker is present, that is to say direct fusion of the two portions, there will be a direct link between the N-terminus of the full ectodomain and the C-terminus of the antibody constant regions C.sub.H2-C.sub.H3. For example, in fusing the Fc variant SEQ ID NO:9 to the SEQ ID NO:43 via the intrinsically disordered linker with SEQ ID NO:36, which is part of the TβRII-ED (i.e., no additional “linker” amino acids added), one connects the glycine at the last position of SEQ ID NO:9 to the isoleucine at the first position of SEQ ID NO:43.

(62) A common practice when producing fusion constructs is to introduce glycine or glycine-serine linkers (such as GGGGS, or [G.sub.4S].sub.n) between the fused components. As taught in the above paragraph, the polypeptide fusions of the present invention can be produced by direct linkage without use of any additional amino-acid sequence except those present in the Fc portion and in the receptor ectodomain portion. One thus can refrain from utilizing foreign sequences as linkers, providing an advantage due to their potential for undesired immunogenicity and their added molecular weight. Entropic factors are also a potential liability for glycine and glycine-serine linkers, which are highly flexible and may become partially restricted upon target binding, hence causing a loss of entropy unfavourable to binding affinity. Therefore, only the flexible, intrinsically disordered N-terminal regions of the TβSR receptor ectodomains were employed as natural linkers in embodiments of the present invention. However, the particular amino acid compositions and lengths of these intrinsically disordered linkers (e.g., SEQ ID NO:36, 37, 70, 76, 82) precluded accurate prediction of whether the resulting direct-fusion constructs will have the required geometry and favourable molecular interactions for correct binding to their intended dimeric ligands.

(63) The first and second portions of the polypeptide construct are, in embodiments, connected by natural intrinsically disordered polypeptide linkers selected from the group consisting of SEQ ID NO:36, 37, 38, 53, 70, 76, 82, and a sequence substantially identical thereto.

(64) In embodiments, when multiple TβSR-ED structured regions are present, these can be fused directly or they can be connected by natural intrinsically disordered polypeptide linkers between the ectodomains, such as SEQ ID NO:40, 41, 42, 55, 58, 59, 60, 72, 78, 84, 87, and a sequence substantially identical thereto.

(65) Non-limiting examples of full-length polypeptide constructs of the present invention that comprise the two aforementioned portions are selected from the group consisting of SEQ ID NO: 91-120, and a sequence substantially identical thereto.

(66) It is particularly important to note that, in the polypeptide constructs of the present invention, the N-terminus of the second portion is linked to the C-terminus of the first portion (see for example FIGS. **2**A, C, D, E, F, and G).

(67) Some of the polypeptide constructs of the present invention display significantly greater potency of TGF-β neutralization compared to that of the TGF-β superfamily receptor ectodomain alone; for example, the polypeptide construct may be between at least 50-fold and 1×10.sup.6-fold more potent. For example, the polypeptide constructs of the present invention may have a TGF-β

neutralization potency selected from the group consisting of at least 50-, 75-, 100-, 150-, 200 -300-, 400-, 500-, 600, 1000 -1500-, 2000-, 3000-, 4000-, 5000-, 6000-, 7000-, 8000-, 9000, 10000-, 20000-, 30000-, 40000-, 50000-, 60000-, 70000-, 80000-, 90000-, 100000-, 150000-, 200000-, 250000-, 300000-, 350000-, 400000-, 450000-, 500000-, 550000-600000-, 650000-, 700000-, 750000-, 800000-, 850000-, 900000-, 950000, or 1000000-fold, more potent than the TβSR-ED alone, or any amount there between. In one example, the potency of the construct is at least 100-fold greater than the receptor ectodomain alone.

(68) Additionally, when the polypeptide constructs of the present invention include a TβSR-ED that binds TGF-β, the polypeptide construct may neutralize, to varying extents, all three isotypes of TGF-β (that is, TGF-β1, TGF-β2, and TGF-β).

(69) The polypeptide constructs of the present invention have, as assessed in cell-based assays, TGF-β neutralizing potencies that are significantly higher (100-fold or more) than those of bivalent comparator polypeptides, i.e. non-Fc-fused T22d35 and T2m-Fc. Within the series of polypeptide constructs of the present invention, those that contain two or more copies of the TβRII ectodomain fused to the C-terminus of the Fc constant region have potencies that are higher than those constructs that contain only one copy, as assessed in cell based assays. Additionally, within the series of polypeptide constructs of the present invention, if the first portion within the construct is “headless”, i.e. does not contain a Fab region, the potencies of the constructs are increased by engineering (optimizing) the number of cysteines in the hinge region at the “revealed” N-terminus. Engineering of the cysteine residues at the N-termini of “headless” constructs also markedly reduces the aggregation propensity of the constructs. Lastly, within the series of polypeptide constructs of the present invention, in vivo in tumor models, cysteine optimized “headless” constructs exhibit higher anti-tumor immuno-modulatory potencies than constructs in which the first portion is a full-sized antibody.

(70) The polypeptide construct of the present invention is expressed as a single polypeptide chain. Once expressed, the polypeptide construct of the present invention forms a dimer wherein the C.sub.H2 and C.sub.H3 domains of the respective polypeptide constructs interact to form a properly assembled Fc region such as occurs when the expressed products are secreted into the culturing medium. For example, and without wishing to be limiting, examples of dimerized polypeptide constructs of the present invention are shown in FIGS. **2**A and C-G. In one example, homodimers may be formed by identical polypeptide constructs. Alternatively, heterodimers may be formed by two different polypeptide constructs; thus, a heterodimer may be formed by two Fc region polypeptide constructs that have been engineered to induce heterodimerization and inhibit homodimerization.

(71) The first portion of the polypeptide construct described above may further comprise, at its N-terminus, any suitable antigen-binding antibody fragment known in the art. For example, and without wishing to be limiting in any manner, the first portion of the polypeptide construct may comprise C.sub.H2 and C.sub.H3 domains and one selected from the group consisting of a single-chain Fv (scFv; a molecule consisting of V.sub.L and V.sub.H connected with a peptide linker) and a single-domain antibody (sdAb, a fragment composed of a single V.sub.L or a single V.sub.H; see for example FIG. **1**C). In other instances, the antigen-binding fragment may be formed by combining the polypeptide construct with a second polypeptide chain. For example, the first portion of the polypeptide construct may comprise C.sub.H2 and C.sub.H3 domains along with a C.sub.H1 and V.sub.H domains, which when combined with a second polypeptide comprising C.sub.L and V.sub.L form a full-size antibody (i.e., Fc and Fab; see for example FIG. **1**D). In another example, the first portion of the polypeptide may comprise C.sub.H2 and C.sub.H3 domains along with V.sub.H, which when combined with a second polypeptide comprising a V.sub.L forms an Fc fused to a Fv.

(72) The combination of constant domains and antigen-binding fragment may be naturally-occurring, or may be obtained by manipulation of a naturally-occurring antibody or by using

recombinant methods. The polypeptide constructs such as those just described may require a sequence selected from the group consisting of linker sequences, disulfide bonds, hinge region sequences, and other type of covalent bond to link them to the C.sub.H2 and C.sub.H3 domains; those of skill in the art will be familiar with various suitable approaches.

(73) In alternative constructs of the present invention, the polypeptide construct comprises an antibody Fc fragment linked at the C-terminus of each heavy chain to at least one TGF-β superfamily receptor ectodomain (TβSR-ED), as described above and as illustrated in FIG. **2**(A,D,E). The construct may further comprise an antigen-binding fragment at the N-terminus of the Fc; the antigen-binding fragment may be selected from the group consisting of a Fv, scFv, Fab, or sdAb, also as described above. In the polypeptide constructs as described above, the TGF-β receptor ectodomain does not interfere in the native function or specificity of the antigen-binding fragment.

(74) The antigen-binding antibody fragment described above, when present, may be directed to any suitable antigen. In certain limited embodiments, the antigen-binding antibody or fragment does not bind to an antigen that is PD-L1, EGFR1, her-2, CD4, CD6, CD20, CD25, MUC-1, IL-2, IL-6, or CTLA-4.

(75) The present constructs can further comprise antibody or antibody fragments that target any antigen of interest. They can also comprise the antigen itself, or any other moiety of interest that is genetically encoded. Particular embodiments herein include the EGFR antibody cetuximab and its active fragments, Avastin, Herceptin, Synagis, FC5, or a poly-aspartate bone-localization motif, such a D10, or sequence substantially identical or equivalent thereto.

(76) The present constructs can comprise a binding protein e.g., antibody and binding fragments thereof, that inhibits a checkpoint protein which may be CTLA-4, PD1, PDL1, PDL2, PDL3, PD1, B7-H3, B7-H4, BTLA, HVEM, TIM3, GALS, LAGS, VISTA, KIR, 2B4, CD160, CGEN-15049, CHK 1, CHK2, A2aR, CD28, CD86, or one of the B-7 family ligands or a combination thereof.

(77) Illustrative immune checkpoint inhibitors include Tremelimumab (CTLA-4 blocking antibody), anti-OX40, PD-LI monoclonal Antibody (Anti-B7-HI; MED14736), MK-3475 (PD-1 blocker), Nivolumab (anti-PDI antibody), CT-011 (anti-PDI antibody), BY55 monoclonal antibody, AMP224 (anti-PDLI antibody), BMS-936559 (anti-PDLI antibody), MPLDL3280A (anti-PDLI antibody), MSB0010718C (anti-PDLI antibody) and Yervoy/ipilimumab (anti-CTLA-4 checkpoint inhibitor).

(78) Other antibodies provided by the present constructs can include rituximab, muromonab-CD3, abciximab, daclizumab, basiliximab, palivizumab, infliximab, trastuzumab, gemtuzumab ozogamicin, alemtuzumab, ibritumomab tiuxetan, adalimumab, omalizumab, tositumomab, I-131 tositumomab, efalizumab, bevacizumab, panitumumab, pertuzumab, natalizumab, etanercept, IGN101, volociximab, Anti-CD80 mAb, Anti-CD23 mAb, CAT-3888, CDP-791, eraptuzumab, MDX-010, MDX-060, MDX-070, matuzumab, CP-675,206, CAL, SGN-30, zanolimumab, adecatumumab, oregovomab, EGFR-binding antibodies cetuximab, nimotuzumab, necitumumab, panitumumab, matuzumab, and zalutumumab, as well as ABT-874, denosumab, AM 108, AMG 714, fontolizumab, daclizumab, golimumab, CNTO 1275, ocrelizumab, HuMax-CD20, belimumab, epratuzumab, MLN1202, visilizumab, tocilizumab, ocrerlizumab, certolizumab, eculizumab, pexelizumab, abciximab, ranibizimumab, mepolizumab, TNX-355, or MYO-029.

(79) Still other antibodies that can be included in the present constructs are rituximab, zanolimumab, hA20, AME-133, HumaLYM, trastuzumab, pertuzumab, IMC-3G3, ch806, KSB-102, MR1-1, SC100, SC101, SC103, alemtuzumab, muromonab-CD3, OKT4A, ibritumomab, gemtuzumab, alefacept, abciximab, basiliximab, palivizumab, motavizumab, infliximab, adalimumab, CDP-571, etanercept, ABX-CBL, ABX-IL8, ABX-MA1 pemtumomab, Therex, AS1405, natalizumab, HuBC-1, natalizumab, IDEC-131, VLA-1; CAT-152; J695, CAT-192, CAT-213, BR3-Fc, LymphoStat-B, TRAIL-R1mAb, bevacizumab, ranibizumab, omalizumab, efalizumab, MLN-02, zanolimumab, HuMax-IL 15, HuMax-Inflam, HuMax-Cancer, HuMax-

Lymphoma, HuMax-TAC, clenoliximab, lumiliximab, BEC2, IMC-1C11, DC101, labetuzumab, arcitumomab, epratuzumab, tacatuzumab, MyelomaCide, LkoCide, ProstaCide, ipilimumab, MDX-060, MDX-070, MDX-018, MDX-1106, MDX-1103, MDX-1333, MDX-214, MDX-1100, MDX-CD4, MDX-1388, MDX-066, MDX-1307, HGS-TR2J, FG-3019, BMS-66513, SGN-30, SGN-40, tocilizumab, CS-1008, IDM-1, golimumab, ONTO 1275, ONTO 95, ONTO 328, mepolizumab, MOR101, MOR102, MOR201, visilizumab, HuZAF, volocixmab, ING-1, MLN2201, daclizumab, HCD122, CDP860, PRO542, C14, oregovomab, edrecolomab, etaracizumab, siplizumab, lintuzumab, Hu1D10, Lym-1, efalizumab, ICM3, galiximab, eculizumab, pexelizumab, LDP-01, huA33, WX-G250, sibrotuzumab, Chimeric KW-2871, hu3S193, huLK26; bivatuzumab, ch14.18, 3F8, BC8, huHMFG1, MORAb-003, MORAb-004, MORAb-009, denosumab, PRO-140, 1D09C3, huMikbeta-1, NI-0401, NI-501, cantuzumab, HuN901, 8H9, chTNT-1/B, bavituximab, huJ591, HeFi-1, Pentacea, abagovomab, tositumomab, 105AD7, GMA161 and GMA321.

(80) In other embodiments, the constant domain/first portion of the constructs can comprise a polypeptide having medicinal properties, such as agents that stimulate the immune system, in particular in relation to the ability of the immune system to attack tumor cells. These polypeptides can include cytokines (such as interleukin-2) or growth factors that stimulate immune cells directly or indirectly (i.e. act by providing gas to the immune system), as well as ectodomains or other binding agents that neutralize ligands which inhibit immune cells, either directly or indirectly (i.e. act by releasing a brake on the immune system).

(81) In other embodiments, the constant domain/first portion of the constructs can comprise a polypeptide that does not have active medicinal properties pe se, but rather provides a localization signal. This localization motif will serve to focus the intrinsic TGF-β neutralization activity of the second portion of the construct to a particular region of the body. In one example, the first portion comprised a long poly-aspartate bone-localization motif, preferably D10 or an equivalent bone-localization moiety, which acts to enhance localisation of the construct to bone. By increasing the TGF-β neutralization activity of the construct within bone, more favourable dosing levels and schedules may be required for the treatment of bone-related diseases, such as osteogenesis imperfecta, relative to that required for a similar construct without the D10 motif.

(82) Embodiments exemplifying polypeptide constructs of the present invention that include antigen-binding fragments at the N-terminus of the Fc region (first portion) are selected from the group consisting of SEQ ID NO: 121-131, and a sequence substantially identical thereto.

(83) In other embodiments, polypeptide constructs of the present invention that include other targeting agents, e.g., a poly-aspartate bone-localization motif, at the N-terminus of the Fc region (first portion), are exemplified by SEQ ID NO: 136-150.

(84) The polypeptide construct of the present invention may also comprise additional sequences to aid in expression, detection or purification of a recombinant antibody or fragment thereof. Any such sequences or tags known to those of skill in the art may be used. For example, and without wishing to be limiting, the antibody or fragment thereof may comprise a targeting or signal sequence (for example, but not limited to ompA), a detection/purification tag (for example, but not limited to c-Myc, His.sub.5, His.sub.6, or His.sub.8G), or a combination thereof. In another example, the signal peptide may be MVLQTQVFISLLLWISGAYG (SEQ ID NO:89) or MDWTWRILFLVAAATGTHA (SEQ ID NO:90). In a further example, the additional sequence may be a biotin recognition site such as that described in [WO/1995/04069] or in [WO/2004/076670]. As is also known to those of skill in the art, linker sequences may be used in conjunction with the additional sequences or tags, or may serve as a detection/purification tag.

(85) The present invention also encompasses nucleic acid sequences encoding the molecules as described herein. Given the degeneracy of the genetic code, a number of nucleotide sequences would have the effect of encoding the desired polypeptide, as would be readily understood by a skilled artisan. The nucleic acid sequence may be codon-optimized for expression in various micro-organisms. The present invention also encompasses vectors comprising the nucleic acids as just

described, wherein the vectors typically comprise a promoter and signal sequence that are operably linked to the construct-encoding polynucleotide for driving expression thereof in the selected cellular production host. The vectors can be the same or different provided both result in secretion of the dimeric polypeptide construct.

(86) Furthermore, the invention encompasses cells, also referred to herein as transgenic cellular host, comprising the nucleic acid and/or vector as described, encoding a first polypeptide construct. The host cells may comprise a second nucleic acid and/or vector encoding a second polypeptide construct different from the first polypeptide construct. The co-expression of the first and second polypeptide constructs may lead to the formation of heterodimers.

(87) The present invention also encompasses a composition comprising one or more than one polypeptide construct as described herein. The composition may comprise a single polypeptide construct as described above, or may be a mixture of polypeptide constructs. The composition may also comprise one or more than one polypeptide construct of the present invention linked to one or more than one cargo molecule. For example, and without wishing to be limiting in any manner, the composition may comprise one or more than one polypeptide construct of the present invention linked to a cytotoxic drug in order to generate an antibody-drug conjugate (ADC) in accordance with the present invention.

(88) The composition may also comprise a pharmaceutically acceptable diluent, excipient, or carrier. The diluent, excipient, or carrier may be any suitable diluent, excipient, or carrier known in the art, and must be compatible with other ingredients in the composition, with the method of delivery of the composition, and is not deleterious to the recipient of the composition. The composition may be in any suitable form; for example, the composition may be provided in suspension form, powder form (for example, but limited to lyophilised or encapsulated), capsule or tablet form. For example, and without wishing to be limiting, when the composition is provided in suspension form, the carrier may comprise water, saline, a suitable buffer, or additives to improve solubility and/or stability; reconstitution to produce the suspension is effected in a buffer at a suitable pH to ensure the viability of the antibody or fragment thereof. Dry powders may also include additives to improve stability and/or carriers to increase bulk/volume; for example, and without wishing to be limiting, the dry powder composition may comprise sucrose or trehalose. In a specific, non-limiting example, the composition may be so formulated as to deliver the antibody or fragment thereof to the gastrointestinal tract of the subject. Thus, the composition may comprise encapsulation, time-release, or other suitable technologies for delivery of the antibody or fragment thereof. It would be within the competency of a person of skill in the art to prepare suitable compositions comprising the present compounds.

(89) The constructs of the present invention may be used to treat diseases or disorders associated with over-expression or over-activation of ligands of the TGF-β superfamily. The disease or disorder can be selected from, but not limited to, cancer, ocular diseases, fibrotic diseases, or genetic disorders of connective tissue.

(90) In the field of cancer therapy, it has recently been demonstrated that TGF-β is a key factor inhibiting the antitumor response elicited by immunotherapies, such as immune checkpoint inhibitors (ICI's) (Hahn & Akporiaye, 2006). Specifically, therapeutic response to ICI antibodies results primarily from the re-activation of tumor-localized T-cells. Resistance to ICI antibodies is attributed to the presence of immunosuppressive mechanisms that result in a dearth of T-cells in the tumor microenvironment. Thus, it is now recognized that in order to elicit responses in resistant patients, ICI antibodies need to be combined with agents that can activate T-cells and induce their recruitment into the tumor, i.e. reversing of the “non-T-cell-inflamed” tumor phenotype. One publication noted that overcoming the non-T-cell-inflamed tumor microenvironment is the most significant next hurdle in immuno-oncology (Gajewski, 2015).

(91) We have shown using a proof-of-principle TGF-β trap, T22d35, that blocking of TGF-β effectively reverses the “non-T cell inflamed” tumor phenotype (Zwaagstra et al, 2012). This

positions anti-TGF-β molecules as potential synergistic combinations with ICI's and other immunotherapeutics. In support of this, a 2014 study (Holtzhausen et al., ASCO poster presentation) examined effects of a TGF-β blocker when combined an anti-CTLA-4 antibody in a physiologically-relevant transgenic melanoma model. The study demonstrated that while anti-CTLA-4 antibody monotherapy failed to suppress melanoma progression, the combination of the TGF-β antagonist and anti-CTLA-4 antibody significantly and synergistically suppressed both primary melanoma tumor growth as well as melanoma metastasis. These observations correlated with significant increases in effector T-cells in melanoma tissues.

(92) Fibrotic diseases include those that affect any organ of the body, including, but not limited to kidney, lung, liver, heart, skin and eye. These diseases include, but are not limited to, chronic obstructive pulmonary disease (COPD), glomerulonephritis, liver fibrosis, post-infarction cardiac fibrosis, restenosis, systemic sclerosis, ocular surgery-induced fibrosis, and scarring.

(93) Genetic disorders of connective tissue include, but are not limited to, Marfan syndrome (MFS) and Osteogenesis imperfecta (OI).

(94) The present invention will be further illustrated in the following examples. However, it is to be understood that these examples are for illustrative purposes only and should not be used to limit the scope of the present invention in any manner.

Example 1: Production and Purification of Fusion Molecules

(95) Several fusion molecules comprising full-size antibody (FSA), V.sub.HH-IgG Fc, D10-Fc or “headless” Fc C-terminally-fused to the T22d35 or T2m ectodomains were constructed (Table 1). All constructs comprising a heavy chain included the signal sequence MDWTWRILFLVAAATGTHA (SEQ ID NO:89) at the N-terminus, while constructs comprising a light chain included the signal sequence MVLQTQVFISLLLWISGAYG (SEQ ID NO:90) at the N-terminus. The DNA coding for constructs were prepared synthetically (Biobasic Inc. or Genescript USA Inc.). Constructs comprising FSA, D10-Fc and “headless” Fc were cloned into the EcoR1 (5′ end) and BamH1 (3′ end) sites and those comprising V.sub.HH-IgG Fc were cloned into the HindIII (5′ end) and BamH1 (3′ end) sites of the pTT5 mammalian expression plasmid vector (Durocher et al, 2002).

(96) TABLE-US-00012 TABLE 1 FSA-, V.sub.HH-IgG Fc-, D10-Fc- and Fc-fusion constructs produced. The letter in brackets in the construct column refers to the type of construct as illustrated in FIG. 2. Antibody Construct Construct ED Fusion Scaffold source SEQ ID NO: Cet-T2m (F) T2m FSA (hIgG1) Cetuximab 121, 123 Cet-T22d35 (D) T22d35 FSA (hIgG1) Cetuximab 121, 122 Her-T22d35 (D) T22d35 FSA (hIgG1) Herceptin 124, 125 Ava-T22d35 (D) T22d35 FSA (hIgG1) Avastin 126, 127 Syn-T22d35 (D) T22d35 FSA (hIgG1) Synagis 128, 129 FC5-Fc-T22d35 (E) T22d35 VHH-Fc (mIgG2) FC5-Fc 130 FC5-Fc-T2m (G) T2m VHH-Fc (mIgG2) FC5-Fc 131 D10-Fc-T2m (G) T2m Fc huIgG 136-139 (several variants with differing D10 linkage and IgG isotype) Fc-T22d35 (A) T22d35 Fc huIgG 100, 105 (several variants with differing N-termini and IgG isotype) Fc-T2m (C) T2m Fc huIgG 91-97 (several variants with differing N-termini and IgG isotype) T2m-Fc (R&D) (B) T2m Fc huIgG1 132 T2m-Fc (B) T2m Fc huIgG2 133

(97) The Cet-T2m and Cet-T22d35 constructs were produced by transient co-transfection of Chinese Hamster Ovary (CHO) cells with the heavy chain (HC)-T2m or (HC)-T22d35 construct combined with the Cetuximab light chain (LC) construct which then assembled as the Cetuximab-T22d35 (Cet-T22d35) or Cetuximab-T2m (Cet-T2m) fusion molecules. Briefly, CetHC-T22d35 (SEQ ID NO:122) and CetLC (SEQ ID NO:121) plasmid DNAs (ratio=3:2) were co-transfected into a 10 L Wavebag culture of CHO-3E7 cells in FreeStyle F17 medium (Invitrogen) containing 4 mM glutamine and 0.1% Kolliphor p-188 (Sigma) in a Wavebag maintained at 37° C. Transfection conditions were: DNA (50% HC+LC plasmids, 30% ssDNA, 15% AKT plasmid, 5% GFP plasmid): PEI(polyethylenimine)pro (Polyplus) (ratio=1:2.5). At 24 hours post-transfection, 10%

Tryptone N1feed (TekniScience Inc.) and 0.5 mM Vaporic acid (VPA, Sigma) were added and the temperature was shifted to 32° C. to promote the production and secretion of the fusion proteins and maintained for 15 days post transfection after which the cells were harvested. At final harvest the cell viability was 89.6%. The harvest supernatant (10.8 L) was filtered (0.2 μm) and loaded onto a 55 mL Protein A MabSelect Sure 55 mL column (GE Healthcare). The column was washed with 2 column volumes of PBS and protein was eluted with 3 column volumes of 0.1 M sodium citrate pH 3.6. To maximize the yield, the flow through was reloaded onto the Protein A column and eluted as described above. Eluted fractions were neutralized with 1 M Tris, evaluated by SDS-PAGE and those containing Cet-T22d35 were pooled (FIG. **3**A) and subsequently loaded onto a Hi-load Superdex S200 26/60 size exclusion chromatography (SEC) column (GE Healthcare) equilibrated in formulation buffer (DPBS without Ca.sup.2+, without Mg.sup.2+). Protein was eluted using 1 column volume formulation buffer, collected into successive fractions and detected by UV absorbance at 280 nM (FIG. **3**B). The main peak SEC fractions containing Cet-T22d35 protein were then pooled and concentrated to a concentration of 7.8 mg/mL. The final yield was 533 mg.

(98) Similar transfection, production and purification methods were performed for the other FSA-trap examples listed in Table 1. In the case of the V.sub.HH-Fc IgG-, D10 and “headless” Fc-fusion molecules the composition of the transfection mixture was modified as follows: DNA (80% plasmid construct, 15% AKT plasmid, 5% GFP plasmid): PEIpro (ratio 1:2.5).

(99) The integrity of the pooled Prot-A and SEC fractions of Cet-T22d35 protein was analyzed by SDS-PAGE (4-15% polyacrylamide) under reducing and non-reducing conditions (FIG. **3**C) and by UPLC-SEC (FIG. **3**D). For UPLC-SEC, 2-10 μg of protein in DPBS (Hyclone, minus Ca2+, minus Mg2+) was injected onto a Waters BEH200 SEC column (1.7 μm, 4.6×150 mm, SN:01773430816818) and resolved under a flow rate of 0.4 mL/min for 8.5 min at room temperature, using the Waters Acquity UPLC H-Class Bio-System. Protein peaks were detected at 280 nM (Acquity PDA detector). Coomassie brilliant blue (CBB) staining of the gels shows the CetHC-T22d35 (˜110 Kd) and CetLC bands (˜30 Kd) under reducing conditions while under non-reducing conditions a 250 Kd band is detected which represents the fully assembled and highly pure Cet-T22d35 fusion protein. Additional UPLC-SEC analysis of the SEC purified, pooled ProtA sample confirmed the high degree of purity (99.42%) of the Cet-T22d35 protein and the absence of aggregates. Together, these results demonstrate the manufacturability of the Cet-T22d35 fusion protein.

(100) Similar methods were used to analyse expression levels, purifiability, aggregation levels, and dimeric assembly of several other Fc-ectodomain constructs. The results from these studies are shown in FIG. **3** E to L.

(101) FIG. **3**E to **3**H show the results from the analysis of the hIgG1FcΔK(C)-T2m construct (an example of Type C construct from FIG. **2**). FIG. **3**E shows the (ProtA)-affinity column elution profile. Fraction 12-15 were pooled, subjected to a UPLC-SEC evaluation (FIG. **3**F), further SEC purified to remove aggregates and re-evaluated by UPLC-SEC (FIG. **3**G). This confirmed the high degree of purity of the hIgG1FcΔK(C)-T2m construct and the absence of aggregates. SDS-PAGE (FIG. **3**H) under non-reducing (NR) and reducing (R) conditions shows bands of expected molecular weights, demonstrating the expected assembly of hIgG1FcΔK(C)-T2m as a disulphide linked dimer.

(102) FIGS. **3**I and **3**J compare the level of aggregation of two “headless” Fc-T2m constructs (examples of Type C in FIG. **2**). The Fc-T2m construct is an IgG2-based construct without engineering of the hinge region, thus it contains four cysteine residues, whereas the hIgG2FcΔK(CC)-T2m has been engineered by N-terminal truncation of the hinge region to have only two cysteines in the hinge region. It can be seen that the Fc-T2m construct contains a high level of aggregates after Protein A purification, with a doublet peak remaining even after further SEC purification. In contrast, hIgG2FcΔK(CC)-T2m, which has an engineered N-terminus,

exhibited low levels of aggregates after only Protein A purification. These results demonstrate the advantage of carrying out N-terminal engineering of headless Fc-T2m constructs to reduce aggregation.

(103) FIGS. **3**K and **3**L compare the level of aggregation of two "headless" Fc-T22d35 constructs (examples of Type A in FIG. **2**). The Fc-T22d35 construct is without engineering of the hinge-region cysteine residues whereas the hIgG1 FcΔK(C)-T22d35 construct has been engineered by N-terminal truncation of the hinge to have only one cysteine in the hinge region. It can be seen that, similarly to Fc-T2m, the Fc-T22d35 construct contains a high level of aggregates after Protein A purification, with a doublet peak remaining as detected by UPLC-SEC even after further SEC purification. In contrast, hIgG1FcΔK(C)-T22d35, which has an engineered N-terminus, exhibited lower levels of aggregates after Protein A purification. Further SEC purification yielded a singlet peak as detected by UPLC-SEC, confirming the absence of aggregates. These results demonstrate the advantage of carrying out N-terminal engineering of headless Fc-T22d35 constructs to reduce aggregation.

Example 2: Neutralization and Binding of TGF-β by Fusion Constructs

(104) The TGF-β neutralization potencies of purified Fc-ectodomain fusion constructs were determined and compared to those of non-Fc-fused T22d35. It should be noted that non-Fc-fused T2m does not neutralize any of TGF-β1, -β2, or -β3 (De Crescenzo et al, 2001).

(105) TGF-β neutralization potencies for TGF-β1, -β2 and -β3 were determined for purified fusion constructs using two cell-based signaling assays: 1) the Mv1 Lu cell luciferase reporter assay with Mv1 Lu cells having a PAI-1-luciferase reporter (as described in (Zwaagstra et al, 2012)) and an A549 cell/IL-11 release assay adapted to the MSD (Meso Scale Discovery) platform.

(106) Mv1Lu cell luciferase reporter assay: Briefly, cells were seeded onto 96-well plates (20,000 cells/well) and then treated with T2m, T22d35, or a fusion construct+25 pM TGF-β at 37° C. for 16 h in DM EM, 1% FBS, 0.1% BSA. Cells were then lysed and luciferase activity was measured (Promega Corp.) using a Synergy 2 plate reader (BioTek Instruments Inc.).

(107) A549 cell IL-11 release assay: Human A549 lung cancer cells (ATCC-CCL-185, Cedarlane Burlington ON) were seeded in 96-well plates (5×103 cells/well). The following day 10 pM TGF-β in complete media in the absence or presence of a serial dilution of fusion protein was incubated for 30 min at RT prior to adding to the cells. After 21 h of incubation (37° C., 5% CO2, humidified atmosphere) conditioned medium was harvested and added to MSD Streptavidin Gold plates (Meso Scale Diagnostics, Gaithersburg, MD) that were coated with 2 μg/mL biotinylated mouse anti-human IL-11 antibody (MAB618, R&D Systems, Minneapolis, MN). After 18 h (4° C.) plates were washed with PBS containing 0.02% Tween 20, a 2 μg/mL SULFO-tagged goat anti-human IL-11 antibody (AF-218-NA, R&D Systems Minneapolis, MN) was added and plates were incubated for 1 h at RT. After a final wash, plates were read in a MESO QuickPlex SQ120 machine (Meso Scale Diagnostics, Gaithersburg, MD). IL-11 readouts were expressed as percent IL-11 release compared to control cells treated with TGF-β alone.

(108) In one set of experiments, using the Mv1Lu cell reporter assay, the neutralization potency of Cet-T2m (construct Type F in FIG. **2**), Cet-T22d35 (construct Type D in FIG. **2**) and T**22**d35 (non-Fc-fused) were compared. FIG. **4**A shows representative TGF-β1 (top panel), TGF-β3 (middle panel) and TGF-β2 (bottom panel) neutralization curves for Cet-T2m, Cet-T22d35 and T22d35 while Table 2 summarizes TGF-β1, -β2 and -β3 neutralization IC.sub.50 values. Unexpectedly, the TGF-β1 and TGF-β3 neutralization curves for the Cetuximab fusion constructs indicated extremely high potencies that lie in the picomolar range (determining a single IC.sub.50 value in these experiments is difficult due to the biphasic nature of the curves). The observed TGF-β1 IC.sub.50 value for Cet-T22d35 was in the picomolar range. In contrast, the TGF-β1 IC.sub.50 for non-Fc-fused T22d35 was approximately 1 nM. This illustrates that there is a large increase in T22d35 potency upon fusion to the C-terminus of the Fc region of Cetuximab (due to the biphasic nature of the Cet-T22d35 curve, the fold difference is difficult to determine in these experiments). The TGF-

β1 IC50 value for Cet-T2m was also subpicomolar (but less potent than Cet-T22d35), whereas unfused T2m is not able to detectably neutralize TGF-β1, even at concentrations above 500 nM (De Crescenzo et al, 2001). This demonstrates that, similar to T22d35, a very significant increase in T2m potency occurs upon fusion to the C-terminus of the Fc region of Cetuximab. Both Cet-T22d35 and Cet-T2m neutralized TGF-β2 (IC.sub.50˜nM range), whereas T22d35 and T2m (De Crescenzo et al, 2001) alone did not, even at a concentration of 800 nM, again showing the remarkable increase in neutralization potency that occurs upon fusion of T22d35 or T2m to the C-terminus of an Fc region.

(109) In another set of experiments using the Mv1 Lu cell reporter assay, similar extremely high potencies of TGF-β neutralization were observed for other C-terminus Fc fusion constructs (Table 2), e.g. for constructs in which T22d35 or T2m were fused with FSAs such as Herceptin (Her-T22d35), Avastin (Ava-T22d35) or Synagis (Syn-T22d35) [Type F and D constructs in FIG. **2**], or with the blood-brain barrier crossing Fc-fused FC5 V.sub.HH antibody (FCS-Fc-T22d35 and FC5-Fc-T2m) [Type E and G constructs in FIG. **2**]. In addition, fusion of T22d35 or T2m to the C-terminus of an IgG2-Fc region alone, i.e. an antibody with no Fab region present (Fc-T22d35 and Fc-T2m) [Type A and C constructs in FIG. **2**] resulted in fusion proteins with similarly high neutralization potencies. However, fusing T2m to the N-terminus of an IgG2-Fc (T2m-Fc) generates a fusion protein that does not neutralize TGF-β1 and -β3 in the picomolar range, but rather in the range of 1 nanomolar (0.3 to 15 nM in Table 2) and lacks any activity towards TGF-β2. These results are similar to those obtained with commercially available N-terminally IgG1 Fc-fused TGF-β Type II receptor ectodomain (T2m-Fc (R&D Systems)) (0.3 to 0.5 nM in Table 2). Together, these results thus demonstrate that fusion of TGF-β superfamily receptor ectodomains to the C-terminus of an Fc domain in the context of full-size antibodies, a V.sub.HH-Fc, or an Fc region alone, give rise to unexpectedly high TGF-β neutralization potencies.

(110) TABLE-US-00013 TABLE 2 TGF-β neutralization IC.sub.50 of fusion constructs. It should be noted that T2m does not neutralize any of TGF-β1, -β2, or -β3 (De Crescenzo et al, 2001). It should also be noted that the IC.sub.50 values in the table below are estimates due to the biphasic nature of the curves. TGF-β1 TGF-β3 TGF-β2 Av IC.sub.50 (nM) Av IC.sub.50 (nM) Av IC.sub.50 (nM) Cet-T22d35 0.000, 001 0.000, 002 13.6 Cet-T2m 0.000, 1 0.000, 0015 129.2 T22d35 1.232 0.033 No neutralization Her-T22d35 0.000, 13 0.000, 05 7.89 Ava-T22d35 0.000, 000, 72 0.000, 00038 8.60 Syn-T22d35 0.000, 042 0.000, 0001 35.2 FC5-Fc-T22d35 0.000, 001 0.000, 001 96.2 FC5-Fc-T2m 0.000, 017 0.000, 015 432.9 Fc-T22d35 0.001, 445 0.000, 026 108.4 T2m-Fc (R&D) 0.506 0.323 No neutralization T2m-Fc 14.523 0.276 No neutralization Fc-T2m 0.009, 923 0.000, 766 460.5

(111) In order to confirm the relative potencies of Fc-ectodomain constructs, a second cell-based assay, an A549 cell IL-11 release assay, was used. This IL-11 release assay acts as a model of TGF-β-mediated biological responses that contribute to both tumor metastasis and fibrosis. In the set of experiments shown in FIG. **4**B, the neutralization potencies of T22d35, Fc-T2m, Fc-T22d35, Cet-T22d35, Her-T22d35, Ava-T22d35, Syn-T22d35 and FCS-Fc-T22d35 were compared. It can be seen that the neutralization curves in this assay are not biphasic, making it less challenging than the Mv1Lu assay to determine and compare IC.sub.50 values. All of the constructs in which T22d35 was fused to the C-terminus of an Fc region exhibited IC.sub.50 values in the range of 5 pM, corroborating the extremely high potency observed for these constructs in the Mv1Lu assay. It can also be seen in FIG. **4**B that the IC.sub.50 value for non-Fc-fused T22d35 was ˜0.5 nM. This indicates that a ˜100-fold increase in potency occurs upon fusion of T22d35 to the C-terminus of an Fc region. The IC.sub.50 value for Fc-T2m was 0.05 nM. This indicates that constructs with two ectodomains in the C-terminal portion may be more potent than a construct with one ectodomain in the C-terminal portion (as was observed in the Mv1Lu assay, FIG. **4**A), however, it should be noted that Fc-T2m does not have an optimized N-terminus.

(112) An additional set of experiments in which the A549 cell IL-11 release assay was used to

compare TGF-β neutralization potencies is shown in FIG. **4**C. Here, the potencies of several "headless"-T2m constructs were assessed along with that of non-Fc-fused T22d35. The potency of T22d35 was determined to be ˜0.5 nM, consistent with the data shown in FIG. **4**B. This is illustrative of the robustness of the A549 cell IL-11 release assay. It can also be seen in FIG. **4**C that all of the "headless"-T2m constructs exhibited high potencies with IC.sub.50s in the range of 5 pM (3 to 17 pM). These values are in the same range as those of the T22d35-containing constructs shown in FIG. **4**B, and are 10-fold higher potency than that of the "headless"-T2m containing construct also shown in FIG. **4**B (Fc-T2m). Since all of the T2m-containing constructs in FIG. **4**C have engineered N-termini, whereas Fc-T2m does not, these results indicate that engineering of the cysteine residues of the hinge region of "headless" constructs is able to increase their potency by approximately 10-fold.

(113) We have also compared the potencies of constructs that include three TβRII structured ectodomains with constructs carrying two ectodomains using the A549 cell IL-11 release assay. We observed that the triple-repeat based constructs (SEQ ID NO:111 and SEQ ID NO:116) are potent in neutralizing TGF-β1 in this assay, and typically have improved IC50 values relative to the corresponding double-repeat based constructs (SEQ ID NO:100 and

(114) SEQ ID NO:106, respectively). All constructs involved in this comparative study had the same engineered N-terminus of the Fc portion, hIgG1FcΔK(C).

(115) Binding to TGF-β: Binding of T22d35, Cet-T22d35, and Cet-T2m to TGF-β2 was measured using a competitive SPR binding experiment. In this assay, the molecule of interest was first allowed to bind to a fixed amount of TGF-β in solution. A 2-fold dilution series was prepared in PBS-0.05% Tween, starting with 1000 nM T22d35 trap or 20 nM Cet-T22d35 or Cet-T2m. Each diluted sample was pre-incubated with 1 nM TGF-β2 for 30 min at room temperature to allow binding. The mixture was then flowed over immobilized, pan-specific anti-TGF-β antibody 1D11 (2000 RU 1D11) in order to quantify the amount of ligand left unbound (TβRII ectodomain and 1D11 bind to a similar epitope on TGF-β) using a Biacore T200 instrument. The TGF-β2 binding EC.sub.50 values were determined by plotting the percent free TGF-β versus the protein concentration of the molecule of interest. Binding curves and EC.sub.50 values are shown in FIG. **4**D and Table 3. In the case of TGF-β2 binding, a 100-fold increase in binding was observed between Cet-T22d35 and unfused T22d35 (EC.sub.50˜1 nM versus>100 nM, respectively), indicating that C-terminal fusion of the T22d35 trap to antibody provides a gain in affinity for the TGF-β2 isoform. This correlates with the ability of Cet-T22d35 to neutralize TGF-β2 in the 10 nM range, and the inability of unfused T22d35 to neutralize TGF-β2, as observed in the Mv1 Lu-Luc cell reporter assay.

(116) TABLE-US-00014 TABLE 3 EC.sub.50 of Cetuximab-trap binding to TGF-β in solution. EC.sub.50 is the Effective Concentration at which 50% of TGF-β is bound and is given in nM. Note: T2m alone shows an IC50 of greater than 1000 nM (Zwaagstra et al, 2012), and thus is not considered neutralizing. Trap variant EC.sub.50 for TGF-β2 T22d35 >100 Cetuximab-T2m 1.17 Cetuximab-T22d35 0.50

Example 3A: Validation of Antibody Binding

(117) The ability of an antibody alone or in a fusion construct of Example 1 to bind to its intended target antigen was evaluated using surface plasmon resonance (SPR).

(118) Direct binding of Cet-T22d35 or Cetuximab to the EGF receptor extracellular domain (EGFR-ED) was quantified by SPR using a BIAcore T200 instrument, performed in the standard manner. Briefly, Cet-T22d35 or Cetuximab alone were captured on the SPR CM5 chip using immobilized anti-human IgG Fc-specific antibody (2000 RU). Variable concentrations of EGFR-ED in PBS-0.05% Tween were then flowed over the capture surface at 100 μl/min and 25° C. The resulting sensorgrams (data not shown) were analyzed using the Biacore T200 evaluation software. The K.sub.D values of Cet-T22d35 and Cetuximab were very similar (847 and 708 pM, respectively), indicating that fusion of T22d35 to the Fc portion of Cetuximab does not appreciably

alter binding to EGFR-ED, compared to the non-fused FSA. Similar SPR methods and analyses were performed for other antibody-trap fusion examples, compared with their corresponding target antigens (see Table 4).

(119) From Table 4 it is evident for each exemplified construct that fusion of a TGF-β superfamily receptor ectodomain(s) to the C-terminus of the Fc region of an antibody did not significantly alter antigen-binding affinities and K.sub.D values of the antibody. This indicates that the ectodomain(s) can be readily fused to any antibody without compromising the ability the antibody to bind its target antigen.

(120) TABLE-US-00015 TABLE 4 SPR determination of antigen-binding affinity of T22d35 fusion constructs or antibodies alone. NOTE: FC-5-Fc, FC5-Fc-T2m, and FC-5-Fc-T22d35 binding affinity was assessed via the transwell functional assay (see Example 3C). Antigen K.sub.a (1/Ms) K.sub.d (1/s) K.sub.D (M) Cetuximab- EGFR 1.22 × 10.sup.6 8.65 × 10.sup.−4 7.08 × 10.sup.−10 T22d35 Cetuximab EGFR 1.03 × 10.sup.6 8.45 × 10.sup.−4 8.47 × 10.sup.−10 Her-T22d35 Her2 8.30 × 10.sup.4 5.30 × 10.sup.−5 6.37 × 10.sup.−10 Herceptin Her2 6.88 × 10.sup.4 5.03 × 10.sup.−5 7.33 × 10.sup.−10 Syn-T22d35 RSV-F 3.55 × 10.sup.4 1.42 × 10.sup.−3 4.10 × 10.sup.−9 Synagis RSV-F 2.57 × 10.sup.4 1.68 × 10.sup.−3 6.60 × 10.sup.−9

Example 3B: Validation of Cetuximab Function

(121) The ability of Cetuximab to maintain its therapeutic function (i.e. inhibition of EGF-induced EGFR phosphorylation and signaling) when fused to either T2m or T22d35 (Example 1) was evaluated.

(122) Phosphorylation of EGFR: The ability of Cetuximab-comprising constructs to inhibit EGF-induced phosphorylation of EGFR in human lung cancer A549 cells was evaluated. A549 cells were seeded in 24-well plates (100,000 cells/well) and either mock treated (−) or pre-treated with Cetuximab, Cet-T2m, or Cet-T22d35 (all at 10, 1 or 0.1 nM) or T22d35 (10 nM) at 37° C. for 3 h, then treated with 50 ng/mL EGF at 37° C. for 10 min. Whole cell lysates were prepared and resolved by SDS-PAGE, western blotted and probed with anti-phosphoTyrosine antibody (Clone 4G10, Millipore 05-321). As shown in FIG. **5**A, Cetuximab and Cet-T2m and Cet-T22d35 inhibited EGFR phosphorylation to similar extents, whereas T22d35 had no effect, relative to the +EGF control. These results thus confirm that the TGF-β superfamily receptor ectodomain moieties in Cetuximab-T2m and Cetuximab-T22d35 fusion proteins do not interfere with the function of the Cetuximab antibody (i.e. inhibition of EGF induced EGFR signaling).

(123) Inhibition of EGFR signaling: Inhibition of autocrine EGFR signaling results in varying degrees of cytotoxicity in EGFR-expressing cells treated with Cetuximab. Cytotoxicity of Cet-T22d35 was compared to Cetuximab and T22d35 in human breast cancer cells (MDA-MB-468) and immortalized keratinocyte cells (HaCaT). These cells exhibited significant Cetuximab cytotoxicity, due to their intrinsic dependence on the EGF signaling pathway for basal growth.

(124) The cells were seeded onto 96-well plates (MDA-MB-468, 2300 cells/well; HaCaT, 1500 cells/well) and then treated with different doses of inhibitor at 37° C. for 5 days. Cell viability was measured using sulforhodamine reagent to determine the percentage of viable cells relative to mock-treated controls. Results are shown in FIG. **5**B and Table 5. The IC.sub.50 values for Cet-T22d35 and Cetuximab were similar in both cell lines (0.2-1.4 nM range), while T22d35 resulted in no cytotoxicity. These results further confirm that the TGF-β superfamily receptor ectodomain moiety in Cetuximab-T22d35 does not interfere with the function of the Cetuximab antibody.

(125) TABLE-US-00016 TABLE 5 Cetuximab-T22d35 cytotoxicity in MDA-MB-468 and HaCat cells. The cytotoxic potency IC.sub.50 values are given in nM. MDA-MB-468 IC.sub.50 HaCaT IC.sub.50 Cetuximab 0.50 0.33 Cetuximab-T22d35 1.42 0.22 T22d35 0 0

Example 3C: Validation of FC5 Function

(126) The ability of FC5 V.sub.HH to maintain its function (i.e. transmigrate the blood-brain barrier) when fused with the T2m or T22d35 moieties (see description in Example 2 and activities in Table 2) was evaluated.

(127) Briefly, SV40-immortalized Adult Rat Brain Endothelial Cells (Sv-ARBEC) were used to generate an in vitro blood-brain barrier (BBB) model as described (Garberg et al, 2005; Haqqani et al, 2013). Sv-ARBEC cells (80,000 cells/membrane) were seeded on 0.1 mg/mL rat tail collagen type I-coated tissue culture inserts (pore size-1 μm; surface area 0.9 cm.sup.2, Falcon) in 1 ml of growth medium. The bottom chamber of the insert assembly contained 2 ml of growth medium supplemented with the immortalized neonatal rat astrocytes-conditioned medium in a 1:1 (v/v) ratio. Equimolar amounts (5.6 μM) of positive (FC5-Fc) control; negative controls (A20.1); and T22d35, T2m, FC5-Fc-T22d35 or FC5-Fc-T2m were tested for their ability to cross the Sv-ARBEC cell monolayer. Following exposure of equimolar amounts of the proteins to the luminal side of the BBB, samples were taken after 15, 30, and 60 min from the abluminal side. The protein content of each sample was then quantified by mass spectrometry (multiple reaction monitoring-isotype labeled internal standards; MRM-ILIS) as described by (Haqqani et al, 2013) (see method description below).

(128) Quantified values can be directly plotted or the P.sub.app (apparent permeability coefficient) values can be determined using the following formula

(129) $\text{Papp} = \frac{dQr / dt}{A \times C_0}$

(130) The P.sub.app value is commonly used to determine the specific permeability of a molecule, and is a measure of transport across the brain endothelial monolayer. [Qr/dt=cumulative amount in the receiver (bottom) compartment versus time; A=area of the cell monolayer; C0=initial concentration of the dosing solution (top chamber)].

(131) FIG. **6** shows the results of the experiment. The P.sub.app value of FC5-Fc-T22d35 was similar to the control FC5-Fc, indicating it was transported efficiently and that the fused T22d35 did not interfere with transport. The P.sub.app value for FC5-Fc-T2m was approximately 50% less, compared to FC5-Fc-T22d35 and FC5-Fc, indicating somewhat reduced permeability. Nevertheless, the level of transport of FC5-Fc-T2m was about 4-fold greater than the negative controls (T2m, T22d35, and antibody A20.1).

Example 4: Inhibition of Epithelial to Mesenchymal Transition

(132) Treatment of A549 cells with EGF plus TGF-β results in a strong epithelial to mesenchymal transition (EMT). The EMT is phenotypically characterized by changes in cell morphology (tight cellular junctions with “cobble-stone” appearance converts to elongated cells, see FIG. **7**A) and changes in the adherin junction proteins E-cadherin and N-cadherin. The ability of the fusion constructs to block EMT was assessed in A549 cells by western blotting (E-cadherin) and flow cytometry (E-cadherin and N-cadherin).

(133) Briefly, for the western blot analysis, A549 cells were seeded in 24-well plates (8000 cells/well) and then treated with EGF (50 ng/mL)+TGF-β1 (50 pM) at 37° C. for 3 days in the presence of Cet-T22d35, Cetuximab, or T22d35 (0, 0.05, 0.5, 5, 50, or 500 nM). Whole cell lysates were prepared and resolved by SDS-PAGE. The proteins were transferred to nitrocellulose and then probed with an E-cadherin antibody (BD Transduction laboratories Biosciences) (FIG. **7**B). The E-Cadherin positive bands in the Western blot were quantified by densitometer detection and ImageJ analysis (FIG. **7**C). EGF+TGF-β treatment resulted in an EMT, as indicated by the disappearance of E-cadherin (compare non-treated and EGF+TGF-β lanes in the absence of inhibitors). Cet-T22d35 blocked the EMT (E-cadherin disappearance) in a dose-dependent manner whereas 500 nM Cetuximab or T22d35 treatments only modestly blocked the EMT (E-cadherin levels ˜20-25% of the non-treated control level).

(134) The ability of Cet-T22d35, Cetuximab and T22d35 to block the EGF+TGF-β EMT response was further examined by flow cytometry using A549 cells treated with Cet-T22d35 or Cetuximab, (all at 50, 5, 0.5 nM) or the Cetuximab+T22d35 combination (50 nM+100 nM, 5 nM+10 nM, or 0.5 nM+1 nM, respectively) and evaluating the EMT associated changes in E-cadherin and N-cadherin cell surface expression levels (FIG. **7**D). In this experiment the molar amounts of the molecules of interest used in ‘T22d35 alone’ and ‘Cetuximab+T22d35’ groups were two-fold

higher than for Cet-T22d35 in order to correspond with a 2:1 trap/antibody ratio in the Cet-T22d35 fusion molecule. A549 cells were seeded in 6-well plates (30,000 cells/well) and pre-treated with the inhibitors at 37° C. for 1 h, followed by added treatment with EGF (10 ng/mL)+TGF-β (10 pM) and incubation at 37° C. for 3 days. Cells were then dissociated from the plate using 1 mL Dissociation Buffer (Sigma)/well, centrifuged at 2000 rpm for 2 min and re-suspended in 100 μl RPMI-5 media at 4° C. AlexaFluor488-E-cadherin (Santa Cruz, SC21791) and AlexaFluor647-N-cadherin (BD Biosciences, 563434) antibodies (1/25 v/v dilutions) were added and samples were incubated at 4° C. for 1 h. Cells were then centrifuged, washed once in RPMI-5, and re-suspended in 400 μl RPMI-5 containing 15 μg/mL propidium iodide (Life Technologies) at 4° C. Mean fluorescent intensities (MFI) were measured by flow cytometry (BD LS RII flow cytometer, BD Biosciences) to quantify E-cadherin and N-cadherin levels. The results show that Cet-T22d35 was more effective in preventing down-regulation of E-cadherin (FIG. **7**D, top panel) and up-regulation of N-cadherin (FIG. **7**D, bottom panel), as a measure of blocking EMT, compared to Cetuximab, T22d34 or the Cetuximab+T22d35 combination at each respective dose, and is most notable at the lowest dose used (0.5 nM).

Example 5: Pharmacokinetic (PK) Studies on Constructs with and without a Lysine Residue at the Fusion Site Between the C-Terminus of the Fc Region and the N-Terminus of the Ectodomain

(135) PK studies were carried out in normal, healthy mice to determine whether fusion of T22d35 to an antibody increased its half-life in vivo, and whether removal of a lysine at the fusion site within constructs reduced the amount of cleavage occurring in vivo.

(136) Results from Cet-T22d35 (construct Type D in FIG. **2**—containing a lysine at the fusion site): A single bolus of Cet-T22d35 protein (10 mgs/Kg) formulated in DPBS was intravenously injected (IV) into the tail vein of normal Balb/c mice and serum samples were collected from the submandibular vein at selected time points (0.5, 1, 2, 4, 8, 14, 24, 48, 96 h). Blood samples were centrifuged at 2000 g at 4° C. for 10 min and the serum supernatant was removed and stored frozen at −80° C., prior to analyses. The samples were thawed at 4° C. and analyzed via mass spectrometry (multiple reaction monitoring-isotype labeled internal standards; MRM-LIS) in order to measure the levels of both the Cetuximab and T22d35 trap moieties. Briefly, 20 μl of sample was thawed and treated with mild detergents (0.1% RapiGest SF, Waters; 5.5 mM TCEP) at 95° C. for 10 min. The sample was cooled to room temperature and Iodoacetamide (IAA) in 50 mM Ammonium Bicarbonate was added to a final concentration of 10 mM IAA, followed by incubation for 40 min in the dark. DTT (10 mM final) was then added and the sample was incubated at room temperature for 15 min, followed by trypsin digestion (Sigma, 0.8 mg/mL final) at 37° C. for 18 h. A mixture of 5 μM each of isotope-labeled trap and cetuximab peptides (formulated in 30% acetonitrile, 0.1% formic acid) were added to final concentrations of 1 μM, as internal standards for quantification. The isotope-labeled peptides were 13C/15N-(H2N-LPYHDFILEDAASPK-OH; SEQ ID NO:134) and 13C/15N-(H2N-ALPAPIEK-OH; SEQ ID NO:135) for T22d35 and Cetuximab, respectively (NewEngland Peptide). Trifluoroacetic acid was then added (0.5% final), followed by incubation at 37° C. for 30 min. The samples were centrifuged at 13000 rpm for 20 min and the supernatant was used analyzed via MRM-ILIS using an Agilent 1260 HPLC system coupled with Agilent QQQ6410B at 55° C. The PK profiles seen in FIG. **8**A show that the levels of the Cetuximab and T22d35 moieties diverge at early time points (<10 h) after injection, indicating different kinetics and suggesting possible cleavage of T22d35 from the Cet-T22d35 protein in vivo. Nevertheless, analysis of these curves using a two compartmental model (Phoenix WinNonlin SoftwareVersion 6.3) indicated that the average terminal half-life (T½β) of the T22d35 component was 45.8 h. This represents a 7.6-fold increase compared to the previously determined half-life for unfused T22d35 (T½β˜6 h). As well, the PK profile shows that the Cetuximab moiety was maintained in the blood, with a T½β=262.5 h, indicating that the Cetuximab moiety has a long circulating half-life.

(137) Results from several constructs with lysine deleted at the fusion site:

(138) The same methods that were used in the PK study of Cet-T22d35 (FIG. **8**A) were applied to assess the PK of CetΔK-T2m (Type F in FIG. **2**) as well as several "headless" T2m constructs (Type C in FIG. **2**; hIgG1FcΔK(SS)-T2m, hIgG1FcΔK(ΔC)-T2m, and hIgG2FcΔK(SS)-T2m), all of which have the lysine deleted at the fusion site. The data shown in FIG. **8**B indicate that no detectable cleavage of these constructs is occurring in vivo since the levels of the Fc moieties (closed symbols) and ectodomain moieties (open symbols) do not diverge over time. Additionally, all of the constructs exhibit similar long circulating half-lives with T½ βs of approximately 100 h. This represents an improvement to the half-life of the ectodomain moiety of the construct which has a lysine at the fusion site, presented in FIG. **8**A (45.8 h).

Example 6: Efficacy Studies Comparing the Effect of "Headless" Fc-T2m and FSA-T2M Constructs on Tumor Growth (A) and T-Cell Function (B and C) in an Immune-Competent Syngeneic Triple Negative Breast Cancer (4T1) Model

(139) A FSA-T2m construct (Cet-T2m—Type F in FIG. **2**) and three headless constructs, all with engineered N-termini, (hIgG1FcΔK(CC)-T2m, hIgG1 FcΔK(C)-T2m, and hIgG2FcΔK(CC)-T2m —Type C in FIG. **2**) were evaluated for their ability to inhibit tumor growth and to affect T-cell function in a syngeneic tumor model derived from 4T1 triple negative breast cancer cells. The results presented in FIG. **9** show the effect on tumor growth (A) and T-cell function (B, C). The effects of these Fc-fused ectodomain constructs were compared to those of a pan-specific neutralizing anti-TGF-β antibody, 1 D11 and a non-Fc-fused ectodomain construct, T22d35.

(140) The protocols used for these syngeneic mouse model studies are described in (Zheng et al, 2013). Briefly, female BALB/c (H-2Kd) mice 6 weeks of age were purchased from The Jackson Laboratories and kept in filter-top cages. The 4T1 breast cancer cells and B16F10 cells were purchased from American type culture collection and cultured in RPMI-1640 supplemented with 2 mmol/L L-glutamine, 100 U/mL penicillin, 100 microgm/L streptomycin, 50 micromol/L 2-mercaptoethanol, and 10% fetal calf serum. Mice were inoculated subcutaneously in the left flank with 100 uL sterile saline containing 5×10.sup.4 4T1 cells. Tumors were grown to ˜100 mm.sup.3, as measured by caliper, then mice were randomized and divided into the six treatment groups (8 animals/group) (Day 0). Treatments commenced on Day 1 and continued for 15 days with the animals being dosed at 5 mg/kg twice per week such that they received a total of 4 doses. Tumor growth was monitored by caliper measurements 3× per week. Animals were euthanized by exsanguination under anaesthesia on Day 15; T cells were isolated from draining lymph nodes and assessed for their capacity to kill mouse 4T1 and B16F10 tumour cells ex vivo. The capacity of T cells from mice treated with or without test agents to lyse target 4T1 tumor cells was measured using a CytoTox 96 nonradioactive cytotoxicity assay kit (Promega) according to the manufacturer's instructions. Briefly, naïve target 4T1 cells or melanoma cell line B16F10 cells are plated and incubated for 4 hr with CD8+ effector T cells isolated from 4T1 tumor-bearing mice using CD8 magnetic MicroBeads (BD Bioscience). The isolated CD8+ cells are confirmed, by flow cytometry, to be over 85% CD8+. A range of ratios of effectors to target cells is tested (100:1, 50:1, 25:1). Lactate dehydrogenase (LDH) release in response to effector T cells is measured in the buffer bathing target cells. Target cells incubated in the absence of effector cells are used as a comparator to control for spontaneous LDH release. Released LDH in culture supernatants is detected after a 30-min incubation using a coupled enzymatic assay. The intensity of the color formed is proportional to the number of lysed cells. Cytotoxic activity of CTL is calculated using the following formula:

Cytotoxic activity %=[(absorbance)−(spontaneous effector cell LDH release)−(spontaneous target cell LDH release)]/[(maximal LDH release)−(spontaneous target cell LDH release)]×100

(141) The results presented in FIG. **9**A show the effect of the Fc-fused ectodomain constructs listed above on 4T1 tumor growth (the pan-specific neutralizing anti-TGF-β antibody, 1D11, and a non-Fc-fused ectodomain construct, T22d35, being tested as comparators). As can be seen in FIG. **9**A, all of the Fc-fused ectodomain constructs reduced tumor growth relative to the saline treatment

when tested for significance by t-test. The 1D11 and T22d35 comparator treatments were observed to be less effective relative to the Fc-fused ectodomain treatments, and not significantly different from the saline control. These results demonstrate that constructs with an ectodomain fused to the C-terminus of an Fc region have significant anti-tumor potency, with the efficacy being higher than the 1D11 and T22d35 comparators. There was no significant difference between the FSA-T2m construct (Cet-T2m) and the constructs that have no Fab, i.e. are “headless” (hIgG1 FcΔK(CC)-T2m, hIgG1 FcΔK(C)-T2m, and hIgG2FcΔK(CC)-T2m) with respect to their effect on tumor growth. The high anti-tumor potency of these Fc-ectodomain fusions relative to comparators likely results from high potency neutralization of TGF-β combined with a favourable circulating half-life.

(142) To investigate whether these Fc-ectodomain fusions exhibit an immuno-modulatory effect in vivo on cytotoxic T lymphocyte cells (CTLs) present in tumor draining lymph nodes, lymph nodes were removed from mice treated with or without test agents; T-cells were then isolated and tested for their capacity to lyse target 4T1 tumor cells (and B16F10 cells as a test of tumor specificity) using the methods described above.

(143) As shown in FIG. **9**B, treatment of the animals with Fc-ectodomain fusions significantly increased the ability of draining lymph node T-cells to lyse target 4T1 tumor cells ex vivo. It can be seen that this immuno-stimulatory effect is specific to 4T1 cells since the T-cells were not able to effectively lyse B16F10 melanoma cells (FIG. **9**C; maximal lysis of ˜15% for B16F10 cells and ˜80% for 4T1 cells). When administered at the same 5 mg/kg dose as the Fc-ectodomain fusions, the non-Fc-fused comparator molecule, T22d35, had no effect above saline. This is consistent with its lack of effect on tumor volume. Although the 1D11 antibody had no statistically significant effect on tumor volume (FIG. **9**A), it did increase the ability of lymph node T-cells to lyse 4T1 tumor cells.

(144) Interestingly, with respect to the Fc-ectodomain fusions of this invention, the most potent constructs were the hIgG1FcΔK(CC)-T2m and hIgG2FcΔK(CC)-T2m constructs; both of these constructs containing two cysteines in the engineered hinge region. These constructs were more potent than the construct with one cysteine in the engineered hinge region, hIgG1FcΔK(C)-T2m, as well as being more potent than the full-size antibody-T2m construct, Cet-T2m. The difference in potency between constructs with one versus two hinge region cysteines may result from the construct with one cysteine having a lower relative stability. The lower potency of the full size antibody construct relative to the headless constructs with two hinge region cysteines may result from a difference in molecular weight, with the smaller constructs being able to penetrate the tumor microenvironment more effectively.

Example 7: In Vitro and In Vivo Studies Illustrating Enhanced Bone Localization of Constructs Containing a Deca-Aspartate Motif for Bone Targeting at the N-Terminus of the Fc Region

(145) In vivo studies were carried out to investigate whether the addition of a 10 amino acid long poly-aspartate bone-localization motif (D10) to the N-terminus of the Fc region of constructs will promote their localization to bone. Optical imaging of D10-hIgG1 Fc-T2m fusions: Upon arrival, male Balb/c mice were housed 3 mice/cage. On the day of the experiment, animals were shaved dorsal and ventral and treated with the hair removal cream, NAIR®. Mice were injected with a single intravenous bolus of 10 mg/kg of two CF770 labeled constructs with a deca-aspartate motif for bone targeting (D10) at their N-termini (D10-hIgG1FcΔK(CC)-T2m, D10-GSL-hIgG1FcΔK(CC)-T2m) or with a control construct without the D10 motif (hIgG1FcΔK(CC)-T2m) and whole body bio-distribution followed using both in vivo and ex-vivo near infrared imaging. Imaging was conducted with a small-animal time-domain eXplore Optix pre-clinical imager MX3 (Advanced Research Technologies, ART) at various time points (prescan, 5 mins, 3 hr, 6 hr, 24 h, 48 h, 72 h, 96 h and 120 h).

(146) The small animal time-domain eXplore Optix preclinical imagers consists of a 785-nm pulsed laser diode with a repetition frequency of 80 MHz and a time resolution of 12.5 ps light pulse was used for excitation. The fluorescence emission beyond 813 nm was collected by a highly

sensitive time-correlated single photon counting system and detected through a fast photomultiplier tube. The data were recorded as temporal point-spread functions (TPSF) and the images were presented as fluorescence intensity maps using ART Optix Optiview analysis software 3.02.
(147) For in vivo optical imaging, mice were first anesthetized using isofluorane (1.5-2%), positioned on the animal stage within a chamber which allows for gaseous anesthesia and maintenance of animal temperature at 36° C. The scanning of the mouse at each time point lasted up to 20 mins using a 2.5 mm step size and the mouse is placed back in its home cage between imaging time points.
(148) At the end of the imaging protocol (120 hrs) animals were sacrificed by intracardiac perfusion using heparnized saline with deep anesthesia. The organs (brain, heart, lungs, liver, kidney, spleen and right and left leg bones) were imaged ex-vivo using a 1.0 mm step size using the eXplore Optix pre-clincal imager MX3.
(149) Data analysis was done using eXplore Optix Optiview analysis software 3.02 (Advanced Research Technologies, Montreal, QC) to estimate the fluorescence total and average fluorescence intensity in region of interest containing the ex-vivo organs.
(150) The results shown in FIGS. **10**A and B demonstrate that the fusion of the deca-aspartate D10 motif on the N-termini of the fusion constructs had no impact on their ability to neutralize TGF-β, i.e. the IC.sub.50 of the construct lacking the D10 motif (hIgG1FcΔK(CC)-T2m) was 3 nM, which is the same as the value determined in FIG. **4**C, while the IC.sub.50s of the D10 containing constructs, D10-hIgG1FcΔK(CC)-T2m and D10-GSL-hIgG1FcΔK(CC)-T2m, were very similar at 4-5 nM. The results in FIGS. **10**A and B also indicate that labeling with the CF770 dye reduced the ability of the constructs to neutralize TGF-β by approximately 4-fold. Since dye conjugation occurs at lysine residues, and since it is know that lysines are at the binding interface between the Type II ectodomain and TGF-β, it is not entirely surprising that labeling reduced neutralization potency. In any case, since this is a comparative study of differences in in vivo localization promoted by the D10 peptide, it was felt that partially active constructs would be informative.
(151) The results shown in FIGS. **10**C and D demonstrate that the addition of the D10 peptide to the N-termini of the constructs greatly enhanced bone localization. Images taken 120 h post-injection of the CF770 labeled fusions show a clear accumulation of the D10-fusions in the vertebrae. Further ex vivo imaging of the brain, heart, lungs, liver, kidneys, spleen, and the left and right legs 120 h post-injection confirmed the specific accumulation of the D10-fusions in the bones. The fluorescent signals observed in the kidneys and liver were similar for all fusions indicating that accumulation in these organs was not affected by the presence of the D10 sequence. These results indicate that the TGF-β neutralization activity of constructs may be increased within bone through the addition of the D10 peptide. This could result in more favourable dosing levels and schedules for the treatment of bone-related diseases, such as osteogenesis imperfecta, relative to that required for a similar construct without the D10 motif.
(152) The embodiments and examples described herein are illustrative and are not meant to limit the scope of the invention as claimed. Variations of the foregoing embodiments, including alternatives, modifications and equivalents, are intended by the inventors to be encompassed by the claims. Furthermore, the discussed combination of features might not be necessary for the inventive solution.
(153) TABLE-US-00017 LISTING OF SEQUENCES SEQ ID NO: Sequence Description 1 APELLGGPSVFLFPPKPKDTLMISR Human IgG1 Fc TPEVTCVVVDVSHEDPEVKFNWYVD region GVEVHNAKTKPREEQYNSTYRVVSV LTVLHQDWLNGKEYKCKVSNKALPA PIEKTISKAKGQPREPQVYTLPPSR DELTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFF LYSKLTVDKSRWQQGNVFSCSVKHE ALHNHYTQKSLSLSPGK 2 APPVAGPSVFLFPPKPKDTLMISRT Human IgG2 Fc PEVTCVVVDVSHEDPEVQFNWYVDG region VEVHNAKTKPREEQFNSTFRVVSVL

TVVHQDWLNGKEYKCKVSNKGLPAP IEKTISKTKGQPREPQVYTLPPSRE
EKTKNQVSLTCLVKGFYPSDISVEW ESNGQPENNYKTTPPMLDSDGSFFL
YSKLTVDKSRWQQGNVFSCSVKHEA LHNHYTQKSLSLSPGK 3
APELLGGPSVFLFPPKPKDTLMISR Human IgG3 Fc
TPEVTCVVVDVSHEDPEVQFKWYVD region GVEVHNAKTKPREEQYNSTFRVVSV
LTVLHQDWLNGKEYKCKVSNKALPA PIEKTISKTKGQPREPQVYTLPPSR
EEMTKNQVSLTCLVKGFYPSDIAVE WESSGQPENNYNTTPPMLDSDGSFF
LYSKLTVDKSRWQQGNIFSCSVMHE ALHNRFTQKSLSLSPGK 4
APEFLGGPSVFLFPPKPKDTLMISR Human IgG4 Fc
TPEVTCVVVDVSQEDPEVQFNWYVD region GVEVHNAKTKPREEQFNSTYRVVSV
LTVLHQDWLNGKEYKCKVSNKGLPS SIEKTISKAKGQPREPQVYTLPPSQ
EEMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFF
LYSRLTVDKSRWQEGNVFSCSVKHE ALHNHYTQKSLSLSLGK 5 EPKSCDKTHTCPPCP
Human IgG1 hinge region 6 ERKCCVECPPCP Human IgG2 hinge region 7
ELKTPLCDTTHTCPRCPEPKSCDTP Human IgG3 PPCPRCPEPKSCDTPPPCPRCPEPK
hinge region SCDTPPPCPRCP 8 ESKYGPPCPSCP Human IgG4 hinge region 9
APELLGGPSVFLFPPKPKDTLMISR hIgG1FcΔK-AC TPEVTCVVVDVSHEDPEVKFNWYVD
Fc variant GVEVHNAKTKPREEQYNSTYRVVSV LTVLHQDWLNGKEYKCKVSNKALPA
PIEKTISKAKGQPREPQVYTLPPSR DELTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYKTTPPVLDSDGSFF LYSKLTVDKSRWQQGNVFSCSVKHE
ALHNHYTQKSLSLSPG 10 PPCPAPELLGGPSVFLFPPKPKDTL hIgG1FcΔK-C Fc
MISRTPEVTCVVVDVSHEDPEVKFN variant WYVDGVEVHNAKTKPREEQYNSTYR
VVSVLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKAKGQPREPQVYTL
PPSRDELTKNQVSLTCLVKGFYPSD IAVEWESNGQPENNYKTTPPVLDSD
GSFFLYSKLTVDKSRWQQGNVFSCS VKHEALHNHYTQKSLSLSPG 11
DKTHTCPPCPAPELLGGPSVFLFPP hIgG1FcΔK-CC KPKDTLMISRTPEVTCVVVDVSHED
Fc variant PEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYK
CKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSRDELTKNQVSLTCLVK
GFYPSDIAVEWESNGQPENNYKTTP PVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMHEALHNHYTQKSLSLSP G 12 EPKSSDKTHTCPPCPAPELLGGPSV
hIgG1FcΔK-S Fc FLFPPKPKDTLMISRTPEVTCVVVD variant
VSHEDPEVKFNWYVDGVEVHNAKTK PREEQYNSTYRVVSVLTVLHQDWLN
GKEYKCKVSNKALPAPIEKTISKAK GQPREPQVYTLPPSRDELTKNQVSL
TCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKS
RWQQGNVFSCSVMHEALHNHYTQKS LSLSPG 13 EPKSSDKTHTCPPCPAPELLGGPSV
hIgG1ΔK-SS Fc FLFPPKPKDTLMISRTPEVTCVVVD variant
VSHEDPEVKFNWYVDGVEVHNAKTK PREEQYNSTYRVVSVLTVLHQDWLN
GKEYKCKVSNKALPAPIEKTISKAK GQPREPQVYTLPPSRDELTKNQVSL
TCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKS
RWQQGNVFSCSVMHEALHNHYTQKS LSLSPG 14 EPKSSDKTHTSPPSPAPELLCGPSV
hIgG1FcΔK-SSS FLFPPKPKDTLMISRTPEVTCVVVD Fc variant
VSHEDPEVKFNWYVDCVEVHNAKTK PREEQYNSTYRVVSVLTVLHQDWLN
GKEYKCKVSNKALPAPIEKTISKAK GQPREPQVYTLPPSRDELTKNQVSL
TCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKS
RWQQCNVFSCSVMHEALHNHYTQKS LSLSPC 15 APPVAGPSVFLFPPKPKDTLMISRT
hIgG2FcΔK-AC PEVTCVVVDVSHEDPEVQFNWYVDG Fc variant
VEVHNAKTKPREEQFNSTFRVVSVL TVVHQDWLNGKEYKCKVSNKGLPAP
IEKTISKTKGQPREPQVYTLPPSRE EMTKNQVSLTCLVKGFYPSDISVEW
ESNGQPENNYKTTPPMLDSDGSFFL YSKLTVDKSRWQQGNVFSCSVMHEA
LHNHYTQKSLSLSPG 16 PPCPAPPVACPSVFLFPPKPKDTLM hIgG2FcΔK-C Fc

ISRTPEVTCVVVDVSHEDPEVQFNW variant YVDGVEVHNAKTKPREEQFNSTFRV
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PSREEMTKNQVSLTCLVKGFYPSDI SVEWESNGQPENNYKTTPPMLDSDG
SFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSPG 17
VECPPCPAPPVAGPSVFLFPPKPKD hIgG2FcΔK-CC TLMISRTPEVTCVVVDVSHEDPEVQ
Fc variant FNWYVDGVEVHNAKTKPREEQFNST FRVVSVLTVVHQDWLNGKEYKCKVS
NKGLPAPIEKTISKTKGQPREPQVY TLPPSREEMTKNQVSLTCLVKGFYP
SDISVEWESNGQPENNYKTTPPMLD SDGSFFLYSKLTVDKSRWQQGNVFS
CSVMHEALHNHYTQKSLSLSPG 18 ERKCCVECPPCPAPPVAGPSVFLFP hIgG2Fc-CCCC
PKPKDTLMISRTPEVTCVVVDVSHE Fc variant DPEVQFNWYVDGVEVHNAKTKPREE
QFNSTFRVVSVLTVVHQDWLNGKEY KCKVSNKGLPAPIEKTISKTKGQPR
EPQVYTLPPSREEKTKNQVSLTCLV KGFYPSDISVEWESNGQPENNYKTT
PPMLDSDGSFFLYSKLTVDKSRWQQ GNVFSCSVKHEALHNHYTQKSLSLS PGK 19
ERKSSVECPPCPAPPVAGPSVFLFP hIgG2FcΔK-SS PKPKDTLMISRTPEVTCVVVDVSHE
Fc variant DPEVQFNWYVDGVEVHNAKTKPREE QFNSTFRVVSVLTVVHQDWLNGKEY
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KGFYPSDISVEWESNGQPENNYKTT PPMLDSDGSFFLYSKLTVDKSRWQQ
GNVFSCSVNHEALHNHYTQKSLSLS PG 20 ERKSSVESPPCPAPPVAGPSVFLFP
hIgG2FcΔK-SSS PKPKDTLMISRTPEVTCVVVDVSHE Fc variant
DPEVQFNWYVDGVEVHNAKTKPREE QFNSTFRVVSVLTVVHQDWLNGKEY
KCKVSNKGLPAPIEKTISKTKGQPR EPQVYTLPPSREEMTKNQVSLTCLV
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DPEVQFNWYVDGVEVHNAKTKPREE QFNSTFRVVSVLTVVHQDWLNGKEY
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GNVFSCSVMHEALHNHYTQKSLSLS PG 22 APELLGGPSVFLFPPKPKDTLMISR
hIgG3FcΔK-AC TPEVTCVVVDVSHEDPEVQFKWYVD Fc variant
GVEVHNAKTKPREEQYNSTFRVVSV LTVLHQDWLNGKEYKCKVSNKALPA
PIEKTISKTKGQPREPQVYTLPPSR EEMTKNQVSLTCLVKGFYPSDIAVE
WESSGQPENNYNTTPPMLDSDGSFF LYSKLTVDKSRWQQGNIFSCSVMHE
ALHNRFTQKSLSLSPG 23 PRCPAPELLGGPSVFLFPPKPKDTL hIgG3FcΔK-C Fc
MISRTPEVTCVVVDVSHEDPEVQFK variant WYVDGVEVHNAKTKPREEQYNSTFR
VVSVLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKTKGQPREPQVYTL
PPSREEMTKNQVSLTCLVKGFYPSD IAVEWESSGQPENNYNTTPPMLDSD
GSFFLYSKLTVDKSRWQQGNIFSCS VKHEALHNRFTQKSLSLSPG 24
DTPPPCPRCPAPELLGGPSVFLFPP hIgG3FcΔK-CC KPKDTLMISRTPEVTCVVVDVSHED
Fc variant PEVQFKWYVDGVEVHNAKTKPREEQ YNSTFRVVSVLTVLHQDWLNGKEYK
CKVSNKALPAPIEKTISKTKCQPRE PQVYTLPPSREEMTKNQVSLTCLVK
CFYPSDIAVEWESSGQPENNYNTTP PMLDSDCSFFLYSKLTVDKSRWQQG
NIFSCSVMHEALHNRFTQKSLSLSP G 25 EPKSSDTPPPCPRCPAPELLGGPSV hIgG3FcΔK-
S Fc FLFPPKPKDTLMISRTPEVTCVVVD variant VSHEDPEVQFKWYVDGVEVHNAKTK
PREEQYNSTFRVVSVLTVLHQDWLN GKEYKCKVSNKALPAPIEKTISKTK
GQPREPQVYTLPPSREEMTKNQVSL TCLVKGFYPSDIAVEWESSGQPENN
YNTTPPMLDSDGSFFLYSKLTVDKS RWQQGNIFSCSVMHEALHNRFTQKS LSLSPG 26
EPKSSDTPPPSPRCPAPELLGGPSV hIgG3FcΔK-SS FLFPPKPKDTLMISRTPEVTCVVVD
Fc variant VSHEDPEVQFKWYVDCVEVHNAKTK PREEQYNSTFRVVSVLTVLHQDWLN
GKEYKCKVSNKALPAPIEKTISKTK GQPREPQVYTLPPSREEMTKNQVSL
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RWQQGNIFSCSVMHEALHNRFTQKS LSLSPG 27 EPKSSDTPPPSPRSPAPELLGGPSV
hIgG3FcΔK-SSS FLFPPKPKDTLMISRTPEVTCVVVD Fc variant
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TCLVKGFYPSDIAVEWESSGQPENN YNTTPPMLDSDGSFFLYSKLTVDKS
RWQQGNIFSCSVMHEALHNRFTQKS LSLSPG 28 APEFLGGPSVFLFPPKPKDTLMISR
hIgG4FcΔK-AC TPEVTCVVVDVSQEDPEVQFNWYVD Fc variant
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ALHNHYTQKSLSLSLG 29 PSCPAPEFLGGPSVFLFPPKPKDTL hIgG4FcΔK-C Fc
MISRTPEVTCVVVDVSQEDPEVQFN variant WYVDGVEVHNAKTKPREEQFNSTYR
VVSVLTVLHQDWLNGKEYKCKVSNK GLPSSIEKTISKAKGQPREPQVYTL
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GSFFLYSRLTVDKSRWQEGNVFSCS VMHEALHNHYTQKSLSLSLG 30
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Fc variant EDPEVQFNWYVDGVEVHNAKTKPRE EQFNSTYRVVSVLTVLHQDWLNGKE
YKCKVSNKGLPSSIEKTISKAKGQP REPQVYTLPPSQEEMTKNQVSLTCL
VKGFYPSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLYSRLTVDKSRWQ
EGNVFSCSVMHEALHNHYTQKSLSL SLG 31 ESKYGPPCPPCPAPEFLGGPSVFLF
hIgG4FcΔK-CC- PPKPKDTLMISRTPEVTCVVVDVSQ 228P Fc variant
EDPEVQFNWYVDGVEVHNAKTKPRE EQFNSTYRVVSVLTVLHQDWLNGKE
YKCKVSNKGLPSSIEKTISKAKGQP REPQVYTLPPSQEEMTKNQVSLTCL
VKGFYPSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLYSRLTVDKSRWQ
EGNVFSCSVMHEALHNHYTQKSLSL SLG 32 ESKYGPPCPPCPAPEFLGGPSVFLF
hIgG4FcΔK-CC- PPKPKDTLMISRTPEVTCVVVDVSQ 228P-409K Fc
EDPEVQFNWYVDGVEVHNAKTKPRE variant EQFNSTYRVVSVLTVLHQDWLNGKE
YKCKVSNKGLPSSIEKTISKAKGQP REPQVYTLPPSQEEMTKNQVSLTCL
VKGFYPSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLYSKLTVDKSRWQ
EGNVFSCSVMHEALHNHYTQKSLSL SLG 33 ESKYCPPSPSCPAPEFLCCPSVFLF
hIgG4FcΔK-S Fc PPKPKDTLMISRTPEVTCVVVDVSQ variant
EDPEVQFNWYVDGVEVHNAKTKPRE EQFNSTYRVVSVLTVLHQDWLNGKE
YKCKVSNKGLPSSIEKTISKAKGQP REPQVYTLPPSQEEMTKNQVSLTCL
VKGFYPSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLYSRLTVDKSRWQ
EGNVFSCSVMHEALHNHYTQKSLSL SLG 34 ESKYGPPSPSSPAPEFLGGPSVFLF
hIgG4FcΔK-SS PPKPKDTLMISRTPEVTCVVVDVSQ Fc variant
EDPEVQFNWYVDCVEVHNAKTKPRE EQFNSTYRVVSVLTVLHQDWLNGKE
YKCKVSNKGLPSSIEKTISKAKGQP REPQVYTLPPSQEEMTKNQVSLTCL
VKGFYPSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLYSRLTVDKSRWQ
EGNVFSCSVMHEALHNHYTQKSLSL SLG 35 QLCKFCDVRFSTCDNQKSCMSNCSI TβRII-
ED TSICEKPQEVCVAVWRKNDENITLE structured domain
TVCHDPKLPYHDFILEDAASPKCIM KEKKKPCETFFMCSCSSDECNDNII F 36
IPPHVQKSVNNDMIVTDNNGAVKFP TβRII-ED N-term unstructured region and natural
linker 37 IPPHVQKSDVEMEAQKDEIICPSCN TβRIIb-ED N-term
RTAHPLRHINNDMIVTDNNCAVKFP unstructured region and natural linker 38
IPPHVQKSDVEMEAQKDEIIAPSAN TβRIIb-ED Cys-
RTAHPLRHINNDMIVTDNNGAVKFP mutated N-term unstructured region and natural
linker 39 SEEYNTSNPD TβRII-ED C-term unstructured region and natural linker 40
SEEYNTSNPDIPPHVQKSVNNDMIV TβRII-ED natural TDNNGAVKFP linker 41
SEEYNTSNPDIPPHVQKSDVEMEAQ TβRIIb-ED natural

KDEIICPSCNRTAHPLRHINNDMIV linker TDNNGAVKFP 42
SEEYNTSNPDIPPHVQKSDVEMEAQ TβRIIb-ED Cys- KDEIIAPSANRTAHPLRHINNDMIV
mutated linker TDNNGAVKFP 43 IPPHVQKSVNNDMIVTDNNGAVKFP TβRII-ED
QLCKFCDVRFSTCDNQKSCMSNCSI monomer, also TSICEKPQEVCVAVWRKNDENITLE
termed T2 or T2m TVCHDPKLPYHDFILEDAASPKCIM
KEKKKPGETFFMCSCSSDECNDNII FSEEYNTSNPD 44
IPPHVQKSDVEMEAQKDEIICPSCN TpbRIIb-ED RTAHPLRHINNDMIVTDNNGAVKFP
monomer, also QLCKFCDVRFSTCDNQKSCMSNCSI termed T2b
TSICEKPQEVCVAVWRKNDENITLE TVCHDPKLPYHDFILEDAASPKCIM
KEKKKPGETFFMCSCSSDECNDNII FSEEYNTSNPD 45
IPPHVQKSDVEMEAQKDEIIAPSAN TβRIIb-ED RTAHPLRHINNDMIVTDNNGAVKFP
monomer Cys- QLCKFCDVRFSTCDNQKSCMSNCSI mutated in the
TSICEKPQEVCVAVWRKNDENITLE linker region, TVCHDPKLPYHDFILEDAASPKCIM
also KEKKKPGETFFMCSCSSDECNDNII termed T2b.sup.AA FSEEYNTSNPD 46
IPPHVQKSVNNDMIVTDNNGAVKFP TβRII-ED dimer,
QLCKFCDVRFSTCDNQKSCMSNCSI also termed T2-
TSICEKPQEVCVAVWRKNDENITLE T2 or T22d35 TVCHDPKLPYHDFILEDAASPKCIM
KEKKKPGETFFMCSCSSDECNDNII FSEEYNTSNPDIPPHVQKSVNNDMI
VTDNNGAVKFPQLCKFCDVRFSTCD NQKSCMSNCSITSICEKPQEVCVAV
WRKNDENITLETVCHDPKLPYHDFI LEDAASPKCIMKEKKKPGETFFMCS
CSSDECNDNIIFSEEYNTSNPD 47 IPPHVQKSDVEMEAQKDEIICPSCN TβRIIb-ED dimer,
RTAHPLRHINNDMIVTDNNGAVKFP also termed T2-
QLCKFCDVRFSTCDNQKSCMSNCSI T2b TSICEKPQEVCVAVWRKNDENITLE
TVCHDPKLPYHDFILEDAASPKCIM KEKKKPGETFFMCSCSSDECNDNII
FSEEYNTSNPDIPPHVQKSDVEMEA QKDEIICPSCNRTAHPLRHINNDMI
VTDNNGAVKFPQLCKFCDVRFSTCD NQKSCMSNCSITSICEKPQEVCVAV
WRKNDENITLETVCHDPKLPYHDFI LEDAASPKCIMKEKKKPGETFFMCS
CSSDECNDNIIFSEEYNTSNPD 48 IPPHVQKSDVEMEAQKDEIICPSCN TβRIIb-ED dimer
RTAHPLRHINNDMIVTDNNGAVKFP Cys-mutated in QLCKFCDVRFSTCDNQKSCMSNCSI
the linker region, TSICEKPQEVCVAVWRKNDENITLE also termed T2-
TVCHDPKLPYHDFILEDAASPKCIM T2b.sup.AA KEKKKPGETFFMCSCSSDECNDNII
FSEEYNTSNPDIPPHVQKSDVEMEA QKDEIIAPSANRTAHPLRHINNDMI
VTDNNGAVKFPQLCKFCDVRFSTCD NQKSCMSNCSITSICEKPQEVCVAV
WRKNDENITLETVCHDPKLPYHDFI LEDAASPKCIMKEKKKPGETFFMCS
CSSDECNDNIIFSEEYNTSNPD 49 IPPHVQKSVNNDMIVTDNNGAVKFP TβRII-ED trimer,
QLCKFCDVRFSTCDNQKSCMSNCSI also termed T2-
TSICEKPQEVCVAVWRKNDENITLE T2-T2 TVCHDPKLPYHDFILEDAASPKCIM
KEKKKPGETFFMCSCSSDECNDNII FSEEYNTSNPDIPPHVQKSVNNDMI
VTDNNGAVKFPQLCKFCDVRFSTCD NQKSCMSNCSITSICEKPQEVCVAV
WRKNDENITLETVCHDPKLPYHDFI LEDAASPKCIMKEKKKPGETFFMCS
CSSDECNDNIIFSEEYNTSNPDIPP HVQKSVNNDMIVTDNNGAVKFPQLC
KFCDVRFSTCDNQKSCMSNCSITSI CEKPQEVCVAVWRKNDENITLETVC
HDPKLPYHDFILEDAASPKCIMKEK KKPGETFFMCSCSSDECNDNIIFSE EYNTSNPD 50
IPPHVQKSVNNDMIVTDNNGAVKFP TβRIIb-ED trimer,
QLCKFCDVRFSTCDNQKSCMSNCSI also termed T2-
TSICEKPQEVCVAVWRKNDENITLE T2b-T2b TVCHDPKLPYHDFILEDAASPKCIM
KEKKKPGETFFMCSCSSDECNDNII FSEEYNTSNPDIPPHVQKSDVEMEA
QKDEIICPSCNRTAHPLRHINNDMI VTDNNGAVKFPQLCKFCDVRFSTCD
NQKSCMSNCSITSICEKPQEVCVAV WRKNDENITLETVCHDPKLPYHDFI
LEDAASPKCIMKEKKKPGETFFMCS CSSDECNDNIIFSEEYNTSNPDIPP

HVQKSDVEMEAQKDEIICPSCNRTA HPLRHINNDMIVTDNNGAVKFPQLC
KFCDVRFSTCDNQKSCMSNCSITSI CEKPQEVCVAVWRKNDENITLETVC
HDPKLPYHDFILEDAASPKCIMKEK KKPGETFFMCSCSSDECNDNIIFSE EYNTSNPD 51
IPPHVQKSVNNDMIVTDNNGAVKFP TβRIIb-ED trimer
QLCKFCDVRFSTCDNQKSCMSNCSI Cys-mutated in TSICEKPQEVCVAVWRKNDENITLE
the linker regions, TVCHDPKLPYHDFILEDAASPKCIM also termed T2-
KEKKKPGETFFMCSCSSDECNDNII T2b.sup.AA-T2b.sup.AA
FSEEYNTSNPDIPPHVQKSDVEMEA QKDEIIAPSANRTAHPLRHINNDMI
VTDNNGAVKFPQLCKFCDVRFSTCD NQKSCMSNCSITSICEKPQEVCVAV
WRKNDENITLETVCHDPKLPYHDFI LEDAASPKCIMKEKKKPGETFFMCS
CSSDECNDNIIFSEEYNTSNPDIPP HVQKSDVEMEAQKDEIIAPSANRTA
HPLRHINNDMIVTDNNGAVKFPQLC KFCDVRFSTCDNQKSCMSNCSITSI
CEKPQEVCVAVWRKNDENITLETVC HDPKLPYHDFILEDAASPKCIMKEK
KKPGETFFMCSCSSDECNDNIIFSE EYNTSNPD 52 ALQCFCHLCTKDNFTCVTDGLCFVS
TβRI-ED VTETTDKVIHNSMCIAEIDLIPRDR structured domain
PFVCAPSSKTGSVTTTYCCNQDHCN KIEL 53 AALLPGAT TβRI-ED N-term unstructured
region and natural linker 54 PTTVKSSPGLGPVE TβRI-ED C-term unstructured region
and natural linker 55 PTTVKSSPGLGPVEAALLPGAT TβRI-ED natural linker 56
AALLPGATALQCFCHLCTKDNFTCV TβRI-ED TDGLCFVSVTETTDKVIHNSMCIAE
monomer, also IDLIPRDRPFVCAPSSKTGSVTTTY termed T1 or T1m
CCNQDHCNKIELPTTVKSSPGLGPV E 57 AALLPGATALQCFCHLCTKDNFTCV TβRI-ED
dimer, TDGLCFVSVTETTDKVIHNSMCIAE also termed T1-
IDLIPRDRPFVCAPSSKTGSVTTTY T1 CCNQDHCNKIELPTTVKSSPGLGPV
EAALLPGATALQCFCHLCTKDNFTC VTDGLCFVSVTETTDKVIHNSMCIA
EIDLIPRDRPFVCAPSSKTGSVTTT YCCNQDHCNKIELPTTVKSSPGLGP VE 58
PTTVKSSPGLGPVEIPPHVQKSVNN TβRI-TβRII-ED DMIVTDNNGAVKFP natural linker 59
PTTVKSSPGLGPVEIPPHVQKSDVE TβRI-TβRIIb-ED MEAQKDEIICPSCNRTAHPLRHINN
natural linker DMIVTDNNGAVKFP 60 SEEYNTSNPDAALLPGAT TβRII-TβRI-ED natural
linker 61 AALLPGATALQCFCHLCTKDNFTCV TβRI-TβRII-ED
TDGLCFVSVTETTDKVIHNSMCIAE dimer T1-T2 IDLIPRDRPFVCAPSSKTGSVTTTY
CCNQDHCNKIELPTTVKSSPGLGPV EIPPHVQKSVNNDMIVTDNNGAVKF
PQLCKFCDVRFSTCDNQKSCMSNCS ITSICEKPQEVCVAVWRKNDENITL
ETVCHDPKLPYHDFILEDAASPKCI MKEKKKPGETFFMCSCSSDECNDNI
IFSEEYNTSNPD 62 AALLPGATALQCFCHLCTKDNFTCV TβRI-TβRII-ED
TDGLCFVSVTETTDKVIHNSMCIAE dimer T1-T2b IDLIPRDRPFVCAPSSKTGSVTTTY
CCNQDHCNKIELPTTVKSSPGLCPV EIPPHVQKSDVEMEAQKDEIICPSC
NRTAHPLRHINNDMIVTDNNGAVKF PQLCKFCDVRFSTCDNQKSCMSNCS
ITSICEKPQEVCVAVWRKNDENITL ETVCHDPKLPYHDFILEDAASPKCI
MKEKKKPGETFFMCSCSSDECNDNI IFSEEYNTSNPD 63
IPPHVQKSVNNDMIVTDNNGAVKFP TβRI-TβRII-ED QLCKFCDVRFSTCDNQKSCMSNCSI
dimer T2-T1 TSICEKPQEVCVAVWRKNDENITLE TVCHDPKLPYHDFILEDAASPKCIM
KEKKKPGETFFMCSCSSDECNDNII FSEEYNTSNPDAALLPGATALQCFC
HLCTKDNFTCVTDGLCFVSVTETTD KVIHNSMCIAEIDLIPRDRPFVCAP
SSKTGSVTTTYCCNQDHCNKIELPT TVKSSPGLGPVE 64
AALLPGATALQCFCHLCTKDNFTCV TβRI-TβRII-ED TDGLCFVSVTETTDKVIHNSMCIAE
trimer T1-T2-T2 IDLIPRDRPFVCAPSSKTGSVTTTY CCNQDHCNKIELPTTVKSSPGLGPV
EIPPHVQKSVNNDMIVTDNNGAVKF PQLCKFCDVRFSTCDNQKSCMSNCS
ITSICEKPQEVCVAVWRKNDENITL ETVCHDPKLPYHDFILEDAASPKCI
MKEKKKPGETFFMCSCSSDECNDNI IFSEEYNTSNPDIPPHVQKSVNNDM
IVTDNNGAVKFPQLCKFCDVRFSTC DNQKSCMSNCSITSICEKPQEVCVA

VWRKNDENITLETVCHDPKLPYHDF ILEDAASPKCIMKEKKKPGETFFMC
SCSSDECNDNIIFSEEYNTSNPD 65 AALLPGATALQCFCHLCTKDNFTCV TβRI-TβRII-ED
TDGLCFVSVTETTDKVIHNSMCIAE trimer T1-T2-T2b IDLIPRDRPFVCAPSSKTGSVTTTY
CCNQDHCNKIELPTTVKSSPGLGPV EIPPHVQKSVNNDMIVTDNNGAVKF
PQLCKFCDVRFSTCDNQKSCMSNCS ITSICEKPQEVCVAVWRKNDENITL
ETVCHDPKLPYHDFILEDAASPKCI MKEKKKPGETFFMCSCSSDECNDNI
IFSEEYNTSNPDIPPHVQKSDVEME AQKDEIICPSCNRTAHPLRHINNDM
IVTDNNGAVKFPQLCKFCDVRFSTC DNQKSCMSNCSITSICEKPQEVCVA
VWRKNDENITLETVCHDPKLPYHDF ILEDAASPKCIMKEKKKPGETFFMC
SCSSDECNDNIIFSEEYNTSNPD 66 AALLPGATALQCFCHLCTKDNFTCV TβRI-TβRII-ED
TDGLCFVSVTETTDKVIHNSMCIAE trimer T1-T2- IDLIPRDRPFVCAPSSKTGSVTTTY
T2b.sup.AA CCNQDHCNKIELPTTVKSSPGLGPV EIPPHVQKSDVEMEAQKDEIICPSC
NRTAHPLRHINNDMIVTDNNGAVKF PQLCKFCDVRFSTCDNQKSCMSNCS
ITSICEKPQEVCVAVWRKNDENITL ETVCHDPKLPYHDFILEDAASPKCI
MKEKKKPGETFFMCSCSSDECNDNI IFSEEYNTSNPDIPPHVQKSDVEME
AQKDEIIAPSANRTAHPLRHINNDM IVTDNNGAVKFPQLCKFCDVRFSTC
DNQKSCMSNCSITSICEKPQEVCVA VWRKNDENITLETVCHDPKLPYHDF
ILEDAASPKCIMKEKKKPGETFFMC SCSSDECNDNIIFSEEYNTSNPD 67
IPPHVQKSVNNDMIVTDNNGAVKFP TβRI-TβRII-ED QLCKFCDVRFSTCDNQKSCMSNCSI
trimer T2-T2-T1 TSICEKPQEVCVAVWRKNDENITLE TVCHDPKLPYHDFILEDAASPKCIM
KEKKKPGETFFMCSCSSDECNDNII FSEEYNTSNPDIPPHVQKSVNNDMI
VTDNNGAVKFPQLCKFCDVRFSTCD NQKSCMSNCSITSICEKPQEVCVAV
WRKNDENITLETVCHDPKLPYHDFI LEDAASPKCIMKEKKKPGETFFMCS
CSSDECNDNIIFSEEYNTSNPDAAL LPGATALQCFCHLCTKDNFTCVTDG
LCFVSVTETTDKVIHNSMCIAEIDL IPRDRPFVCAPSSKTGSVTTTYCCN
QDHCNKIELPTTVKSSPGLGPVE 68 IPPHVQKSVNNDMIVTDNNGAVKFP TβRI-TβRII-ED
QLCKFCDVRFSTCDNQKSCMSNCSI trimer T2-T2b.sup.AA-
TSICEKPQEVCVAVWRKNDENITLE T1 TVCHDPKLPYHDFILEDAASPKCIM
KEKKKPGETFFMCSCSSDECNDNII FSEEYNTSNPDIPPHVQKSDVEMEA
QKDEIIAPSANRTAHPLRHINNDMI VTDNNGAVKFPQLCKFCDVRFSTCD
NQKSCMSNCSITSICEKPQEVCVAV WRKNDENITLETVCHDPKLPYHDFI
LEDAASPKCIMKEKKKPGETFFMCS CSSDECNDNIIFSEEYNTSNPDAAL
LPGATALQCFCHLCTKDNFTCVTDG LCFVSVTETTDKVIHNSMCIAEIDL
IPRDRPFVCAPSSKTCSVTTTYCCN QDHCNKIELPTTVKSSPGLGPVE 69
TLPFLKCYCSGHCPDDAINNTCITN BMPRIa-ED GHCFAIIEEDDQGETTLASGCMKYE
structured domain GSDFQCKDSPKAQLRRTIECCRTNL CNQYLQPTLP 70
QNLDSMLHGTGMKSDSDQKKSENGV BMPRIa-ED N- TLAPED term unstructured
region and natural linker 71 PWIGPFFDGSIR BMPRIa-ED C- term unstructured region
and natural linker 72 PWIGPFFDCSIRQNLDSMLHGTGMK BMPRIa-ED
SDSDQKKSENGVTLAPED natural linker 73 QNLDSMLHGTGMKSDSDQKKSENGV
BMPRIa-ED TLAPEDTLPFLKCYCSGHCPDDAIN monomer
NTCITNGHCFAIIEEDDQGETTLAS GCMKYEGSDFQCKDSPKAQLRRTIE
CCRTNLCNQYLQPTLPPVVIGPFFD GSIR 74 QNLDSMLHGTGMKSDSDQKKSENGV
BMPRIa-ED TLAPEDTLPFLKCYCSGHCPDDAIN dimer NTCITNGHCFAIIEEDDQGETTLAS
CCMKYEGSDFQCKDSPKAQLRRTIE CCRTNLCNQYLQPTLPPVVIGPFFD
GSIRQNLDSMLHGTGMKSDSDQKKS ENGVTLAPEDTLPFLKCYCSGHCPD
DAINNTCITNCHCFAIIEEDDQCET TLASGCMKYEGSDFQCKDSPKAQLR
RTIECCRTNLCNQYLQPTLPPWIGP FFDGSIR 75 TQECLFFNANWEKDRTNQTGVEPCY
ActRila-ED GDKDKRRHCFATWKNISGSIEIVKQ structured domain
GCWLDDINCYDRTDCVEKKDSPEVY FCCCEGNMCNEKFSYFP 76 AILGRSE ActRIIa-ED

N- term unstructured region and natural linker 77 EMEVTQPTSNPVTPKPPYYNI ActRIIa-ED C- term unstructured region and natural linker 78 EMEVTQPTSNPVTPKPPYYNIAILG ActRIIa-ED RSE natural linker 79 AILGRSETQECLFFNANWEKDRTNQ ActRIIa-ED TGVEPCYGDKDKRRHCFATWKNISG monomer SIEIVKQGCWLDDINCYDRTDCVEK KDSPEVYFCCCEGNMCNEKFSYFPE MEVTQPTSNPVTPKPPYYNI 80 AILGRSETQECLFFNANWEKDRTNQ ActRIIa-ED dimer TGVEPCYCDKDKRRHCFATWKNISG SIEIVKQGCWLDDINCYDRTDCVEK KDSPEVYFCCCEGNMCNEKFSYFPE MEVTQPTSNPVTPKPPYYNIAILGR SETQECLFFNANWEKDRTNQTGVEP CYGDKDKRRHCFATWKNISGSIEIV KQGCWLDDINCYDRTDCVEKKDSPE VYFCCCEGNMCNEKFSYFPEMEVTQ PTSNPVTPKPPYYNI 81 RECIYYNANWELERTNQSGLERCEG ActRIIb-ED EQDKRLHCYASWRNSSGTIELVKKG structured domain CWLDDFNCYDRQECVATEENPQVYF CCCEGNFCNERFTHLP 82 SGRGEAET ActRIIb-ED N- term unstructured region and natural linker 83 EAGGPEVTYEPPPTAPT ActRIIb-ED C- term unstructured region and natural linker 84 EAGGPEVTYEPPPTAPTSGRGEAET ActRIIb-ED natural linker 85 SGRGEAETRECIYYNANWELERTNQ ActRIIb-ED SGLERCEGEQDKRLHCYASWRNSSG monomer TIELVKKGCWLDDFNCYDRQECVAT EENPQVYFCCCECNFCNERFTHLPE AGGPEVTYEPPPTAPT 86 SGRGEAETRECIYYNANWELERTNQ ActRIIb-ED dimer SGLERCEGEQDKRLHCYASWRNSSG TIELVKKGCWLDDFNCYDRQECVAT EENPQVYFCCCEGNFCNERFTHLPE AGGPEVTYEPPPTAPTSGRGEAETR ECIYYNANWELERTNQSGLERCEGE QDKRLHCYASWRNSSGTIELVKKGC WLDDFNCYDRQECVATEENPQVYFC CCEGNFCNERFTHLPEAGGPEVTYE PPPTAPT 87 EMEVTQPTSNPVTPKPPYYNIQNLD ActRIIa-BMPRIa-SMLHGTCMKSDSDQKKSENCVTLAP ED natural linker ED 88 AILGRSETQECLFFNANWEKDRTNQ ActRIIa-BMPRIa-TGVEPCYGDKDKRRHCFATWKNISG ED dimer SIEIVKQGCWLDDINCYDRTDCVEK KDSPEVYFCCCEGNMCNEKFSYFPE MEVTQPTSNPVTPKPPYYNIQNLDS MLHGTGMKSDSDQKKSENGVTLAPE DTLPFLKCYCSGHCPDDAINNTCIT NGHCFAIIEEDDQGETTLASGCMKY EGSDFQCKDSPKAQLRRTIECCRTN LCNQYLQPTLPPWIGPFFDGSIR 89 MDWTWRILFLVAAATCTHA Signal peptide 90 MVLQTQVFISLLLWISGAYG Signal peptide 91 APELLGGPSVFLFPPKPKDTLMISR hIgG1FcΔK-AC- TPEVTCVVVDVSHEDPEVKFNWYVD T2m fusion GVEVHNAKTKPREEQYNSTYRVVSV LTVLHQDWLNGKEYKCKVSNKALPA PIEKTISKAKGQPREPQVYTLPPSR DELTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFF LYSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSPGIPPHVQKSV NNDKIVTDNNGAVKFPQLCKFCDVR FSTCDNQKSCKSNCSITSICEKPQE VCVAVWRKNDENITLETVCHDPKLP YHDFILEDAASPKCIMKEKKKPGET FFMCSCSSDECNDNIIFSEEYNTSN PD 92 PPCPAPELLGGPSVFLFPPKPKDTL hIgG1FcΔK-C- MISRTPEVTCVVVDVSHEDPEVKFN T2m fusion WYVDGVEVHNAKTKPREEQYNSTYR VVSVLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKAKGQPREPQVYTL PPSRDELTKNQVSLTCLVKGFYPSD IAVEWESNGQPENNYKTTPPVLDSD GSFFLYSKLTVDKSRWQQGNVFSCS VKHEALHNHYTQKSLSLSPGIPPHV QKSVNNDMIVTDNNGAVKFPQLCKF CDVRFSTCDNQKSCMSNCSITSICE KPQEVCVAVWRKNDENITLETVCHD PKLPYHDFILEDAASPKCIMKEKKK PGETFFMCSCSSDECNDNIIFSEEY NTSNPD 93 DKTHTCPPCPAPELLGGPSVFLFPP hIgG1FcΔK-CC- KPKDTLMISRTPEVTCVVVDVSHED T2m fusion PEVKFNWYVDGVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSRDELTKNQVSLTCLVK

GFYPSDIAVEWESNGQPENNYKTTP PVLDSDGSFFLYSKLTVDKSRWQQG
NVFSCSVMHEALHNHYTQKSLSLSP GIPPHVQKSVNNDMIVTDNNGAVKF
PQLCKFCDVRFSTCDNQKSCMSNCS ITSICEKPQEVCVAVWRKNDENITL
ETVCHDPKLPYHDFILEDAASPKCI MKEKKKPGETFFMCSCSSDECNDNI
IFSEEYNTSNPD 94 APPVAGPSVFLFPPKPKDTLMISRT hIgG2FcΔK-AC-
PEVTCVVVDVSHEDPEVQFNWYVDG T2m fusion VEVHNAKTKPREEQFNSTFRVVSVL
TVVHQDWLNGKEYKCKVSNKGLPAP IEKTISKTKGQPREPQVYTLPPSRE
EMTKNQVSLTCLVKGFYPSDISVEW ESNGQPENNYKTTPPMLDSDGSFFL
YSKLTVDKSRWQQGNVFSCSVKHEA LHNHYTQKSLSLSPGIPPHVQKSVN
NDMIVTDNNGAVKFPQLCKFCDVRF STCDNQKSCMSNCSITSICEKPQEV
CVAVWRKNDENITLETVCHDPKLPY HDFILEDAASPKCIMKEKKKPGETF
FMCSCSSDECNDNIIFSEEYNTSNP D 95 PPCPAPPVAGPSVFLFPPKPKDTLM hIgG2FcΔK-
C- ISRTPEVTCWVDVSHEDPEVQFNWY T2m fusion
VDGVEVHNAKTKPREEQFNSTFRVV SVLTVVHQDWLNGKEYKCKVSNKGL
PAPIEKTISKTKGQPREPQVYTLPP SREEMTKNQVSLTCLVKGFYPSDIS
VEWESNGQPENNYKTTPPMLDSDGS FFLYSKLTVDKSRWQQGNVFSCSVM
HEALHNHYTQKSLSLSPGIPPHVQK SVNNDMIVTDNNGAVKFPQLCKFCD
VRFSTCDNQKSCMSNCSITSICEKP QEVCVAVWRKNDENITLETVCHDPK
LPYHDFILEDAASPKCIMKEKKKPG ETFFMCSCSSDECNDNIIFSEEYNT SNPD 96
APPVAGPSVFLFPPKPKDTLMISRT hIgG2FcΔK-CC-
PEVTCVVVDVSHEDPEVQFNWYVDG T2m fusion VEVHNAKTKPREEQFNSTFRVVSVL
TVVHQDWLNGKEYKCKVSNKGLPAP IEKTISKTKGQPREPQVYTLPPSRE
EMTKNQVSLTCLVKGFYPSDISVEW ESNGQPENNYKTTPPMLDSDGSFFL
YSKLTVDKSRWQQGNVFSCSVMHEA LHNHYTQKSLSLSPGIPPHVQKSVN
NDMIVTDNNGAVKFPQLCKFCDVRF STCDNQKSCMSNCSITSICEKPQEV
CVAVWRKNDENITLETVCHDPKLPY HDFILEDAASPKCIMKEKKKPGETF
FMCSCSSDECNDNIIFSEEYNTSNP D 97 MDWTWRILFLVAAATGTHAERKCCV hIgG2Fc-
CCCC- ECPPCPAPPVAGPSVFLFPPKPKDT T2 fusion, also
LMISRTPEVTCVVVDVSHEDPEVQF termed Fc-T2m
NWYVDGVEVHNAKTKPREEQFNSTF RVVSVLTVVHQDWLNGKEYKCKVSN
KGLPAPIEKTISKTKGQPREPQVYT LPPSREEMTKNQVSLTCLVKCFYPS
DISVEWESNCQPENNYKTTPPMLDS DCSFFLYSKLTVDKSRWQQGNVFSC
SVKHEALHNHYTQKSLSLSPGKIPP HVQKSVNNDMIVTDNNGAVKFPQLC
KFCDVRFSTCDNQKSCKSNCSITSI CEKPQEVCVAVWRKNDENITLETVC
HDPKLPYHDFILEDAASPKCIMKEK KKPGETFFMCSCSSDECNDNIIFSE EYNTSNPD 98
ESKYGPPCPPCPAPEFLGGPSVFLF hIgG4FcΔK-CC- PPKPKDTLMISRTPEVTCVVVDVSQ
228P-T2m fusion EDPEVQFNWYVDGVEVHNAKTKPRE
EQFNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKGLPSSIEKTISKAKGQP
REPQVYTLPPSQEEMTKNQVSLTCL VKGFYPSDIAVEWESNGQPENNYKT
TPPVLDSDGSFFLYSRLTVDKSRWQ EGNVFSCSVMHEALHNHYTQKSLSL
SLGIPPHVQKSVNNDMIVTDNNCAV KFPQLCKFCDVRFSTCDNQKSCMSN
CSITSICEKPQEVCVAVWRKNDENI TLETVCHDPKLPYHDFILEDAASPK
CIMKEKKKPGETFFMCSCSSDECND NIIFSEEYNTSNPD 99
ESKYGPPCPPCPAPEFLGGPSVFLF hIgG4FcΔK-CC- PPKPKDTLMISRTPEVTCVVVDVSQ
228P-409K-T2m EDPEVQFNWYVDGVEVHNAKTKPRE fusion
EQFNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKGLPSSIEKTISKAKGQP
REPQVYTLPPSQEEMTKNQVSLTCL VKGFYPSDIAVEWESNGQPENNYKT
TPPVLDSDGSFFLYSKLTVDKSRWQ EGNVFSCSVMHEALHNHYTQKSLSL
SLGIPPHVQKSVNNDMIVTDNNGAV KFPQLCKFCDVRFSTCDNQKSCKSN
CSITSICEKPQEVCVAVWRKNDENI TLETVCHDPKLPYHDFILEDAASPK

CIMKEKKKPGETFFMCSCSSDECND NIIFSEEYNTSNPD 100
PPCPAPELLGGPSVFLFPPKPKDTL hIgG1FcΔK-C- MISRTPEVTCVVVDVSHEDPEVKFN
T22d35 fusion WYVDGVEVHNAKTKPREEQYNSTYR
VVSVLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKAKGQPREPQVYTL
PPSRDELTKNQVSLTCLVKGFYPSD IAVEWESNGQPENNYKTTPPVLDSD
GSFFLYSKLTVDKSRWQQGNVFSCS VKHEALHNHYTQKSLSLSPGIPPHV
QKSVNNDMIVTDNNGAVKFPQLCKF CDVRFSTCDNQKSCMSNCSITSICE
KPQEVCVAVWRKNDENITLETVCHD PKLPYHDFILEDAASPKCIMKEKKK
PGETFFMCSCSSDECNDNIIFSEEY NTSNPDIPPHVQKSVNNDMIVTDNN
GAVKFPQLCKFCDVRFSTCDNQKSC MSNCSITSICEKPQEVCVAVWRKND
ENITLETVCHDPKLPYHDFILEDAA SPKCIMKEKKKPGETFFMCSCSSDE
CNDNIIFSEEYNTSNPD 101 DKTHTCPPCPAPELLGGPSVFLFPP hIgG1FcΔK-CC-
KPKDTLMISRTPEVTCVVVDVSHED T22d35 fusion
PEVKFNWYVDCVEVHNAKTKPREEQ YNSTYRVVSVLTVLHQDWLNCKEYK
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fusion from R&D, CKFCDVRFSTCDNQKSCMSNCSITS also termed T2m-
ICEKPQEVCVAVWRKNDENITLETV Fc (R&D) CHDPKLPYHDFILEDAASPKCIMKE
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also termed T2m- PQEVCVAVWRKNDENITLETVCHDP Fc
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NGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQEGNVFSCSVMHEALH
NHYTQKSLSLSLGIPPHVQKSVNND MIVTDNNGAVKFPQLCKFCDVRFST
CDNQKSCMSNCSITSICEKPQEVCV AVWRKNDENITLETVCHDPKLPYHD
FILEDAASPKCIMKEKKKPGETFFM CSCSSDECNDNIIFSEEYNTSNPDI
PPHVQKSVNNDMIVTDNNGAVKFPQ LCKFCDVRFSTCDNQKSCMSNCSIT
SICEKPQEVCVAVWRKNDENITLET VCHDPKLPYHDFILEDAASPKCIMK
EKKKPGETFFMCSCSSDECNDNIIF SEEYNTSNPDIPPHVQKSVNNDMIV
TDNNGAVKFPQLCKFCDVRFSTCDN QKSCMSNCSITSICEKPQEVCVAVW
RKNDENITLETVCHDPKLPYHDFIL EDAASPKCIMKEKKKPGETFFMCSC
SSDECNDNIIFSEEYNTSNPD

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Claims

1. A polypeptide construct comprising: a first portion comprising the second constant domain (CH2) and/or third constant domain (CH3) of an antibody heavy chain, and a second portion comprising at least two TGF-β superfamily receptor ectodomains (TβSR-ED) linked in tandem, wherein the N-terminus of the second portion is linked to the C-terminus of the first portion, wherein the at least two TβSR-EDs are TGF-β receptor II ectodomains (TβR-II-EDs), and wherein the polypeptide construct has neutralization activity against TGF-β2.
2. The polypeptide construct of claim 1, wherein the second portion comprises two TβSR-ED.
3. The polypeptide construct of claim 1, wherein the first portion further comprises a CH1, a CH1 and VH, or CH1 and scFv.
4. The polypeptide construct of claim 1, wherein the polypeptide construct shows longer in vivo half-life compared to the half-life of the second portion alone.
5. The polypeptide construct of claim 1, wherein the polypeptide construct forms a dimeric polypeptide.
6. The polypeptide construct of claim 1, wherein the construct comprises an antibody or an antigen binding fragment thereof, or a targeting moiety.
7. The polypeptide construct of claim 6, wherein the antibody or the antigen binding fragment thereof binds to an antigen that is not PD-L1, CD4, CD6, CD20, CD25, MUC-1, IL-2, IL-6, or CTLA-4.
8. The polypeptide construct according to claim 6, wherein the targeting moiety comprises a poly-aspartate sequence motif for bone targeting.
9. A composition comprising one or more than one independently selected polypeptide construct of claim 1 and a pharmaceutically-acceptable carrier, diluent, or excipient.
10. The polypeptide construct of claim 1, wherein the N-terminus of the second portion is directly linked to the C-terminus of the first portion, and wherein the C-terminal residue of the amino acid sequence of the first portion is not a lysine residue.
11. The polypeptide construct of claim 1, wherein each of the at least two TβSR-EDs comprises the amino acid sequence set forth in SEQ ID NO: 35.
12. The polypeptide construct of claim 11, wherein the at least two TβSR-EDs are joined by a linker.
13. The polypeptide construct of claim 12, wherein the linker comprises the amino acid sequence set forth in SEQ ID NO: 39.
14. The polypeptide construct of claim 1, wherein the second portion comprises the amino acid sequence set forth in SEQ ID NO: 46.