

TRANSFORMING GROWTH FACTOR BETA (TGF β) BINDING AGENTS AND USES
THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 63/038,290, filed June 12, 2020, the content of which is incorporated by reference herein in its entirety.

REFERENCE TO SEQUENCE LISTING SUBMITTED ELECTRONICALLY

[0002] This application incorporates by reference in its entirety the Computer Readable Form (CRF) of a Sequence Listing submitted herewith. The Sequence Listing text file submitted herewith, entitled "14247-632-228_SEQ_LISTING.txt", was created on June 7, 2021, and is 247,915 bytes in size.

FIELD

[0003] The present disclosure relates to TGF β binding agents comprising TGF β receptor ectodomain (TGF β R-ECD)-derived fusion molecules and uses thereof for binding and neutralizing TGF β ligands, particularly for the treatment of diseases or conditions associated with TGF β .

BACKGROUND

[0004] Transforming growth factor beta (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. TGF β has been implicated in the pathogenesis of multiple human disorders (Akhurst, R.J. and Hata, A., 2012; Akhurst, R.J., 2017). For instance, TGF β and activin ligands play critical pathogenic roles in many diseases including fibrosis and cancer. Examples of TGF β -associated disorders include hematologic malignancies, solid tumors, bone marrow failure states, and a wide variety of disorders characterized by uncontrolled fibrosis such as pulmonary, liver, renal and vascular fibrosis, pulmonary arterial hypertension, and systemic sclerosis (SSc; also called scleroderma) (Nanthakumar, D.B. et al., 2015; Meng, X.-M. et al., 2016).

[0005] Persistent activation of TGF β signaling plays a central role in the pathogenesis of fibrosis (Varga, J. and Whitfield, M.L., 2009). TGF β canonical signaling stimulates the transition of fibroblasts to myofibroblasts (Desmouliere, A. et al., 1993; Midgley, A.C. et al., 2013) and

plays a critical role in the production and deposition of collagen and other components of the extracellular matrix (ECM) (Prud'homme, G.J., 2007) as well as the induction of other mediators involved in fibrosis (Todd, N.W. et al., 2015). In patients with fibrotic disease such as scleroderma and idiopathic pulmonary fibrosis (IPF), TGF β increases collagen deposition in the skin and/or lung, and stimulates fibroblast activation into myofibroblasts in the skin (Prud'homme, G.J., 2007; Lafyatis, R., 2014; Kissin, E.Y. et al., 2006). In addition, non-canonical TGF β pathways also contribute to the maintenance of the fibrotic phenotype (Leask, A., 2008). Hence, the TGF β signaling pathway has emerged as the most obvious target for therapeutic intervention in fibrosis (Varga, J. and Whitfield, M.L., 2009; Hunzelmann, N. and Krieg, T., 2010; Varga, J. and Pasche, B., 2008).

[0006] TGF β is also 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 epithelial tumor cells, leading to aggressive metastasis. TGF β also promotes tumorigenesis by acting as a powerful suppressor of the immune response in the tumor microenvironment. In fact, TGF β is recognized to be 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. The importance of TGF β 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 efforts have been invested in devising anti-tumor therapeutic approaches that involve TGF β inhibition. These approaches include the use of polypeptide fusions based on a TGF β receptor ectodomain that binds or "traps" the TGF β ligand (see for example, WO01/83525; WO2005/028517; WO2008/113185; WO2008/157367; WO2010/0031168; WO2010/099219; WO2012/071649; WO2012/142515; WO2013/000234; WO2018/158727; US5693607; US2005/0203022; US2007/0244042; US8318135; US8658135; US8815247; US2015/0225483; and US2015/0056199).

[0007] One approach to developing therapeutic agents that inhibit TGF β function has been to use antibodies or soluble decoy receptors (also termed receptor ectodomain (ECD)-based ligand traps) to bind and sequester ligand, thereby blocking access of ligand to its cell surface receptors.

In general, receptor ECD-based traps are a class of therapeutic agents that are able to sequester selectively ligands, and that can be optimized using protein-engineering approaches.

[0008] Previously, it was shown that single-chain, bivalent TGF β traps having two TGF β receptor Type II (TGF β RII) ectodomains linked as a doublet can neutralize members of the TGF β superfamily of ligands (WO2008/113185, WO2010031168). In those cases, bivalency was achieved by covalently linking two TGF β RII ectodomains using the intrinsically disordered regions (IDR) that flank the structured, ligand-binding domain of the TGF β RII ectodomain. It was further shown that potency increases when such bivalent doublets are joined in tandem to a multimerization domain such as an Fc component at the N- or C-terminus (WO2017/037634, WO2018/158727).

[0009] To date, most therapeutic approaches to neutralizing TGF β have focused on the TGF β 1 isoform, especially in immune-oncology. This is because TGF β 1 is the predominantly expressed isoform in the immune system (Li, M.O. et al., 2006) as well as in many types of human tumors (Martin, C.J. et al., 2020). Although the intended target has usually been the TGF β 1 isoform, most therapeutic agents under development generally inhibit other TGF β isoforms with varying potencies. For example, fresolimumab is a monoclonal antibody that is a pan-inhibitor of all three TGF β isoforms. Although it neutralizes all isoforms, it inhibits the TGF β 1 isoform $\sim$ 7-fold more potently than the TGF β 3 isoform, and $\sim$ 14-fold more potently than the TGF β 2 isoform (Grutter, C. et al., 2008). This monoclonal antibody has been tested in clinical trials in cancer patients (Morris, J.C. et al., 2014; Lacouture, M.E. and Morris, J.C., 2015) and in patients with glomerulosclerosis (Vincenti, F. et al., 2017).

[0010] The TGF β 2 isoform has been implicated in cardiac homeostasis (Roberts, A.B. et al., 1992; Herbertz, S. et al., 2015), control of tumor dormancy (Bragado, P. et al., 2013), and the positive regulation of hematopoiesis (Langer, J.C. et al., 2004), suggesting that this isoform should be spared from neutralization since it plays beneficial roles.

[0011] Therefore, it would be useful to provide TGF β RII-ECD-based traps having tailored isoform specificity in order to maximize therapeutic efficacy in particular disease indications, while minimizing adverse effects. In particular, it may be useful to provide traps that neutralize TGF β 3 with potencies similar to that of TGF β 1.

SUMMARY

[0012] There are provided herein tetravalent TGF β receptor-ectodomain based traps having a tailored isoform-specificity profile for neutralization of TGF β ligands, and methods of use thereof in the treatment of diseases and conditions associated with TGF β . Tetravalent TGF β binding agents provided herein comprise two polypeptides assembled via a multimerization domain, each polypeptide having two TGF β II receptor (TGF β R) ligand-binding domains linked as a doublet. TGF β binding agents provided herein have been designed to tailor TGF β isoform specificity, in order to maximize therapeutic efficacy in specific disease indications while minimizing adverse effects.

[0013] The present technology is based, at least in part, on the inventors' realization that a TGF β ligand trap with an isoform specificity that is differentiated from other known agents in development can be advantageous for the treatment of certain TGF β -associated diseases and conditions. Recent reports have indicated an important role for the TGF β 3 isoform in certain TGF β -associated conditions, such as fibrosis. For example, a recent report identified TGF β 3 as a key therapeutic target in kidney fibrosis by demonstrating that the specific downregulation of TGF β 3 by miR-29 counteracts renal fibrosis (Wang, H. et al., 2019). An important role for the TGF β 3 isoform in immunity was also suggested by recent reports on the production of TGF β 3 by immune cells (Komai, I.D. and Okamura, T., 2018). With respect to SSc, a genome-wide association study in African American patients identified TGF β 3 as a novel SSc susceptibility gene (Gourh, P. et al., 2017).

[0014] With respect to the TGF β 2, the implication of this isoform in cardiac homeostasis (Roberts, A.B. et al., 1992; Herbertz, S. et al., 2015), control of tumor dormancy (Bragado, P. et al., 2013), and the positive regulation of hematopoiesis (Langer, J.C. et al., 2004), has suggested that it may be desirable to avoid neutralization of this isoform.

[0015] Taken together, such findings suggest that neutralizing TGF β 1 and TGF β 3 to a similar extent may be beneficial for treatment of certain disorders, particularly those in which TGF β 3 is implicated. Achieving approximately equal inhibition of TGF β 1 and TGF β 3 may be useful, in some cases, to ensure both TGF β 1 and TGF β 3 can be neutralized effectively, which may prevent compensatory mechanisms that could occur when one of these isoforms is neutralized preferentially, and/or which may maximize efficacy. It is also desirable to inhibit TGF β 1 and

TGF β 3 similarly without neutralizing TGF β 2 signaling, since it may be beneficial to avoid neutralization of this isoform.

[0016] In a broad aspect, there are provided herein novel polypeptide constructs useful for inhibiting an effect of a Transforming Growth Factor Beta (TGF β) isoform. Polypeptides in accordance with the present disclosure comprise a TGF β -binding region and a multimerization domain, wherein the N-terminus of the multimerization domain is joined to the C-terminus of the TGF β -binding region. The TGF β -binding region comprises two TGF β receptor ligand-binding domains (TGF β R-LBDs) joined together by a first linker and joined to the multimerization domain by a second linker. In another broad aspect, there are provided TGF β binding agents comprising two such polypeptide chains assembled via the multimerization domains, thereby forming a tetravalent molecule having a particular inhibition specificity for TGF β ligands (TGF β 1, TGF β 2 and TGF β 3).

[0017] Without wishing to be limited by theory, the present invention is based, at least in part, on the finding that modifying one or more of the linkers in such a TGF β ligand trap (e.g., the linker joining two TGF β R-LBDs together and/or the linker joining the TGF β R-LBDs to the multimerization domain) differentially affects inhibition potency of the binding agent for different TGF β isoforms. It is shown herein that, in some cases, modifying one or both linker(s) can lower or equalize the TGF β 3:TGF β 1 IC₅₀ ratio (indicating similar or equalized inhibition potency for both isoforms), without increasing undesired inhibition of TGF β 2, and without significantly reducing the overall potency (e.g., IC₅₀ remains in the low picomolar range).

[0018] TGF β binding agents provided herein generally comprise a first polypeptide and a second polypeptide that are associated together via the multimerization domain, each polypeptide comprising, in an N- to C- terminal orientation: an N-terminal region; a first TGF β receptor ligand-binding domain ((TGF β R-LBD); a first linker; a second TGF β R-LBD; a second linker; and a multimerization domain. An embodiment of a TGF β binding agent is shown schematically in FIG. 1 (which shows an embodiment where the TGF β binding agent is a homodimer, i.e., the first and second polypeptides are the same). The first polypeptide and the second polypeptide may be bound to each other through their respective multimerization domains, e.g., by disulfide bonds (cysteine bridges), coiled coil interactions, and the like.

[0019] TGF β binding agents of the present technology have been designed to lower or equalize the TGF β 3:TGF β 1 IC₅₀ ratio. That is, they have been designed to display reduced preferential inhibition of TGF β 1 compared to other known TGF β traps. Accordingly, TGF β binding agents provided herein are characterized by their specificity profile for isoform inhibition: specifically, the relative inhibition potency for the TGF β 1 and TGF β 3 isoforms (expressed herein as TGF β 3:TGF β 1 IC₅₀ ratio) is no more than about 2.5:1, and the activities of both TGF β 3 and TGF β 1 isoforms are inhibited at much greater potency than that of the TGF β 2 isoform (e.g., in the picomolar range for TGF β 3 and TGF β 1, and nanomolar for TGF β 2).

[0020] Additionally, in some embodiments the polypeptides and TGF β binding agents of the present technology may provide certain advantages, in addition to tailored isoform specificity. For example and without limitation, the polypeptides and TGF β binding agents may provide improved manufacturability, due for example to reduced glycosylation, increased homogeneity, ease of expression, and the like. Thus in some embodiments, polypeptides and TGF β binding agents of the present technology provide one or more of the following advantages, relative to previous TGF β binding agents: improved therapeutic effect for specific disease indications, for example TGF β 3-mediated conditions; reduced glycosylation; increased homogeneity; improved manufacturability; and increased production.

[0021] In some embodiments of polypeptides and TGF β binding agents of the present technology, the first linker and the second linker are designed so as to provide the desired relative isoform-specificity of inhibition. For example, in some embodiments the lengths of the first linker and the second linker are selected such that the TGF β 3:TGF β 1 IC₅₀ ratio is no more than about 2.5:1, and both TGF β 3 and TGF β 1 isoform activity are inhibited at much greater potency than TGF β 2 isoform activity (e.g., in the picomolar range for TGF β 3 and TGF β 1, and nanomolar for TGF β 2).

[0022] In some embodiments of polypeptides and TGF β binding agents of the present technology, the first linker and the second linker are selected so that the TGF β 3:TGF β 1 IC₅₀ ratio is about 2.5:1 or less. In some embodiments, the TGF β 3:TGF β 1 IC₅₀ ratio is less than about 2.5:1, about 2.3:1 or less, about 2:1 or less, about 1.8:1 or less, about 1.5:1 or less, about 1.3:1 or less, about 1.1:1 or less, about 1:1 or less, about 0.8:1 or less, or about 0.5:1 or less. In some

embodiments, the TGF β 3:TGF β 1 IC₅₀ ratio for the TGF β binding agent is from about 1:1 to about 2:1, or is about 1.1:1, 1.2:1, 1.3:1, 1.4:1, 1.5:1, 1.6:1, 1.7:1, 1.8:1, or 1.9:1. In a particular embodiment, the TGF β 3:TGF β 1 IC₅₀ ratio for the TGF β binding agent is from about 1:1 to about 1.5:1 or from about 1.4:1 to about 1.6:1, or about 1.4:1, 1.5:1, or 1.6:1. In some such embodiments, the TGF β binding agent inhibits both TGF β 1 isoform activity and TGF β 3 isoform activity with at least 20-fold, 100-fold, 200-fold, 300-fold, 400-fold, 500-fold, 600-fold, 700-fold, 800-fold, 900-fold, or 1000-fold greater potency than TGF β 2 isoform activity.

[0023] In some embodiments, the first linker is 33 amino acids or shorter. In an embodiment, the first linker is from about 10 to 33 amino acids long. In embodiments, the first linker may be from about 15 to 33 amino acids long, or from about 18 to about 30 amino acids long. In an embodiment, the first linker is 16, 18, 30, or 32 amino acids long. In a particular embodiment, the first linker is 16 amino acids long. In another particular embodiment, the first linker is 18 amino acids long. In another particular embodiment, the first linker is 30 amino acids long. In another particular embodiment, the first linker is 32 amino acids long.

[0024] In some embodiments, the second linker is 10 amino acids or longer. In an embodiment, the second linker is from about 10 to about 35 amino acids long. In embodiments, the second linker may be from about 10 to about 34 or from about 15 to about 34 amino acids long. In an embodiment, the second linker is 16, 30, 32, or 34 amino acids long. In a particular embodiment, the second linker is 30 amino acids long. In another particular embodiment, the second linker is 16 amino acids long. In another particular embodiment, the second linker is 32 amino acids long. In another particular embodiment, the second linker is 34 amino acids long.

[0025] In an embodiment, the first linker is 18 amino acids and the second linker is 16 amino acids. In another embodiment, the first linker is 18 amino acids and the second linker is 30 amino acids. In another embodiment, the first linker is 18 amino acids and the second linker is 10 amino acids. In another embodiment, the first linker is 18 amino acids and the second linker is 32 amino acids. In another embodiment, the first linker is 18 amino acids and the second linker is 34 amino acids. In another embodiment, the first linker is 16 amino acids and the second linker is 18 amino acids. In another embodiment, the first linker is 16 amino acids and the second linker is 16 amino acids. In another embodiment, the first linker is 16 amino acids and the second linker is 30 amino acids. In another embodiment, the first linker is 16 amino acids and the second linker is 32 amino

acids. In another embodiment, the first linker is 26 amino acids and the second linker is 26 amino acids. In another embodiment, the first linker is 32 amino acids and the second linker is 32 amino acids. In another embodiment, the first linker is 32 amino acids and the second linker is 34 amino acids. It should be understood that many other permutations are possible, as long as the desired isoform-specificity of inhibition is achieved.

[0026] In some embodiments, one or more of the first linker and the second linker comprises or consists of an IDR linker, an IDR linker variant, a hybrid linker, a hybrid linker variant, a truncated linker, a truncated linker variant or an elongated linker disclosed herein. For example, one or more of the first linker and the second linker may independently comprise or consist of the amino acid sequence set forth in any one of SEQ ID NOs: 4 or 8-26, or a sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical thereto. In some embodiments, the first linker comprises or consists of the amino acid sequence set forth in any one of SEQ ID NOs: 8, 9, 10, 11, 12, 13, 14, 16, 21, 22, 23, and 26, or a sequence at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical thereto. In some embodiments, the second linker comprises or consists of the amino acid sequence set forth in any one of SEQ ID NOs: 4, 9, 11, 15, 17, 18, 19, 20, 22, 23, 24, 25, and 26, or a sequence at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical thereto.

[0027] In a representative embodiment, the first linker comprises or consists of the amino acid sequence set forth in SEQ ID NO: 12 and/or the second linker comprises or consists of the amino acids sequence set forth in SEQ ID NO: 11. In another representative embodiment, the first linker comprises or consists of the amino acid sequence set forth in SEQ ID NO: 8 and/or the second linker comprises or consists of the amino acids sequence set forth in SEQ ID NO: 9. It should be understood that other embodiments using combinations of linkers provided herein are encompassed, as long as the desired isoform-specificity of inhibition is achieved.

[0028] In some embodiments of polypeptides and TGF β binding agents of the present technology, the N-terminal region comprises or consists of an IDR linker, an IDR linker variant, a hybrid linker, a hybrid linker variant, a truncated linker, a truncated linker variant or an elongated linker. For example, the N-terminal region may comprise or consist of the amino acid sequence set forth in SEQ ID NO: 3, or a sequence at least 90%, at least 95%, at least 96%, at least 97%, at

least 98%, or at least 99% identical thereto.

[0029] In some embodiments of polypeptides and TGF β binding agents of the present technology, the first TGF β R-LBD and/or the second TGF β R-LBD comprises or consists of the amino acid sequence set forth in SEQ ID NO: 2, or a sequence at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical thereto. In some embodiments, the first TGF β R-LBD and the second TGF β R-LBD are the same or substantially the same. In other embodiments, the first TGF β R-LBD and the second TGF β R-LBD may have different amino acid sequences.

[0030] In some embodiments of polypeptides and TGF β binding agents of the present technology, the multimerization domain allows dimerization of two polypeptides in accordance with the present disclosure, in a non-covalent manner, e.g., by coiled-coil interactions, and the like.

[0031] In other embodiments, the multimerization domain allows dimerization of two polypeptides in accordance with the present disclosure in a covalent manner, e.g., by disulfide bridging, and the like.

[0032] In some embodiments, the multimerization domain comprises one or more constant region of an antibody, e.g., the second constant domain (C_{H2}) and/or the third constant domain (C_{H3}) of an antibody heavy chain, or an Fc region of an antibody heavy chain. The antibody may be, for example and without limitation, an IgG antibody such as an IgG1, IgG2, IgG3 or IgG4 antibody. In particular embodiments, the antibody is a human antibody, e.g., the multimerization domain comprises a constant region of the heavy chain of a human IgG1, IgG2, IgG3 or IgG4. In some embodiments, the multimerization domain has at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity with a human IgG1, IgG2, IgG3 or IgG4 constant region. In a particular embodiment, the multimerization domain comprises or consists of an Fc region of a human IgG1 antibody. In another particular embodiment, the multimerization domain comprises or consists of an Fc region of a human IgG4 antibody.

[0033] In some embodiments, the multimerization domain comprises one or more cysteine residue for crosslinking of a first polypeptide construct with a second polypeptide construct. For example, the multimerization domain may include at least two cysteine residues for forming a

disulfide bridge between two polypeptide constructs, thereby forming a dimer.

[0034] In some embodiments, the multimerization domain is engineered to reduce aggregation or to modulate stability of a dimer or multimer of the polypeptide construct. For example, an Fc region may contain one or more amino acid substitution that reduces aggregation and/or increases stability of the TGF β binding agent compared to naturally occurring Fc sequences. In some embodiments, the multimerization domain is selected to provide one or more effector function such as antibody dependent cellular cytotoxicity (ADCC), complement activation (complement dependent cytotoxicity or CDC), opsonization, and the like.

[0035] In some embodiments, the multimerization domain comprises or consists of the amino acid sequence set forth in any one of SEQ ID NOs: 49-80 or a sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical thereto. In a particular embodiment, the multimerization domain comprises or consists of the amino acid sequence set forth in SEQ ID NO:49, or a sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical thereto. In another particular embodiment, the multimerization domain comprises or consists of the amino acid sequence set forth in SEQ ID NO:50, or a sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical thereto.

[0036] In some embodiments of polypeptides and TGF β binding agents of the present technology, the TGF β binding region (comprising the N-terminal domain, the two LBDs, and the two linkers) comprises or consists of the sequence set forth in any one of SEQ ID NOs: 27-48, or a sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical thereto. In a particular embodiment, the TGF β -binding region comprises or consists of the amino acid sequence set forth in SEQ ID NO: 27. In another particular embodiment, the TGF β -binding region comprises or consists of the amino acid sequence set forth in SEQ ID NO: 29. In another particular embodiment, the TGF β -binding region comprises or consists of the amino acid sequence set forth in SEQ ID NO: 32. In another particular embodiment, the TGF β -binding region comprises or consists of the amino acid sequence set forth in SEQ ID NO: 41.

[0037] In another particular embodiment, the TGF β -binding region comprises or consists of

the amino acid sequence set forth in SEQ ID NO: 40.

[0038] In some embodiments of polypeptides and TGF β binding agents of the present technology, the polypeptide construct comprises or consists of the amino acid sequence set forth in any one of SEQ ID NOs: 81 to 103 and 105, or a sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical thereto. In a particular embodiment, the polypeptide construct comprises or consists of the amino acid sequence set forth in SEQ ID NO: 81. In another particular embodiment, the polypeptide construct comprises or consists of the amino acid sequence set forth in SEQ ID NO: 84. In another particular embodiment, the polypeptide construct comprises or consists of the amino acid sequence set forth in SEQ ID NO: 87. In another particular embodiment, the polypeptide construct comprises or consists of the amino acid sequence set forth in SEQ ID NO: 96.

[0039] In another particular embodiment, the polypeptide construct comprises or consists of the amino acid sequence set forth in SEQ ID NO: 95, or a sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical thereto. In another particular embodiment, the polypeptide construct comprises or consists of the amino acid sequence set forth in SEQ ID NO: 95.

[0040] In some embodiments, the polypeptide construct provided herein is a polypeptide construct comprising N-terminus to C-terminus: (i) an amino acid sequence consisting of the amino acid sequence of SEQ ID NO:40; and (ii) an Fc region of human IgG1.

[0041] In some embodiments of the present technology, a TGF β binding agent is heterodimeric, that is, the first and the second polypeptide are different. In such embodiments, the first and the second polypeptide may differ by one or more region or domain, e.g., by the sequence of the first linker, the second linker, the LBD, the multimerization domain, etc., as well as combinations thereof. Thus each of the following may independently be the same or different in the two polypeptides: the N-terminal region; the first linker; the second linker; the first LBD; the second LBD; and the multimerization domain. Many combinations are possible, as long as the desired isoform-specificity of inhibition is provided.

[0042] In some embodiments of the TGF β binding agent, the first polypeptide construct and the second polypeptide construct comprises or consists of the sequence set forth in SEQ ID NO:95,

or a sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical thereto. In some embodiments of the TGF β binding agent, the first polypeptide construct and the second polypeptide construct comprises or consists of the sequence set forth in SEQ ID NO:95.

[0043] In some embodiments of the TGF β binding agent, the different TGF β -binding regions comprise or consist of the amino acid sequence set forth in SEQ ID NO:95, or a sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical thereto.

[0044] In some embodiments of the TGF β binding agent, the TGF β binding agent comprises:
a first polypeptide construct comprising from N-terminus to C-terminus: (i) an amino acid sequence consisting of the amino acid sequence of SEQ ID NO:40; and (ii) a first Fc region of human IgG1, and

a second polypeptide construct comprising from N-terminus to C-terminus: (i) an amino acid sequence consisting of the amino acid sequence of SEQ ID NO:40; and (ii) a second Fc region of human IgG1;

wherein the first polypeptide construct and the second polypeptide construct are linked together through the first and second Fc region of human IgG1.

[0045] In some embodiments, the inhibitory potency of the TGF β binding agent for both TGF β 1 isoform activity and TGF β 3 isoform activity is greater than for TGF β 2 isoform activity; and wherein the relative inhibitory potency of the TGF β binding agent for TGF β 3 isoform activity compared to TGF β 1 isoform activity (IC₅₀ ratio for TGF β 3 TGF β 1) is about 2.5:1 or less.

[0046] In some embodiments, the TGF β binding agent provided herein is a homodimer of the polypeptide construct provided herein.

[0047] In alternate embodiments, a TGF β binding agent is homodimeric, that is, the first and the second polypeptide are the same or substantially the same.

[0048] In some embodiments, the polypeptide or the TGF β binding agent may be conjugated with a targeting agent, a therapeutic moiety, a detectable moiety and/or a diagnostic moiety.

[0049] In another broad aspect, there are provided nucleic acids encoding the polypeptides and TGF β binding agents of the present technology. Vectors and plasmids comprising such nucleic

acids and/or for expression of the polypeptides and TGF β binding agents are also provided. For example, in an embodiment there is provided a nucleic acid having the sequence set forth in any one of SEQ ID NOs: 106-109, and vectors and plasmids comprising these nucleic acids. In another embodiment, there is provided a nucleic acid having at least 80% sequence identity, at least 85% sequence identity, at least 90% sequence identity, at least 95% sequence identity, at least 98% sequence identity, or at least 99% sequence identity to SEQ ID NOs: 106-109, or capable of hybridizing thereto under conditions of high stringency. Cells expressing the polypeptides and TGF β binding agents of the present technology are also provided.

[0050] In further aspects, there are provided methods of manufacturing the polypeptides and TGF β binding agents of the present technology, comprising expressing one or more polypeptide provided herein in a cell, followed by isolation and/or purification thereof. In some embodiments, polypeptide constructs and TGF β binding agents are expressed in a form that is secretable by a cell, e.g., using a signal peptide at the N-terminus, allowing recovery of the polypeptide or TGF β binding agent from the culture medium.

[0051] In another broad aspect, there are provided pharmaceutical compositions comprising the polypeptide construct or the TGF β binding agent according to the present disclosure and a pharmaceutically acceptable carrier, diluent or excipient. In some embodiments, pharmaceutical compositions are formulated for administration by injection or infusion, e.g., for intravenous, subcutaneous, intraperitoneal, or intramuscular administration. In some embodiments, pharmaceutical compositions are provided in unit dosage form.

[0052] In yet another broad aspect, there are provided methods of preventing or treating a TGF β -associated disease or condition, the methods comprising administering a therapeutically effective amount of the polypeptide, TGF β binding agent or pharmaceutical composition of the present technology to a subject, such that the TGF β -associated disease or condition is prevented or treated.

[0053] Examples of TGF β -associated diseases or conditions that may be prevented or treated in accordance with the present disclosure include, for example and without limitation: fibrosis (e.g., fibrotic disease, fibrotic scarring, fibroproliferative disorders); cancer (e.g., malignancies, solid tumors, metastasis); and bone marrow failures (e.g., Shwachman-Bodian-Diamond

syndrome, Fanconi anemia). In some embodiments, the polypeptide or TGF β binding agent described herein is used for the treatment or prevention of fibrosis, including for example and without limitation, fibrotic disease of tissues and/or organs, and fibrotic scarring, e.g., pulmonary fibrosis (e.g., idiopathic pulmonary fibrosis), renal fibrosis, liver fibrosis (e.g., hepatic cirrhosis), systemic sclerosis, scleroderma, skin fibrosis, heart fibrosis, myelofibrosis, etc.

[0054] In some embodiments, there are provided methods of preventing or treating a disease or condition mediated by TGF β 1 and/or TGF β 3, the methods comprising administering a therapeutically effective amount of the polypeptide, TGF β binding agent or pharmaceutical composition of the present technology to a subject, such that the disease or condition mediated by TGF β 1 and/or TGF β 3 is treated. In an embodiment, there is provided a method of preventing or treating a disease or condition mediated by TGF β 3 in a subject in need thereof, the method comprising administering a therapeutically effective amount of the polypeptide, TGF β binding agent or pharmaceutical composition of the present technology to the subject, such that the disease or condition mediated by TGF β 3 is prevented or treated.

[0055] In some embodiments, there are provided methods of preventing or treating fibrosis in a subject in need thereof, the method comprising administering a therapeutically effective amount of the polypeptide, TGF β binding agent or pharmaceutical composition of the present technology to the subject, such that fibrosis is prevented or treated.

[0056] In a further broad aspect, there are provided kits and packages for treating a TGF β -associated disease or condition in a subject in need thereof, comprising a polypeptide, a TGF β binding agent or a pharmaceutical composition in accordance with the present disclosure; optionally one or more additional component such as acids, bases, buffering agents, inorganic salts, solvents, antioxidants, preservatives, or metal chelators, and/or tools for administration thereof such as syringes, needles, and the like. Instructions for administration or use may also be included.

[0057] In yet another aspect, provided herein are methods of manufacturing of the polypeptide construct or the TGF β binding agent provided herein, comprising culturing the host cell as provided herein under conditions suitable for protein expression; and harvesting the polypeptide construct or the TGF β binding agent.

[0058] In yet another aspect, provided herein are polypeptide constructs or TGF β binding

agents produced by the manufacturing methods provided herein.

[0059] Further scope, applicability and advantages of the present disclosure will become apparent from the non-restrictive detailed description given hereinafter. It should be understood, however, that this detailed description, while indicating exemplary embodiments of the disclosure, is given by way of example only, with reference to the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

[0060] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[0061] For a better understanding of the technology and to show more clearly how it may be carried into effect, reference will now be made by way of example to the accompanying drawings, which illustrate aspects and features according to non-limiting embodiments of the present technology.

[0062] **FIG. 1** shows a schematic structure of the domain organization of tetravalent TGF β binding agents, in accordance with certain embodiments. The embodiment shown here is a homodimer of a first polypeptide (Left side) and a second polypeptide (Right side) linked by a disulfide bridge in the multimerization domain (shown as two lines). Ligand-binding domains (LBDs) are shown as circles, multimerization domains are shown as ovals, and N-terminal regions and linkers are shown as rectangles. In embodiments where the binding agent is a heterodimer, the first and second polypeptides are different in one or more region or portion (not shown).

[0063] **FIG. 2A** shows an overlay of the monomeric structures of TGF β 1 (blue) and TGF β 3 (green).

[0064] **FIG. 2B** shows an overlay of the TGF β 1 dimer (blue) and the TGF β 3 dimer (green). The corresponding monomers in the area are superposed to show the monomer difference in the dimer angle.

[0065] **FIG. 2C** shows a representative model of T22d35-Fc-IgG1-v1(CC) (SEQ ID NO: 6) bound to TGF β ligand showing the second ligand binding domain, second linker, and multimerization domain (Fc) regions.

[0066] **FIG. 3A** shows polyacrylamide gel electrophoresis analysis under non-reducing conditions of representative TGF β binding agents. After expression and purification, 2 μ g of each protein was loaded on the gel, as indicated: Ctl: Control; p61: Protein 61; p96: Protein 96; p101: Protein 101; p107: Protein 107; p112: Protein 112.

[0067] **FIG. 3B** shows polyacrylamide gel electrophoresis analysis under reducing conditions of representative TGF β binding agents. After expression and purification, 2 μ g of each protein was loaded on the gel, as indicated: Ctl: Control; p61: Protein 61; p96: Protein 96; p101: Protein 101; p107: Protein 107; p112: Protein 112.

[0068] **FIG. 4A** shows representative results in the A549/IL-11 cell-based assay for inhibition of TGF β 1 for Proteins 61, 96, 101, 107, and 112, and Control, as indicated. The table lists the calculated IC₅₀ values calculated in Graphpad Prism. Error bars indicate standard error of the mean (SEM).

[0069] **FIG. 4B** shows representative results in the A549/IL-11 cell-based assay for inhibition of TGF β 3 for Proteins 61, 96, 101, 107, and 112, and Control, as indicated. The table lists the calculated IC₅₀ values calculated in Graphpad Prism. Error bars indicate standard error of the mean (SEM).

[0070] **FIG. 5A** shows polyacrylamide gel electrophoresis analysis under non-reducing conditions of the following representative TGF β binding agents: p112, p111, p108, p105, p104, p101, p99, and p71. Error bars indicate standard error of the mean (SEM).

[0071] **FIG. 5B** shows polyacrylamide gel electrophoresis analysis under reducing conditions of the following representative TGF β binding agents: p112, p111, p108, p105, p104, p101, p99, and p71. Error bars indicate standard error of the mean (SEM).

[0072] **FIG. 6A** shows representative results in the A549/IL-11 cell-based assay for inhibition of TGF β 1 for Proteins 113, 115, and 116, and Control, as indicated. The table lists the calculated IC₅₀ values calculated in Graphpad Prism. Error bars indicate standard error of the mean (SEM).

[0073] **FIG. 6B** shows representative results in the A549/IL-11 cell-based assay for inhibition of TGF β 3 for Proteins 113, 115, and 116, and Control, as indicated. The table lists the calculated IC₅₀ values calculated in Graphpad Prism. Error bars indicate standard error of the mean (SEM).

[0074] **FIG. 7A** shows representative results in the A549/IL-11 cell-based assay for inhibition of TGFβ1 for Proteins 101, 129, and 130, and Control, as indicated. The table lists the calculated IC₅₀ values calculated in Graphpad Prism. Error bars indicate standard error of the mean (SEM).

[0075] **FIG. 7B** shows representative results in the A549/IL-11 cell-based assay for inhibition of TGFβ3 for Proteins 101, 129, and 130, and Control, as indicated. The table lists the calculated IC₅₀ values calculated in Graphpad Prism. Error bars indicate standard error of the mean (SEM).

[0076] **FIG. 8A** shows representative results in the A549/IL-11 cell-based assay for inhibition of TGFβ1 for Proteins 101, 131, 132 and 133, and Control, as indicated. The table lists the calculated IC₅₀ values calculated in Graphpad Prism. Error bars indicate standard error of the mean (SEM).

[0077] **FIG. 8B** shows representative results in the A549/IL-11 cell-based assay for inhibition of TGFβ3 for Proteins 101, 131, 132 and 133, and Control, as indicated. The table lists the calculated IC₅₀ values calculated in Graphpad Prism. Error bars indicate standard error of the mean (SEM).

[0078] **FIG. 9A** shows representative results in the A549/IL-11 cell-based assay for inhibition of TGFβ1 for Proteins 96, 134, and 135, and Control, as indicated. The table lists the calculated IC₅₀ values calculated in Graphpad Prism. Error bars indicate standard error of the mean (SEM).

[0079] **FIG. 9B** shows representative results in the A549/IL-11 cell-based assay for inhibition of TGFβ3 for Proteins 96, 134, and 135, and Control, as indicated. The table lists the calculated IC₅₀ values calculated in Graphpad Prism. Error bars indicate standard error of the mean (SEM).

[0080] **FIG. 10A** shows representative results in the A549/IL-11 cell-based assay for inhibition of TGFβ1 for Proteins 101 and 128, and Control, as indicated. The table lists the calculated IC₅₀ values calculated in Graphpad Prism. Error bars indicate standard error of the mean (SEM).

[0081] **FIG. 10B** shows representative results in the A549/IL-11 cell-based assay for inhibition of TGFβ3 for Proteins 101 and 128, and Control, as indicated. The table lists the calculated IC₅₀ values calculated in Graphpad Prism. Error bars indicate standard error of the mean (SEM).

[0082] FIG. 11 shows representative results in the A549/IL-11 cell-based assay for inhibition of TGF β 2 for Proteins 61, 96 and 101, and Control, as indicated. The table lists the calculated IC₅₀ values calculated in Graphpad Prism. Error bars indicate standard error of the mean (SEM).

DETAILED DESCRIPTION

[0083] The present technology is explained in greater detail below. This description is not intended to be a detailed catalog of all the different ways in which the technology may be implemented, or all the features that may be added to the instant technology. For examples, features illustrated with respect to one embodiment may be incorporated into other embodiments, and features illustrated with respect to a particular embodiment may be deleted from that embodiment. In addition, numerous variations and additions to the various embodiments suggested herein will be apparent to those skilled in the art in light of the instant disclosure, which variations and additions do not depart from the present technology. Hence, the following description is intended to illustrate some particular embodiments of the technology, and not to exhaustively specify all permutations, combinations and variations thereof.

Definitions

[0084] In order to provide a clear and consistent understanding of the terms used in the present specification, a number of definitions are provided below. Moreover, unless defined otherwise, all technical and scientific terms as used herein have the same meaning as commonly understood to one of ordinary skill in the art to which this invention pertains.

[0085] The use of the terms “a” and “an” and “the” when used in conjunction with the term “comprising” in the claims and/or the specification may mean “one”, but it is also consistent with the meaning of “one or more”, “at least one”, and “one or more than one”. Similarly, the term “another” may mean at least a second or more. These terms are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context.

[0086] As used herein, the terms “comprising” (and any form of comprising, such as “comprise” and “comprises”), “having” (and any form of having, such as “have” and “has”), “including” (and any form of including, such as “include” and “includes”) or “containing” (and any form of containing, such as “contain” and “contains”), are inclusive or open-ended and do not

exclude additional, unrecited elements or process steps. The term “consisting of” is to be construed as close-ended.

[0087] The term “about” is used to indicate that a value or quantity refers to the actual given value and also the approximation of such given value that would reasonably be inferred based on the ordinary skill in the art, including equivalents and approximations due to the experimental and/or measurement conditions for such given value. For example, the term “about” in the context of a given value or range refers to a value or range that is within 20%, preferably within 15%, more preferably within 10%, more preferably within 9%, more preferably within 8%, more preferably within 7%, more preferably within 6%, and more preferably within 5% of the given value or range.

[0088] The expression “and/or” where used herein is to be taken as specific disclosure of each of the specified features or components with or without the other. For example “A and/or B” is to be taken as specific disclosure of each of (i) A, (ii) B, and (iii) A and B, just as if each is set out individually herein. Unless specifically stated or obvious from context, as used herein the term “or” is understood to be inclusive and covers both “or” and “and”. For example, an embodiment of “a composition comprising A or B” would typically present an aspect with a composition comprising both A and B. “Or” should, however, be construed to exclude those aspects presented that cannot be combined without contradiction (e.g., a composition pH that is between 9 and 10 or between 7 and 8).

[0089] It is to be understood herein that terms such as “from 1 to 20” include any individual values comprised within and including 1 and 20. Therefore, the term “from 1 to 20” includes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 and/or 20. Terms such as “from 1 to 20” also include any individual sub-ranges comprised within and including from 1 to 20. The term “from 1 to 20” therefore also includes sub-ranges such as “from 1 to 9”, “from 2 to 9”, “from 3 to 5”, from 5 to 9”, “from 5 to 20”, “from 8 to 20” etc. The same applies for similar expressions such as and not limited to “from 1 to 19”, “from 1 to 18”, “from 1 to 10”, “from 1 to 9”, “from 5 to 15”, etc.

[0090] It is to be understood herein that terms such as “from about 15 to about 35” include any individual values comprised within and including 15 and 35. Therefore, terms such as “from about 15 to about 35” include any number between and including 15 and 35 such as 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34 and/or 35. Terms such as “from

about 15 to about 35” also include any individual sub-ranges comprised within and including from 15 to 35, “from about 16 to about 34”, “from about 16 to about 24”, from about 24 to about 34” and the like. The term “about” in the context of the number of amino acids means that the specified number of amino acids is specifically encompassed and allows a variation of +/- 2 in the number of amino acid residues. As such, the terms such as “from about 15 to about 35” also includes “from 13 to 37”, “from 13 to 35”, “from 17 to 37”, from 17 to 35”, etc. The same applies for similar expressions such as and not limited to “from about 16 to about 34”, “from about 16 to about 24”, from about 24 to about 34” and the like.

[0091] It is to be understood herein that terms such as “at least 80% identical” include any individual values comprised within and including from 80% to 100% and including 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% and 100%. The term “at least 80% identical” also includes any individual sub-ranges comprised within and including from 80% to 100%, such as for example, “from 85% to 99%”, “from 97% to 100%”, “from 90% to 100%”, etc. The same applies for similar expressions such as, and not limited to, expressions such as “at least 70% identical”, “at least 90% identical”, and the like.

[0092] As used herein, the term “inhibition potency” refers to effectiveness of a substance in inhibiting a specific biological or biochemical function such as, without limitation, binding between a protein receptor and its ligand, or activation of a cell receptor by its ligand. In some embodiments, potency of inhibition is determined by measuring the IC₅₀ of an inhibitor for a particular ligand or substrate. In that case, relative inhibition potency for different inhibitors and/or ligands may be assessed by comparing IC₅₀ values. For example, relative inhibition potency of 3:1 means the ratio of IC₅₀ values is 3:1. The terms “inhibition potency”, “inhibitory potency”, “potency of inhibition” and “neutralization potency” are used interchangeably herein.

[0093] As used herein, the term “IC₅₀” refers to the half maximal inhibitory concentration (i.e., the concentration of a substance that is required for 50% inhibition in vitro). It is a measure of the potency or effectiveness of a substance in inhibiting a specific biological or biochemical function. IC₅₀ values are typically expressed as molar concentration. The IC₅₀ of an inhibitor can be determined by constructing a dose-response curve and examining the effect of different concentrations of inhibitor on the specific biological or biochemical function in question.

[0094] As used herein, the term “avidity” refers to the overall strength of binding interactions between a protein receptor and its ligand. Avidity generally refers to the accumulated strength of multiple, individual non-covalent binding interactions, such as between a protein receptor and its ligand, and is distinct from “affinity”, which describes the strength of a single binding interaction. It should be understood that avidity is rarely the mere sum of its constituent affinities as many factors (such as local concentration or proximity, multimerization, 3D structure or conformation, etc.) can affect biomolecular interactions.

[0095] As used herein, the term “functionally equivalent” refers to variant sequences that have the same or substantially the same biological activity or function as the original sequence from which it is derived, e.g., no significant change in physiological, chemical, physico-chemical or functional properties compared to the original sequence. The term “substantially identical” refers to sequences that are functionally equivalent to the original or reference sequence and have a high degree of sequence identity thereto. Generally, a substantially identical sequence is at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identical to the original or reference sequence and has the same function. In some cases when referring to nucleic acid sequences, a substantially identical sequence hybridizes to the original sequence under high stringency conditions, for example at salt and temperature conditions substantially equivalent to 0.5 X SSC to about 5 X SSC and 65 °C for both hybridization and wash. In general, variant sequences that are substantially identical or functionally equivalent to sequences provided in accordance with the present disclosure are meant to be encompassed.

[0096] As used herein, the term “multimerization domain” refers to an amino acid sequence that allows polypeptide chains to assemble into a multimer. The term “multimer” refers to a molecule made from multiple monomers. The term “multimer” encompasses, without limitation, dimers, trimers, 4-mers, 5-mers, 6-mers, 8-mers, 10-mers, etc.

[0097] The term “dimeric” refers to the presence of two polypeptides as described herein in the TGF β binding agent. “Homodimeric” means the two polypeptides have the same sequence, whereas “heterodimeric” means the two polypeptides have different sequences.

[0098] The term “doublet” refers to the presence of two copies of the TGF β R ligand binding domain (LBD) linked together in tandem in the polypeptide.

[00099] The term “tetraivalent” refers to the presence of four copies of TGF β R ligand binding domain (LBD) in the TGF β binding agent.

Polypeptides and TGF β binding agents

[00100] There are provided herein novel polypeptide constructs comprising a TGF β binding region and a multimerization domain, and TGF β binding agents comprising two such polypeptide constructs assembled via the multimerization domain. The TGF β binding region comprises two TGF β RII-LBDs linked in tandem by a first linker, and it is linked to the multimerization domain by a second linker. Polypeptide constructs and TGF β binding agents of the present disclosure are optimized by improving their association with TGF β . Specifically, the linkers are selected to optimize TGF β isoform specificity such that the TGF β 3:TGF β 1 IC₅₀ ratio is no more than about 2.5:1 (indicating similar inhibition potency for both isoforms), without increasing undesired inhibition of TGF β 2, and without significantly reducing the overall potency (e.g., IC₅₀ remains in the picomolar range).

[00101] In exemplary embodiments, the polypeptide constructs and TGF β binding agents of the present disclosure include two polypeptide chains that are associated via an Fc region of an antibody or via a constant CH2 domain, a constant CH3 domain and/or via a combination of CH2 and CH3. The constant region of the antibody may be from a human IgG1, IgG2, IgG3 or IgG4 antibody, or substantially identical thereto. The association of both polypeptide chains generally occurs during expression and secretion of the protein, e.g. in mammalian cells. In some exemplary embodiments, TGF β binding agents may comprise homodimers, i.e., dimers of a polypeptide construct having the sequence set forth in any one of SEQ ID NOs: 81 to 103 and 105, or a sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical thereto. In other embodiments, TGF β binding agents comprise heterodimers, i.e., dimers of two different polypeptide constructs, at least one of the polypeptide constructs having the sequence set forth in any one of SEQ ID NOs: 81 to 103 and 105, or a sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical thereto.

[00102] In general, polypeptide constructs and TGF β binding agents are organized such that the multimerization domain is linked at its N-terminus to the C-terminus of the TGF β

binding region, so that for each polypeptide, the orientation of the construct is, from N-terminus to C-terminus, a single chain of (N-terminal region)-(first TGF β R-LBD)-(first linker)-(second TGF β R-LBD)-(second linker)-multimerization domain.

[00103] In an exemplary embodiment, the multimerization domain allows assembly of two or more polypeptide chains in a covalent manner, for example by disulfide linking between cysteine residues. Alternatively, the multimerization domain may allow polypeptide chains to be assembled in a non-covalent manner such as, for example and without limitation, by coiled-coil structure (De Crescenzo, G. et al., 2004).

[00104] In an embodiment, the multimerization domain is a dimerization domain, i.e., allows assembly of two polypeptide chains, to form a dimer. In accordance with the present disclosure, such dimers generally comprise two polypeptides, each polypeptide including two TGF β R-LBDs linked together and linked to the dimerization domain as described herein, thereby forming a tetravalent TGF β binding agent. Homodimers and heterodimers of polypeptide constructs provided herein are encompassed.

[00105] In some embodiments, the multimerization or dimerization domain of the polypeptide comprises constant regions of an immunoglobulin heavy chain, including for example a CH2 and/or CH3 domain. An Fc portion of an immunoglobulin is typically used. However, a coiled-coil structure has also been found to be suitable for dimerization. Exemplary embodiments of Fc portions include, for example and without limitation, those that have lost their ability to interact with a particular Fc receptor. In additional embodiments, the multimerization domain may comprise an IgG-like dimerization domain, e.g., an IgG1, IgG2, IgG3, or IgG4 dimerization domain. In some embodiments, the multimerization domain may provide one or more effector function such as antibody dependent cellular cytotoxicity (ADCC), complement activation (complement dependent cytotoxicity, CDC), or opsonization.

[00106] In some embodiments, the multimerization or dimerization domain comprises a CH2, a CH3, or a CH2 and a CH3 from an antibody heavy chain that is of human 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. In embodiments, the constant domain in the constructs is CH2 *per se*, or CH3 *per se*, or CH2-CH3. The antibody heavy chain component typically provides for disulfide crosslinking between single chain polypeptide

constructs that are the same or different. In an embodiment, the multimerization domain provides for at least one disulfide link between single chain polypeptide constructs. In another embodiment, the multimerization domain provides for at least two disulfide links between single chain polypeptide constructs. In some cases, the antibody heavy chain also provides for protein A-based isolation of the dimeric polypeptide, e.g. after production in host cells.

[00107] Thus in some embodiments, the multimerization or dimerization domain is an antibody constant domain that provides for cross-linking between two of the present polypeptide constructs. This is achieved when, for example, expressed polypeptide constructs are secreted from their expression host. Thus, production of a single chain polypeptide may provide the construct in a dimeric form in which the two polypeptide chains are cross-linked via disulfide bridges that involve one or more cysteine residues within each of the antibody constant domains present in each of the polypeptides. In some embodiments, the multimerization domain (e.g., the constant region) has no particular activity, other than to act as a structure through which multimers (e.g., dimers) can form. Such minimal constant regions can also be altered to provide some benefit, by incorporating the corresponding hinge regions and optionally changing the cysteine residue composition. For example, 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. One advantage of minimizing the number of cysteine residues is to reduce the propensity for disulfide bond scrambling, which could promote aggregation. It should be noted that not all of the naturally-occurring inter-hinge disulfide bonds need to be formed for Fc dimerization to occur, while noting that the stability of the Fc dimer may depend on the number of inter-molecular disulfide bridges.

[00108] As used herein, the terms "antibody" and "immunoglobulin (Ig)" are used interchangeably to refer to a protein constructed from paired heavy and light polypeptide chains. The structures of an antibody and of each of the domains are well established and familiar to those of skill in the art, and are summarized only briefly here. When an antibody is correctly folded, each chain folds into a number of distinct globular domains joined by more linear polypeptide sequences. In particular, the Ig light chain folds into a variable (VL) and a constant (CL) domain, while the heavy chain folds into a variable (VH) and three constant (CH1, CH2, CH3) domains. Once paired, interaction of the heavy and light chain variable domains (VH and VL) and first constant domain (CL and CH1) results in the formation of a Fab (Fragment, antigen-binding)

containing the binding region (Fv); interaction of two heavy chains results in pairing of CH2 and CH3 domains, leading to the formation of a Fc (Fragment, crystallisable). Characteristics described herein for the CH2 and CH3 domains also apply to the Fc.

[00109] In certain embodiments and aspects of the present disclosure, the multimerization or dimerization domain may have at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% or 100% sequence identity with an IgG1, IgG2, IgG3 or IgG4 constant region or with the CH2 and/or CH3 domain. The IgG1, IgG2, IgG3 or IgG4 may be from a human. In a particular embodiment, the TGF β binding agents described herein include those having a dimerization domain of an IgG1. In another particular embodiment, the TGF β binding agents described herein include those having a dimerization domain of an IgG4.

[00110] A multimerization or dimerization domain may be engineered to reduce aggregation or to modulate stability of a TGF β binding agent formed by the assembly of more than one polypeptide disclosed herein. Fc portions having mutation(s) in, e.g., the hinge region are therefore encompassed by the present disclosure. Exemplary embodiments of Fc variants and modified hinge regions are provided for example in patent applications published under Nos. WO2018/158727 and WO2017/037634. It should be understood that, when the hinge portion of a multimerization or dimerization domain is referenced, the hinge is part of the multimerization domain and is not considered part of the second linker.

[00111] In exemplary embodiments, multimerization or dimerization domains have the sequence set forth in SEQ ID NOs: 49-80, or a functionally equivalent variant thereof, or a sequence at least about 80%, at least about 90%, at least about 95%, at least about 96%, at least about 98%, or at least about 99% identical thereto. In a particular embodiment, the multimerization domain may comprise SEQ ID NO: 49 or a sequence at least about 80%, at least about 90%, at least about 95%, at least about 96%, at least about 98%, or at least about 99% identical thereto. In another embodiment, the multimerization domain may comprise SEQ ID NO: 50 or a sequence at least about 80%, at least about 90%, at least about 95%, at least about 96%, at least about 98%, or at least about 99% identical thereto.

[00112] In accordance with the present disclosure, the first multimerization or dimerization domain and the second multimerization or dimerization domain of a TGF β binding

agent may have the same or substantially the same amino acid sequence in certain embodiments. Alternatively, in some embodiments the multimerization or dimerization domain may be different, as long as multimerization is not adversely affected.

[00113] It should be understood that the multimerization domain is not meant to be particularly limited. Any amino acid sequence that allows association of the polypeptide chains to form a tetravalent TGF β binding agent in accordance with the present disclosure may be used, as long as the desired function and isoform specificity is maintained.

[00114] In accordance with the present disclosure, linkers in the polypeptide constructs and TGF β binding agents may comprise or consist of an IDR linker, an IDR linker variant, a hybrid linker, a hybrid linker variant, a truncated linker, a truncated linker variant or an elongated linker, as disclosed herein.

[00115] The human TGF β RII ectodomain (TGF β RII-ECD; SEQ ID NO: 1) includes a 102 amino acid structured ligand-binding domain (SEQ ID NO: 2; also referred to herein as “TGF β R-LBD”) that is flanked by two intrinsically disordered regions: a region of 24 amino acids at the N-terminal (SEQ ID NO: 3) and a region of 10 amino acids at the C-terminal (SEQ ID NO: 4).

[00116] As used herein, the term “intrinsically disordered region (IDR) linker” refers to a linker comprising or consisting of at least a portion of one or both of the intrinsically disordered regions (IDRs) that flank the structured, ligand-binding domain of the TGF β RII ectodomain. An IDR linker generally possesses substantial sequence identity with at least one sequence of an intrinsically disordered region of the TGF β RII ectodomain, and it may possess substantial sequence identity with both the N- and C-terminal IDRs of the TGF β RII ectodomain or portions thereof. It should be understood that an IDR linker may comprise the entire IDR of the TGF β R or only a portion thereof, or multiple portions linked together.

[00117] In some embodiments, an IDR linker comprises or consists of a portion of one or both of the IDRs (SEQ ID NOS: 3 and 4) of the human TGF β RII-ECD (SEQ ID NO: 1). In embodiments where a portion of each of the two IDRs of the TGF β R is included, the portions may be linked together either directly or via an intervening linker sequence. The portions of the IDRs may include the entire IDR sequence or variants (e.g., substitutions, truncations) thereof.

[00118] In one embodiment, an IDR linker comprises or consists of a portion of each IDR linked directly together. In certain embodiments, the C-terminal IDR or a portion thereof is linked directly to the N-terminus of the N-terminal IDR or a portion thereof. Non-limiting examples of such embodiments include, for example, linkers having the amino acid sequence set forth in SEQ ID NOs: 8-16, 19, 22, 23, and 26.

[00119] In one embodiment, an IDR linker comprises or consists of the sequence set forth in SEQ ID NO: 4.

[00120] In one embodiment, an IDR linker does not consist of the sequence set forth in SEQ ID NO: 7. In such embodiments, polypeptides and TGF β binding agents comprising the sequence set forth in SEQ ID NO: 7 are excluded from the present invention. In some embodiments, IDR linkers that do not provide the desired isoform specificity (e.g., the desired TGF β 3:TGF β 1 IC₅₀ ratio) are excluded from the present invention, as are polypeptides and TGF β binding agents comprising such sequences.

[00121] IDR linker variants, hybrid linkers, hybrid linker variants, truncated linkers, truncated linker variants and elongated linkers are derived from the sequences of IDR linkers disclosed herein.

[00122] As used herein the term “non-IDR linker” means a linker that does not share substantial homology or identity with the intrinsically disordered regions (IDRs) that flank the structured, ligand-binding domain of the TGF β RII ectodomain. In aspects and embodiments described herein, the non-IDR linker may be a flexible linker, including for example, and without limitation glycine and glycine-serine (GS) linkers. It is a common practice when producing fusion constructs to introduce artificial, highly flexible glycine or glycine-serine linkers such as GGGGS or [G4S]_n (where n is 1, 2, 3, 4 or 5 or more, such as 10, 25 or 50) between the various regions of the constructs. However, such artificial linkers can also be disadvantageous due to their potential for undesired immunogenicity and their added molecular weight. Entropic factors are also a potential liability for glycine and GS linkers, which are highly flexible and may become partially restricted upon target binding, causing a loss of entropy that disfavors binding. Therefore in some embodiments, polypeptides and TGF β binding agents of the present disclosure do not include a non-IDR linker, or include at least one IDR linker or IDR linker variant in addition to the non-IDR

linker. Non-limiting examples of non-IDR linkers in accordance with the present disclosure include SEQ ID NOs: 17, 20, 21 and 24.

[00123] In some embodiments, a linker comprises or consists of a mixture of an IDR and a GS linker, such as, for example, the amino acid sequence set forth in SEQ ID NOs: 18 and 25. Such linkers are referred to herein as hybrid linkers. In some embodiments, there are provided linkers in which from 3 to 7 or from 3 to 14 amino acid residues in any one of SEQ ID NOs: 4, 8-16, 19, 22, 23, and 26 have been replaced with an amino acid sequence comprising glycine and/or serine residues (a glycine or a GS linker). These linkers are referred to herein as hybrid linkers. Hybrid linker variants are also encompassed; these are functionally equivalent variants of hybrid linkers that include one or more insertion, deletion, or amino acid substitution, optionally a conservative amino acid substitution. Variants are discussed further below.

[00124] In exemplary embodiments of hybrid linkers and hybrid linker variants, at least 3 consecutive amino acids of IDR linkers or IDR linker variants are replaced with glycine and/or serine residues. In further exemplary embodiments of hybrid linkers or hybrid linker variants, at least 7 consecutive amino acids of IDR linkers or IDR linker variants are replaced with glycine and/or serine residues. In yet further exemplary embodiments of hybrid linkers or hybrid linker variants, two sets of from 3 to 7 consecutive amino acids of IDR linkers or IDR linker variants are replaced with glycine and/or serine residues. The two sets of 3 to 7 consecutive amino acids may be spaced within a linker sequence or may be consecutive.

[00125] Examples of glycine and GS sequences for use in hybrid linkers and hybrid linker variants include, without limitation, GSG, and any one of SEQ ID NOs: 17, 18, 20, 21, 24, and 25.

[00126] In some embodiments, a linker is a truncated linker or a truncated linker variant. Such linkers have a truncation (deletion) of, for example, from 1 to about 20 consecutive amino acids (and any range comprised within 1 and about 20 such as, for example, from 1 to about 10, 1 to about 5, etc.) at either or both the N- or C-terminus of an IDR linker provided herein. In an exemplary embodiment, the amino acid truncation may be at the N-terminus of any one of SEQ ID NO:3 or 8-26. In an exemplary embodiment the truncation may result in the removal of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 residues at the N-terminus. In another exemplary embodiment, the truncation may be at the C-terminus of any one of SEQ ID NOs: 4

and 8-26. In an exemplary embodiment the truncation may result in the removal of 1, 2, 3, 4, 5, 6, 7, 8, or 9 residues at the C-terminus. In another embodiment, the truncation may result in the removal of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 residues at the C-terminus.

[00127] In another embodiment, the truncation may be an internal deletion such as for example a deletion starting at amino acid number 10 or 11 of SEQ ID NO: 7. In an embodiment, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 residues are deleted from SEQ ID NO: 7, including amino acid number 10 and/or 11. In other embodiments, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 residues are deleted internally from any one of SEQ ID NOs: 4 and 8-26.

[00128] In an exemplary embodiment, the amino acid truncation may result in the removal of from 1 to 10 amino acids encompassing the region defined by amino acid residues numbers 11 to 20 of any one of SEQ ID NOs: 7-26.

[00129] Other exemplary and non-limiting embodiments of truncated linkers are provided in SEQ ID NOs: 8-16, 18, 19, 22, 23, and 26.

[00130] The present disclosure further provides truncated linker variants. Such truncated linker variants may comprise an amino acid substitution (conservative or non-conservative) in comparison to the truncated linkers disclosed herein.

[00131] In some embodiments, the first linker comprises or consists of an amino acid sequence having: (a) a deletion of at least one N-terminal amino acid residue in comparison with SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 12 or SEQ ID NO: 8; (b) a deletion of at least one C-terminal amino acid residue in comparison with SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 12 or SEQ ID NO: 8; (c) a deletion of at least one internal amino acid residue in comparison with SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 12 or SEQ ID NO: 8; or (d) one or more substitution in the amino acid sequence in comparison with SEQ ID NO: 3, SEQ ID NO: 7, SEQ ID NO: 12, SEQ ID NO: 8, or of any one of (a) to (c). In an embodiment thereof, the amino acid deletion is a deletion of 16 amino acids of SEQ ID NO: 3.

[00132] In some embodiments, the second linker comprises or consists of an amino acid sequence having: (a) a deletion of at least one N-terminal amino acid residue in comparison

with SEQ ID NO: 7 or SEQ ID NO: 9 or SEQ ID NO: 11; (b) a deletion of at least one C-terminal amino acid residue in comparison with SEQ ID NO: 7 or SEQ ID NO: 9 or SEQ ID NO: 11; (c) a deletion of at least one internal amino acid residue in comparison with SEQ ID NO: 7 or SEQ ID NO: 9 or SEQ ID NO: 11; or (d) one or more substitution in the amino acid sequence in comparison with SEQ ID NO: 4, 7, 9 or 11, or of any one of (a) to (c).

[00133] In some embodiments, a linker is an elongated linker. Such linkers have an addition (elongation) of from 1 to 10 amino acids (and any range comprised within 1 and 10 such as for example, from 1 to 7, from 1 to 5, from 1 to 3, 1, 2, 3 etc.) at either or both the N- or C-terminus of any of an IDR linker, an IDR linker variant, a hybrid linker, a hybrid linker variant, a truncated linker, or a truncated linker variant, as disclosed herein. These additional amino acids may each independently be selected from any amino acid residue.

[00134] In an exemplary embodiment, the linkers disclosed herein may comprise from 1 to 5 additional amino acid residues at their N-terminus. In another exemplary embodiment, the linkers disclosed herein may comprise from 1 to 5 additional amino acid residues at their C-terminus. In a further exemplary embodiment, the linkers disclosed herein may comprise from 1 to 5 additional amino acid residues at both their N-terminus and C-terminus. Such additional amino acid residues may be selected from any amino acid residues and may be either the same or different. Other exemplary and non-limiting embodiments of elongated linkers encompass addition of from 1 to 10 amino acids (and any range comprised within 1 and 10 such as for example, from 1 to 7, from 1 to 5, from 1 to 3, 1, 2, 3 etc.) at either or both the N- or C-terminus of any one of SEQ ID NOs: 4 and 8-26. The added sequence may comprise any amino acid residues.

[00135] Exemplary embodiments of elongated linkers also include those comprising a non-IDR linker portion at either or both of its N- or C-terminus. For example, the IDR linker, the IDR linker variant, the hybrid linker, the hybrid linker variant, the truncated linker or the truncated linker variant may be flanked by at least one non-IDR linker at either or both of its N- and C-terminus. Alternatively, a non-IDR linker may be flanked by at least the IDR linker, the IDR linker variant, the hybrid linker, the hybrid linker variant, the truncated linker or the truncated linker variant at either or both of its N- and C-terminus.

[00136] In some embodiments of the polypeptide constructs and TGF β binding agents of the present disclosure, the N-terminal region comprises or consists of the N-terminal IDR (SEQ ID NO: 3) in the TGF β RII-ECD (SEQ ID NO: 1), or a sequence substantially identical thereto, such as without limitation a truncated or substituted variant thereof. It should be understood that the N-terminal region may be truncated and/or substituted and otherwise modified, as long as desired inhibition potency and specificity are not adversely affected.

[00137] The present disclosure also encompasses variants of the polypeptides and the TGF β binding agents described herein. Variants encompassed by the present disclosure include those having a variation in the amino acid sequence of any one of the elements (first and second TGF β receptor ligand-binding domain (TGF β R-LBD), first linker, second linker, N-terminal region, multimerization domain, etc.) of the polypeptide or TGF β binding agent. Variants of the polypeptide or TGF β binding agent include, for example, those having similar or improved binding affinity, avidity, isoform-specificity, potency of inhibition, stability, manufacturability, and/or reduced aggregation in comparison with the polypeptides and TGF β binding agents disclosed herein.

[00138] A site of interest for substitutional mutagenesis includes the multimerization domain of the polypeptide or TGF β binding agent. Exemplary embodiments of polypeptide or TGF β binding agent variants of the present disclosure may comprise those having a modified IgG1, IgG2, IgG3, or IgG4 constant region or a portion thereof. TGF β binding agents that may comprise an IgG1 constant region (modified or unmodified) are encompassed herewith. TGF β binding agents that may comprise an IgG4 constant region (modified or unmodified) are also encompassed herewith.

[00139] Variants encompassed by the present disclosure include those which may comprise an insertion, a deletion or an amino acid substitution (conservative or non-conservative). These variants may have at least one amino acid residue in its amino acid sequence removed and a different residue inserted in its place.

[00140] In general, a conservative amino acid substitution is the substitution of an amino acid residue for another amino acid residue with similar chemical properties (e.g., size,

charge, or polarity). Conservative substitutions may be made by exchanging an amino acid from one of the groups listed below (group 1 to 6) for another amino acid of the same group.

[00141] Other exemplary embodiments of conservative substitutions are shown in Table 1 under the heading of "preferred substitutions". If such substitutions result in an undesired property, then more substantial changes, denominated "exemplary substitutions" in Table 1, or as further described below in reference to amino acid classes, may be introduced and the products screened.

[00142] It is known in the art that variants may be generated by substitutional mutagenesis and retain the biological activity (i.e., functional equivalence) of the polypeptides of the present disclosure. These variants have at least one amino acid residue in the amino acid sequence removed and a different residue inserted in its place, e.g., one or more conservative amino acid substitution. Examples of substitutions identified as "conservative substitutions" are shown in Table 1. If such substitutions result in a change not desired, then other types of substitutions, denominated "exemplary substitutions" in Table 1, or as further described herein in reference to amino acid classes, are introduced and the products screened.

[00143] Amino acid residues may be divided into groups based on common side chain properties, as follows:

- (group 1) hydrophobic: norleucine, methionine (Met), Alanine (Ala), Valine (Val), Leucine (Leu), Isoleucine (Ile) ;
- (group 2) neutral hydrophilic: Cysteine (Cys), Serine (Ser), Threonine (Thr), Asparagine (Asn), Glutamine (Gln);
- (group 3) acidic: Aspartic acid (Asp), Glutamic acid (Glu);
- (group 4) basic: Histidine (His), Lysine (Lys), Arginine (Arg);
- (group 5) residues that influence chain orientation: Glycine (Gly), Proline (Pro); and
- (group 6) aromatic: Tryptophan (Trp), Tyrosine (Tyr), Phenylalanine (Phe).

[00144] Non-conservative substitutions will entail exchanging a member of one of these classes for another.

Table 1. Exemplary amino acid substitutions.

Original residue	Exemplary substitution	Conservative substitution
Ala (A)	Val, Leu, Ile	Val
Arg (R)	Lys, Gln, Asn	Lys
Asn (N)	Gln, His, Lys, Arg, Asp	Gln
Asp (D)	Glu, Asn	Glu
Cys (C)	Ser, Ala	Ser
Gln (Q)	Asn, Glu	Asn
Glu (E)	Asp, Gln	Asp
Gly (G)	Ala	Ala
His (H)	Asn, Gln, Lys, Arg,	Arg
Ile (I)	Leu, Val, Met, Ala, Phe, norleucine	Leu
Leu (L)	Norleucine, Ile, Val, Met, Ala, Phe	Ile
Lys (K)	Arg, Gln, Asn	Arg
Met (M)	Leu, Phe, Ile	Leu
Phe (F)	Leu, Val, Ile, Ala, Tyr	Tyr
Pro (P)	Ala	Ala
Ser (S)	Thr	Thr
Thr (T)	Ser	Ser
Trp (W)	Tyr, Phe	Tyr
Tyr (Y)	Trp, Phe, Thr, Ser	Phe
Val (V)	Ile, Leu, Met, Phe, Ala, norleucine	Leu

[00145] Generally, the degree of similarity and identity between variable chains is determined herein using the Blast2 sequence program (Tatusova, T.A. and Madden, T.L., 1999) using default settings, i.e., blastp program, BLOSUM62 matrix (open gap 11 and extension gap penalty 1; gapx dropoff 50, expect 10.0, word size 3) and activated filters.

[00146] However, the level of identity may also be determined over the entire length of a given sequence. Percent identity will therefore be indicative of amino acids which are identical in comparison with the original peptide and which may occupy the same or similar position.

Percent similarity will be indicative of amino acids which are identical and those which are replaced with conservative amino acid substitution in comparison with the original peptide at the same or similar position.

[00147] In some embodiments, variants of the present disclosure therefore comprise amino acid sequences which have at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity with an original sequence or a portion of an original sequence.

[00148] In some embodiments, variation in the amino acid sequence occurs in the TGF β receptor ligand-binding domain (TGF β R-LBD) of the polypeptide or TGF β binding agent. In other embodiments, variation may occur outside of the TGF β receptor ligand-binding domain (TGF β R-LBD) of the TGF β binding agent. Variants encompassed by the present disclosure may have a TGF β R-LBD that is identical or substantially identical to the structured ligand-binding domain found in the ectodomain (ECD) of TGF β receptors (including in human, animals etc.). In further embodiments, variation in the amino acid sequence occurs in the multimerization domain. In still other embodiments, variation in the amino acid sequence occurs in the first and/or second linker. It should be understood that variation may occur in multiple regions of the polypeptide or TGF β binding agent, as long as the desired function is maintained.

[00149] In some embodiments, the polypeptide or TGF β binding agent of the present disclosure may be conjugated, for example with a targeting agent, a therapeutic moiety (for therapeutic purposes) or with a detectable moiety (i.e., for detection or diagnostic purposes).

[00150] In an exemplary embodiment, the polypeptide or TGF β binding agent of the present disclosure is conjugated with a therapeutic moiety such as, for example and without limitation, a chemotherapeutic, a cytokine, a cytotoxic agent, an anti-fibrotic drug, an anti-cancer drug (e.g., small molecule), a single chain antibody, and the like.

[00151] In another exemplary embodiment, the polypeptide or TGF β binding agent of the present disclosure is conjugated with a detectable moiety including, for example and without limitation, a moiety detectable by spectroscopic, photochemical, biochemical, immunochemical,

chemical and/or other physical means. A detectable moiety may be coupled either directly or indirectly (for example via a linkage, such as, without limitation, a DOTA or NHS linkage) to the TGF β binding agent using methods well known in the art. A wide variety of detectable moieties may be used, with the choice depending on the sensitivity required, ease of conjugation, stability requirements and available instrumentation. A suitable detectable moiety may include, but is not limited to, a fluorescent label, a radioactive label (for example, without limitation, ^{125}I , In^{111} , Tc^{99} , I^{131} and including positron emitting isotopes for PET scanner etc), a nuclear magnetic resonance active label, a luminescent label, a chemiluminescent label, a chromophore label, an enzyme label (for example and without limitation horseradish peroxidase, alkaline phosphatase, etc.), quantum dots and/or a nanoparticle. A detectable moiety may cause and/or produce a detectable signal thereby allowing for a signal from the detectable moiety to be detected.

[00152] A therapeutic moiety may include, for example and without limitation, Yttrium-90, Scandium-47, Rhenium-186, Iodine-131, Iodine-125, and many others recognized by those skilled in the art (e.g., lutetium (e.g., Lu^{177}), bismuth (e.g., Bi^{213}), copper (e.g., Cu^{67})), 5-fluorouracil, adriamycin, irinotecan, taxanes, pseudomonas endotoxin, ricin, auristatins (e.g., monomethyl auristatin E, monomethyl auristatin F), maytansinoids (e.g., mertansine), and other toxins.

[00153] In another embodiment, a therapeutic moiety may include another therapeutic for a TGF β -associated disease or condition. For example and without limitation, one or more polypeptide construct or TGF β binding agent may be linked to a cytotoxic drug in order to generate an antibody-drug conjugate (ADC).

[00154] A targeting agent may include, for example, an amino acid sequence for delivering the polypeptide or TGF β binding agent to a desired tissue, organ or location in a subject's body. For example and without limitation, a targeting agent may comprise a poly-aspartate sequence motif for bone targeting, or an antibody or antigen-binding fragment.

[00155] In other exemplary embodiments, a targeting agent, therapeutic moiety or diagnostic moiety may comprise, for example and without limitation, an antibody or antigen binding fragment thereof (e.g., single chain antibody), a binding agent having affinity for another member of the TGF β family or for another therapeutic target, a radiotherapy agent, an imaging agent, a fluorescent moiety, a cytotoxic agent, an anti-mitotic drug, a nanoparticle-based carrier, a

polymer-conjugated to drug, nanocarrier, imaging agent, a stabilizing agent, a drug, a nanocarrier and/or a dendrimer.

[00156] It should be understood that the site for conjugation is not particularly limited, as long as the function of the polypeptide or TGF β binding agent is not adversely affected. For example and without limitation, a targeting agent, therapeutic moiety or detectable moiety may be conjugated in the linker portion of a polypeptide or TGF β binding agent (e.g., in a non-IDR linker) or at any other suitable site such as at its N-terminus or in the multimerization domain.

Production of polypeptides and TGF β binding agents

[00157] The polypeptide or TGF β binding agent disclosed herein may be made by a variety of methods familiar to those skilled in the art, including by recombinant DNA methods.

[00158] In order to express the polypeptides or TGF β binding agents, nucleotide sequences able to encode the polypeptide chain described herein may be inserted into an expression vector, i.e., a vector that contains the elements for transcriptional and translational control of the inserted coding sequence in a particular host. These elements may include regulatory sequences, such as enhancers, constitutive and inducible promoters, and 5' and 3' un-translated regions. Methods that are well known to those skilled in the art may be used to construct such expression vectors. These methods include *in vitro* recombinant DNA techniques, synthetic techniques, *in vivo* genetic recombination and the like.

[00159] A variety of expression vector and host cell systems known to those of skill in the art may be used to express the polypeptide chains described herein. These include, but are not limited to, microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with baculovirus vectors; plant cell systems transformed with viral or bacterial expression vectors; and animal cell systems. For long-term production of recombinant proteins in mammalian systems, stable expression in mammalian cell lines may be used. For example, nucleotide sequences able to encode any one of the polypeptide chains described herein may be transformed into cell lines using expression vectors that may contain viral origins of replication and/or endogenous expression elements and a selectable or visible marker gene on the same or on a separate vector. The present disclosure is not to be limited by the vector or host cell

employed. In certain embodiments disclosed herein, nucleic acids able to encode polypeptide chains described herein may be ligated into expression vectors. In the event that the TGF β binding agent is composed of distinct polypeptide chains (i.e., the first polypeptide and the second polypeptide are not identical), each of such polypeptide chain may be ligated into separate vectors or into the same vector. In accordance with the present disclosure, the polypeptide chains of the TGF β binding agent may be encoded by a single vector or by separate vectors (e.g., a vector set). Cells are transformed with the desired vector or vector sets.

[00160] Alternatively, the polypeptide chains may be expressed from an *in vitro* transcription system or a coupled *in vitro* transcription/translation system respectively or any such cell-free system.

[00161] Host cells comprising nucleotide sequences may be cultured under conditions for the transcription of the corresponding RNA (mRNA, etc.) and/or the expression and secretion of the polypeptide(s) from cell culture. In an exemplary embodiment, expression vectors containing nucleotide sequences able to encode the polypeptide chains described herein may be designed to contain signal sequences that direct secretion of the polypeptide through a prokaryotic or eukaryotic cell membrane.

[00162] Due to the inherent degeneracy of the genetic code, DNA sequences that encode the same, substantially the same or a functionally equivalent amino acid sequence may be produced and used. The nucleotide sequences of the present disclosure may be engineered using methods generally known in the art in order to alter the nucleotide sequences for a variety of purposes including, but not limited to, modification of the cloning, processing, and/or expression of the gene product. DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. For example, oligonucleotide-mediated site-directed mutagenesis may be used to introduce mutations that create new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, and so forth. Codon-optimized nucleic acids encoding the polypeptide chains described herein are encompassed by the present disclosure.

[00163] In addition, a host cell strain may be chosen for its ability to modulate expression of the inserted sequences or to process the expressed polypeptide in the desired fashion. Different host cells that have specific cellular machinery and characteristic mechanisms for post-

translational activities (e.g., CHO, HeLa, MDCK, HEK293, and W138) are available commercially and from the American Type Culture Collection (ATCC) and may be chosen to ensure the correct modification and processing of the expressed polypeptide.

[00164] Those of skill in the art will also readily recognize that the nucleic acid and polypeptide sequences may be synthesized, in whole or in part, using chemical or enzymatic methods well known in the art. For example, peptide synthesis may be performed using various solid-phase techniques and machines such as the ABI 431A Peptide synthesizer (PE Biosystems) may be used to automate synthesis. If desired, the amino acid sequence may be altered during synthesis and/or combined with sequences from other proteins to produce a variant protein.

Pharmaceutical compositions

[00165] Pharmaceutical compositions comprising the polypeptides or TGF β binding agents disclosed herein are also encompassed by the present disclosure. The pharmaceutical composition generally comprises the polypeptide or TGF β binding agent disclosed herein and a pharmaceutically acceptable carrier.

[00166] The preparation of pharmaceutical compositions can be carried out as known in the art (see, for example, Remington: The Science and Practice of Pharmacy, 20th Edition, 2000). For example, a therapeutic compound and/or composition, together with one or more solid or liquid pharmaceutical carrier substances and/or additives (or auxiliary substances) and, if desired, in combination with other pharmaceutically active compounds having therapeutic or prophylactic action, are brought into a suitable administration form or dosage form which can then be used as a pharmaceutical in human or veterinary medicine. Pharmaceutical preparations can also contain additives, of which many are known in the art, for example fillers, disintegrants, binders, lubricants, wetting agents, stabilizers, emulsifiers, dispersants, preservatives, sweeteners, colorants, flavorings, aromatizers, thickeners, diluents, buffer substances, solvents, solubilizers, agents for achieving a depot effect, salts for altering the osmotic pressure, coating agents or antioxidants.

[00167] The term "pharmaceutical composition" means a composition comprising a polypeptide or TGF β binding agent as described herein and at least one component comprising pharmaceutically acceptable carriers, diluents, adjuvants, excipients, or vehicles, such as

preserving agents, fillers, disintegrating agents, wetting agents, emulsifying agents, suspending agents, sweetening agents, flavoring agents, perfuming agents, antibacterial agents, antifungal agents, lubricating agents and dispensing agents, depending on the nature of the mode of administration and dosage forms.

[00168] The term "pharmaceutically acceptable carrier" is used to mean any carrier, diluent, adjuvant, excipient, or vehicle, as described herein or as known in the art. Examples of suspending agents include ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth, or mixtures of these substances. Prevention of the action of microorganisms can be ensured by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, and the like. It may also be desirable to include isotonic agents, for example sugars, sodium chloride, and the like. Prolonged absorption of the injectable pharmaceutical form can be brought about by the use of agents delaying absorption, for example, aluminum monostearate and gelatin. Non-limiting examples of suitable carriers, diluents, solvents, or vehicles include water, salt solutions, phosphate buffered saline (PBS), gelatins, oils, alcohols, polyols, suitable mixtures thereof, vegetable oils (such as olive oil), and injectable organic esters such as ethyl oleate. Non-limiting examples of excipients include lactose, milk sugar, sodium citrate, calcium carbonate, and dicalcium phosphate. Non-limiting examples of disintegrating agents include starch, alginic acids, and certain complex silicates. Non-limiting examples of lubricants include magnesium stearate, sodium lauryl sulphate, talc, as well as high molecular weight polyethylene glycols.

[00169] The term "pharmaceutically acceptable" means it is, within the scope of sound medical judgment, suitable for use in contact with the cells of a subject, e.g., humans and animals, without undue toxicity, irritation, allergic response, and the like, and are commensurate with a reasonable benefit/risk ratio.

[00170] A pharmaceutically acceptable carrier may include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. In one embodiment, the carrier is suitable for parenteral administration. The carrier may be suitable for intravenous, intraperitoneal, subcutaneous or intramuscular administration. Alternatively, the carrier may be suitable for sublingual or oral administration. In other embodiments, the carrier is suitable for topical

administration or for administration via inhalation. Pharmaceutically acceptable carriers include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the pharmaceutical compositions provided herein is contemplated. Supplementary active compounds can also be incorporated into the compositions. For example, a pharmaceutical composition provided herein may further comprise at least one additional therapeutic agent, as discussed further below.

[00171] In some embodiments, a pharmaceutical composition provided herein can be administered orally, for example in the form of pills, tablets, lacquered tablets, sugar-coated tablets, granules, hard and soft gelatin capsules, aqueous, alcoholic or oily solutions, syrups, emulsions or suspensions, or rectally, for example in the form of suppositories.

[00172] In other embodiments, a pharmaceutical composition provided herein can be administered parenterally, for example subcutaneously, intramuscularly or intravenously in the form of solutions for injection or infusion. Other suitable administration forms are, for example, percutaneous or topical administration, for example in the form of ointments, creams, tinctures, sprays or transdermal therapeutic systems, or the inhalative administration in the form of nasal sprays or aerosol mixtures, or, for example, microcapsules, implants or wafers.

[00173] Pharmaceutical compositions typically must be sterile and stable under the conditions of manufacture and storage. A composition can be formulated as a solution, microemulsion, liposome, or other ordered structure suitable to high drug concentration. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Prolonged absorption of injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, monostearate salts and gelatin. Moreover, a compound can be administered in a time release formulation, for example in a composition which

includes a slow release polymer. The compound can be prepared with carriers that will protect against rapid release, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, polylactic acid and polylactic, polyglycolic copolymers (PLG).

[00174] Many methods for the preparation of such formulations are generally known to those skilled in the art. Sterile injectable solutions can be prepared by incorporating an active compound, such as a polypeptide or TGF β binding agent provided herein, in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, common methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. Compounds may also be formulated with one or more additional compounds that enhance their solubility.

[00175] It is often advantageous to formulate compositions (such as parenteral compositions) in dosage unit form for ease of administration and uniformity of dosage. The term "unit dosage form" refers to a physically discrete unit suitable as unitary dosages for human subjects and other animals, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect, in association with a suitable pharmaceutical carrier. The specification for the dosage unit forms of the invention may vary and are dictated by and directly dependent on (a) the unique characteristics of the therapeutic compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such a therapeutic compound for the prevention or treatment of a TGF β associated disease or disorder. Dosages are discussed further below.

[00176] In some embodiments, there are provided pharmaceutical compositions that comprise an effective amount of a polypeptide and/or TGF β binding agent described herein, and a pharmaceutically acceptable carrier. In an embodiment, there are provided pharmaceutical compositions for the treatment or prevention of fibrosis, comprising a polypeptide or TGF β

binding agent described herein, and a pharmaceutically acceptable carrier. In another embodiment, there is provided a pharmaceutical composition for the delay of progression of a cancer, for the inhibition of cancer invasion, e.g., malignant glial cell (MGC) invasion, for inhibition of cancer stem cell growth, survival, spheroid formation and/or proliferation, for inhibition of metastasis, for inhibition of cancer recurrence, and/or for overcoming chemoresistance of a cancer, the composition comprising a polypeptide and/or TGF β binding agent described herein, and a pharmaceutically acceptable carrier. In another embodiment, there is provided a pharmaceutical composition for treating or preventing a bone marrow failure state.

[00177] As used herein "pharmaceutically acceptable carrier" or "pharmaceutical carrier" are known in the art and include, but are not limited to, 0.01-0.1 M or 0.05 M phosphate buffer or 0.8 % saline. Additionally, such pharmaceutically acceptable carriers may be aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers such as those based on Ringer's dextrose, and the like. Preservatives and other additives may also be present, such as, for example, antimicrobials, antioxidants, collating agents, inert gases and the like.

[00178] For any compound, the therapeutically effective dose may be estimated initially either in cell culture assays or in animal models such as mice, rats, rabbits, dogs, or pigs. An animal model may also be used to determine the concentration range and route of administration. Such information may then be used to determine useful doses and routes for administration in humans. These techniques are well known to one skilled in the art and a therapeutically effective dose refers to that amount of active ingredient that ameliorates the symptoms or condition. Therapeutic efficacy and toxicity may be determined by standard pharmaceutical procedures in cell cultures or with experimental animals, such as by calculating and contrasting the ED₅₀ (the dose therapeutically effective in 50% of the population) and LD₅₀ (the dose lethal to 50% of the population). Any of the pharmaceutical compositions described

herein may be applied to any subject in need of therapy, including, but not limited to, mammals such as dogs, cats, cows, horses, rabbits, monkeys, and especially humans.

[00179] The pharmaceutical compositions described herein may be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual, or rectal means.

Methods of use

[00180] The polypeptide or TGF β binding agent described herein, and pharmaceutical compositions thereof, are useful for prevention or treatment of a TGF β -associated disease or condition. As such, there are provided methods for prevention or treatment of a TGF β -associated disease or condition in a subject, the methods comprising administering a therapeutically effective amount of the polypeptide, TGF β binding agent or pharmaceutical composition described herein. Polypeptides and TGF β binding agents are generally administered in the form of a pharmaceutical composition. A subject may be in need of such treatment, i.e., having, suspected of having, or at risk of having a disease or condition associated with TGF β (e.g., TGF β 1 and/or TGF β 3).

[00181] As used herein, the term “TGF β -associated disease or condition” refers to diseases or conditions that may be ameliorated through inhibition of TGF β activity, particularly TGF β 1 and/or TGF β 3 activity. TGF β -associated diseases or conditions include, without limitation, diseases or conditions associated with over-expression or over-activation of TGF β ligands, particularly TGF β 1 and/or TGF β 3. In some embodiments, a TGF β -associated disease or condition is mediated by TGF β 1 and/or TGF β 3. In one embodiment, the disease or condition to be treated is mediated by TGF β 3. In another embodiment, the disease or condition to be treated is mediated by a combination of TGF β 1 and TGF β 3. As used herein, the term “amelioration” means to decrease, suppress, attenuate, diminish, arrest, or stabilize the development or progression of a disease.

[00182] Examples of TGF β -associated diseases or conditions that may be prevented or treated in accordance with the present disclosure include, without limitation: fibrosis (e.g., fibrotic disease, fibrotic scarring, fibroproliferative disorders); cancer (e.g., malignancies, solid

tumors, metastasis); bone marrow failures (e.g., Shwachman-Bodian-Diamond syndrome, Fanconi anemia); ocular diseases; and genetic disorders of connective tissue.

[00183] In some embodiments, the polypeptide or TGF β binding agent described herein is used for treatment or prevention of fibrosis, including for example and without limitation, fibrotic disease of tissues and/or organs, fibrotic scarring, and fibroproliferative disorders. Non-limiting examples of fibrotic diseases or conditions that may be treated or prevented include pulmonary fibrosis (e.g., idiopathic pulmonary fibrosis), renal fibrosis, liver fibrosis (e.g., hepatic cirrhosis), systemic sclerosis, scleroderma, skin fibrosis, heart fibrosis, bone marrow fibrosis, and myelofibrosis. In one particular embodiment, systemic sclerosis (SSc) is treated or prevented. In another particular embodiment, scleroderma is treated or prevented. In another particular embodiment, myelofibrosis (MF) is treated or prevented.

[00184] Systemic Sclerosis (SSc, also called scleroderma) is a severely debilitating fibrotic disease. TGF β is a potent profibrotic cytokine that has been shown to be critical for the promotion of several pathological processes including increased collagen deposition in skin and lungs in SSc (Varga, J. and Abraham, D., 2007; Varga, J. and Whitfield, M.L., 2009; Gabrielli, A. et al., 2009; Lafyatis, R., 2014; Allanore, Y. et al., 2015). SSc represents a major unmet therapeutic challenge with the life expectancy of patients with newly diagnosed SSc being approximately eleven years (Mayes, M.D. et al., 2003). A recent clinical study validated TGF β as a driver of fibrosis in SSc human patients with the demonstration of a dramatic reversal in fibrosis following blockade of TGF β by the neutralizing antibody fresolimumab (Rice, L.M. et al., 2015). This clinical proof-of-principal, together with extensive preclinical data demonstrating the importance of TGF β in promoting fibrosis in SSc and other diseases, provides a compelling rationale for the use of TGF β binding agents in accordance with the present disclosure for the treatment of SSc patients.

[00185] In myelofibrosis (MF), bone marrow fibrosis is a hallmark of the disease and its degree correlates with clinical features including anemia. Administration of a TGF β blocking agent has been shown to result in resolution of myelofibrosis in several preclinical studies (Wang, J.C. et al., 2006; Vannucchi, A.M. et al., 2005) and supports a dual pathological role for TGF β in MF, namely promotion of bone marrow fibrosis as well as myeloproliferation. Elevated intraplatelet, peripheral blood mononuclear cell, and megakaryocyte-associated TGF β has been

documented in MF patients. The over-expression of TGF β in clinical samples, together with extensive preclinical data on the effect of TGF β neutralization in MF models, provides a compelling rationale for the use of TGF β binding agents in accordance with the present disclosure for the treatment of MF patients.

[00186] Other exemplary embodiments of fibrosis that may be prevented or treated include, for example and without limitation: interstitial lung disease; human fibrotic lung disease (e.g., obliterative bronchiolitis, idiopathic pulmonary fibrosis, pulmonary fibrosis from a known etiology, tumor stroma in lung disease, systemic sclerosis affecting lungs, Hermansky-Pudlak syndrome, coal worker's pneumoconiosis, asbestosis, silicosis, chronic pulmonary hypertension); AIDS-associated treatable types of fibrosis, including lung fibrosis, cystic fibrosis, liver fibrosis, heart fibrosis, mediastinal fibrosis, retroperitoneal cavity fibrosis, bone marrow fibrosis, skin fibrosis; scleroderma; and systemic sclerosis. Specific forms of fibrosis that can be treated or prevented include those that affect any organ or tissue or cell of the body, such as human tenon's fibroblasts, kidney, lung, intestine, liver, heart, bone marrow, genitalia, skin and eye. These diseases include, but are not limited to, cystic fibrosis, systemic sclerosis, chronic obstructive pulmonary disease (COPD), Dupuytren's contracture, glomerulonephritis, liver fibrosis, post-infarction cardiac fibrosis, restenosis, ocular surgery-induced fibrosis, and scarring. Genetic disorders of connective tissue can also be treated, and include but are not limited to, Marfan syndrome (MFS) and Osteogenesis imperfecta.

[00187] In some embodiments, the polypeptide or TGF β binding agent described herein is used for inhibiting differentiation of fibroblasts into myofibroblasts.

[00188] In some embodiments, the polypeptide or TGF β binding agent described herein is used for treatment or prevention of a fibroproliferative disorder. Fibroproliferative disorders are characterized by proliferation of fibroblasts plus the corresponding overexpression of extracellular matrix such as fibronectin, laminin and collagen.

[00189] In some embodiments, the polypeptide or TGF β binding agent described herein is used for treatment or prevention of cancer, including for example and without limitation, lung cancer, head and neck cancer, melanoma, colon cancer, pancreatic cancer, colorectal cancer, hepatic cancer, breast cancer, epithelial cancer, cholangiocarcinoma, solid tumors, and the like. In some embodiments, the term "prevention" with respect to cancer may include preventing invasion

or metastasis of the main tumor. In some embodiments, the term “treatment” with respect to cancer may include inhibiting TGF β -mediated suppression of the immune response in the tumor microenvironment. With respect to solid tumors, the immunosuppressive role of TGF β in the tumor microenvironment has been clearly demonstrated preclinically. Additionally, it was recently shown in a clinical study that the lack of response to an immune checkpoint inhibitor in patients with bladder cancer is associated with TGF β signaling in the tumor microenvironment, supporting the concept that TGF β restrains anti-tumor immunity, and suggesting that TGF β inhibitors may have single agent activity in some tumor settings, and act in other tumor settings to enhance anti-tumor activity when combined with immune checkpoint inhibitors.

[00190] In some embodiments, the polypeptide construct or TGF β binding agent described herein is used for treatment or prevention of a disease of abnormal cell growth and/or dysregulated apoptosis. Examples of such diseases include, but are not limited to, cancer, mesothelioma, bladder cancer, pancreatic cancer, skin cancer, cancer of the head or neck, cutaneous or intraocular melanoma, ovarian cancer, breast cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, bone cancer, colon cancer, rectal cancer, cancer of the anal region, stomach cancer, gastrointestinal (gastric, colorectal and/or duodenal) cancer, chronic lymphocytic leukemia, acute lymphocytic leukemia, esophageal cancer, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, testicular cancer, hepatocellular (hepatic and/or biliary duct) cancer, primary or secondary central nervous system tumor, primary or secondary brain tumor, Hodgkin's disease, chronic or acute leukemia, chronic myeloid leukemia, lymphocytic lymphoma, lymphoblastic leukemia, follicular lymphoma, lymphoid malignancies of T-cell or B-cell origin, melanoma, multiple myeloma, oral cancer, non-small-cell lung cancer, prostate cancer, small-cell lung cancer, cancer of the kidney and/or ureter, renal cell carcinoma, carcinoma of the renal pelvis, neoplasms of the central nervous system, primary central nervous system lymphoma, non-Hodgkin's lymphoma, spinal axis tumors, brain stem glioma, pituitary adenoma, adrenocortical cancer, gall bladder cancer, cancer of the spleen, cholangiocarcinoma, fibrosarcoma, neuroblastoma, retinoblastoma or a combination thereof.

[00191] In some embodiments, the polypeptide construct or TGF β binding agent described herein is used for treatment or prevention of a disease or disorder selected from the group consisting of bladder cancer, brain cancer, breast cancer, bone marrow cancer, cervical cancer, chronic lymphocytic leukemia, acute lymphocytic leukemia, colorectal cancer, esophageal cancer, hepatocellular cancer, lymphoblastic leukemia, follicular lymphoma, lymphoid malignancies of T-cell or B-cell origin, melanoma, myelogenous leukemia, myeloma, oral cancer, ovarian cancer, non-small-cell lung cancer, prostate cancer, small-cell lung cancer and spleen cancer.

[00192] In some embodiments, the polypeptide construct or TGF β binding agent described herein is used for treatment or prevention of a disease or disorder that is a hematological cancer, such as leukemia, lymphoma, or myeloma. In some embodiments, the cancer is selected from the group consisting of Hodgkin's lymphoma, non-Hodgkin's lymphoma (NHL), cutaneous B-cell lymphoma, activated B-cell lymphoma, diffuse large B-cell lymphoma (DLBCL), mantle cell lymphoma (MCL), follicular center lymphoma, transformed lymphoma, lymphocytic lymphoma of intermediate differentiation, intermediate lymphocytic lymphoma (ILL), diffuse poorly differentiated lymphocytic lymphoma (PDL), centrocytic lymphoma, diffuse small-cleaved cell lymphoma (DSCCL), peripheral T-cell lymphomas (PTCL), cutaneous T-Cell lymphoma, mantle zone lymphoma, low grade follicular lymphoma, multiple myeloma (MM), chronic lymphocytic leukemia (CLL), diffuse large B-cell lymphoma (DLBCL), myelodysplastic syndrome (MDS), acute T cell leukemia, acute myeloid leukemia (AML), acute promyelocytic leukemia, acute myeloblastic leukemia, acute megakaryoblastic leukemia, precursor B acute lymphoblastic leukemia, precursor T acute lymphoblastic leukemia, Burkitt's leukemia (Burkitt's lymphoma), acute biphenotypic leukemia, chronic myeloid lymphoma, chronic myelogenous leukemia (CML), and chronic monocytic leukemia. In a specific embodiment, the disease or disorder is myeloma. In a specific embodiment, the disease or disorder is myelodysplastic syndromes (MDS). In another specific embodiment, the disease or disorder is acute myeloid leukemia (AML). In another specific embodiment, the disease or disorder is chronic lymphocytic leukemia (CLL). In yet another specific embodiment, the myeloma is multiple myeloma (MM).

[00193] In other embodiments, the polypeptide construct or TGF β binding agent described herein is used for treatment or prevention of a disease or disorder that is a solid tumor

malignancy. In some embodiments, the solid tumor malignancy is selected from the group consisting of a carcinoma, an adenocarcinoma, an adrenocortical carcinoma, a colon adenocarcinoma, a colorectal adenocarcinoma, a colorectal carcinoma, a ductal cell carcinoma, a lung carcinoma, a thyroid carcinoma, a nasopharyngeal carcinoma, a melanoma, a non-melanoma skin carcinoma, and a lung cancer.

[00194] In some embodiments, the solid tumor malignancy is an advanced non-CNS-primary solid tumor. In some embodiments, the solid tumor malignancy is selected from a group consisting of gastric/gastroesophageal junction (GEJ) cancer, bladder/urothelial cancer, and non-small-cell lung cancer (NSCLC).

[00195] In some embodiments, the immune checkpoint inhibitor to be administered in combination with the polypeptide or TGF β binding agent described herein can be any pharmaceutical agent that inhibits or blocks the activity of an inhibitory immune checkpoint molecule. In specific embodiments, the activity is binding to the natural binding partner of the immune checkpoint molecule. If the immune checkpoint molecule is a receptor, the activity can be ligand-binding activity. If the immune checkpoint molecule is a ligand, the activity can be receptor-binding activity.

[00196] In specific embodiments, the immune checkpoint inhibitor to be administered in combination with the polypeptide or TGF β binding agent described herein is a negative checkpoint regulator that is involved in T-Cell activation. In certain, more specific embodiments, such a negative checkpoint regulator is Cytotoxic T-lymphocyte antigen-4 (CTLA-4), CD80, CD86, Programmed cell death 1 (PD-1), Programmed cell death ligand 1 (PD-L1), Programmed cell death ligand 2 (PD-L2), Lymphocyte activation gene-3 (LAG-3; also known as CD223), Galectin-3, B and T lymphocyte attenuator (BTLA), T-cell membrane protein 3 (TIM3), Galectin-9 (GAL9), B7-H1, B7-H3, B7-H4, T-Cell immunoreceptor with Ig and ITIM domains (TIGIT/Vstm3/WUCAM/VSIG9), V-domain Ig suppressor of T-Cell activation (VISTA), Glucocorticoid-induced tumor necrosis factor receptor-related (GITR) protein, Herpes Virus Entry Mediator (HVEM), OX40, CD27, CD28, CD137, CGEN-15001T, CGEN-15022, CGEN-15027, CGEN-15049, CGEN-15052, or CGEN-15092. An overview such checkpoint regulators and drugs that target them is set forth in Table 1. In a specific embodiment, the immune checkpoint inhibitor is an inhibitor of PD-1, PD-L1, PD-L2, CTLA-4, LAG3, TIM-3, VISTA, A2AR, B7-H3, B7-H4, BTLA, IDO, or TDO.

[00197] In certain embodiments, the immune checkpoint inhibitor can be an antibody, a small molecule, or an oligonucleotide (such as an aptamer, an shRNA, miRNA, siRNA, or antisense DNA). In specific embodiments, the immune checkpoint inhibitor has been approved by Food and Drug Administration (FDA) in the United States or a foreign counterpart agency for the treatment of the cancer or a disease caused by the pathogen.

[00198] In specific embodiments, the immune checkpoint inhibitor is an antibody that binds to and inhibits the activity of the immune checkpoint. Antibodies that can be the immune checkpoint inhibitor include, but are not limited to, monoclonal antibodies (including Fc-optimized monoclonal antibodies), polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies), antibody fragments retaining antigen-binding activity, such as Fv, Fab, Fab', F(ab')₂, diabodies, linear antibodies, single-chain antibody molecules (e.g., scFv), multispecific antibodies formed from antibody fragments, and fusion proteins containing antibody fragments. In a specific embodiment, the antibody is a monoclonal antibody. Preferably, the antibody is a humanized antibody.

[00199] In certain embodiments, the immune checkpoint inhibitor is an inhibitor of PD-1. In a specific embodiment, the immune checkpoint inhibitor is a monoclonal antibody that binds to and inhibits the activity (e.g., ligand-binding activity) of PD-1.

[00200] In certain embodiments, the monoclonal antibody is selected from the group consisting of nivolumab, pidilizumab, MEDI0680, pembrolizumab, AMP-224, AMP-514, STI-A1110, TSR-042, AUR-012, cemiplimab, spartalizumab, camrelizumab, sintilimab, tislelizumab, and toripalimab.

[00201] In a specific embodiment, the monoclonal antibody is nivolumab, pidilizumab, MEDI0680, or pembrolizumab. In a further specific embodiment, the monoclonal antibody is nivolumab. In another specific embodiment, the immune checkpoint inhibitor that is an inhibitor of PD-1 is AMP-224. In another specific embodiment, the immune checkpoint inhibitor that is an inhibitor of PD-1 is pidilizumab. In another specific embodiment, the immune checkpoint inhibitor that is an inhibitor of PD-1 is pembrolizumab. In another specific embodiment, the immune checkpoint inhibitor that is an inhibitor of PD-1 is MEDI0680. In another specific embodiment, the immune checkpoint inhibitor that is an inhibitor of PD-1 is STI-A1110. In another specific embodiment, the immune checkpoint inhibitor that is an inhibitor of

PD-1 is TSR-042. In another specific embodiment, the immune checkpoint inhibitor that is an inhibitor of PD-1 is AUR-012.

[00202] In certain embodiments, the immune checkpoint inhibitor is an inhibitor of PD-L1. In a specific embodiment, the immune checkpoint inhibitor is a monoclonal antibody that binds to and inhibits the activity (e.g., receptor-binding activity) of PD-L1.

[00203] In certain embodiments, the immune checkpoint inhibitor is selected from the group consisting of mpdl3280A, durvalumab, avelumab, BMS-936559, atezolizumab, RG7446, and STI-A1010.

[00204] In a specific embodiment, the monoclonal antibody is mpdl3280A, durvalumab, avelumab, BMS-936559, or atezolizumab. In another specific embodiment, the immune checkpoint inhibitor that is an inhibitor of PD-L1 is RG7446. In another specific embodiment, the immune checkpoint inhibitor that is an inhibitor of PD-L1 is STI-A1010.

[00205] In certain embodiments, the immune checkpoint inhibitor is an inhibitor of CTLA4 (for example, ipilimumab).

[00206] In certain embodiments, the immune checkpoint inhibitor is an inhibitor of LAG3 (for example, BMS-986016).

[00207] In certain embodiments, immune checkpoint inhibitors to be administered in combination with the polypeptide or TGF β binding agent described herein include but are not limited to: OPDIVO® (nivolumab); YERVOY® (ipilimumab); relatilimab; linrodostat; EMPLICITI® (elotuzumab); BMS-986258; BMS 986315; BMS-986207; BMS-986249; and BMS-986218.

[00208] PD-1 inhibitors useful in the combinations described herein include any molecule capable of inhibiting, blocking, abrogating or interfering with the activity or expression of PD-1. In particular, an anti-PD-1 inhibitor can be a small molecule compound, a nucleic acid, a polypeptide, an antibody, a peptibody, a diabody, a minibody, a single-domain antibody or nanobody, a single-chain variable fragment (ScFv), or a functional fragment or variant thereof. In one instance the PD-1 inhibitor is a small molecule compound (e.g., a compound having a molecule weight of less than about 1000 Da.) In other embodiments, useful PD-1 inhibitors in the combinations described herein include nucleic acids and polypeptides.

[00209] In some embodiments, there are provided methods for preventing or inhibiting recurrence of a cancer after treatment, e.g., after drug treatment or surgical excision. In some embodiments, there are provided methods for delaying the progression of a cancer, wherein cancer re-growth is delayed by more than 30%, or by more than 50%, or by more than 70%, and/or wherein the survival periods of affected subjects are increased. There are further provided methods for enhancing the efficacy of cancer therapies for the treatment of cancer, selected from the group comprising resection, chemotherapy, radiation therapy, immunotherapy, and/or gene therapy, comprising administering a polypeptide or TGF β binding agent as described herein, and simultaneously, separately or sequentially administering said cancer therapy. The term "enhancing the efficacy of a cancer therapy", as used herein, refers to an improvement of conventional cancer treatments and includes reduction of the amount of the anti-cancer composition which is applied during the conventional cancer treatment, e.g. amount of radiation in radiotherapy, of chemotherapeutics in chemotherapy, of immunotherapeutics in immunotherapy or of vectors in gene based therapies, and/or to an increase in efficacy of the conventional therapy and the anti-cancer composition when applied at conventional doses or amounts during the conventional cancer therapy. In one embodiment, enhancing the efficacy of a cancer therapy refers to prolonging the survival rate of subjects receiving the therapy.

[00210] In some embodiments, the polypeptide or TGF β binding agent described herein is used for treating or preventing bone marrow failure in a subject, e.g., a human having or at risk of developing bone marrow failure. Exemplary types of bone marrow failure include, without limitation, SDS (also known as Shwachman-Bodian-Diamond syndrome or SBDS), Fanconi anemia (FA), dyskeratosis congenita (DC), congenital amegakaryocytic thrombocytopenia (CAMT), Blackfan-Diamond anemia (BDA), and reticular dysgenesis (RD). Shwachman-Diamond Syndrome (SDS) patients suffer from bone marrow failure, exocrine pancreatic dysfunction, skeletal anomalies, and increased risk of acute myeloid leukemia. In a particular embodiment, the polypeptide or TGF β binding agent described herein is used for treating or preventing Fanconi anemia (FA) in a subject. In another particular embodiment, the polypeptide or TGF β binding agent described herein is used for treating or preventing Shwachman-Diamond Syndrome (SDS) in a subject.

[00211] Fanconi anemia (FA) is the most common inherited bone marrow failure syndrome. FA patients develop bone marrow failure during the first decade of life due to attrition

of hematopoietic stem and progenitor cells (HSPCs). FA is caused by mutations in one of nineteen Fanconi anemia complementation group (FANC) genes, the products of which cooperate in the FA/BRCA DNA repair pathway. Bone marrow failure in FA may be the result, directly or indirectly, of hyperactivation of growth-suppressive pathways induced, in part, by genotoxic stress. Canonical TGF β pathway-mediated growth suppression of hematopoietic stem cells (HSCs) was recently identified as a cause of bone marrow failure in FA (Rio, P. and Bueren, J.A., 2016; Zhang, H. et al., 2016). Shwachman-Diamond Syndrome (SDS) is another rare bone marrow failure syndrome that is caused by mutations in the SBDS gene (Boocock, G.R. et al., 2003; Rogers, Z.R., 2018). It has been shown that the TGF β pathway is dysregulated in SDS cells. Taken together, such findings provide a compelling rationale for the use of TGF β binding agents in accordance with the present disclosure for the treatment of bone marrow failure syndromes.

[00212] In some embodiments, therefore, there is provided a method for treating or preventing bone marrow failure such as SDS, the method comprising administering an effective amount of a polypeptide or TGF β binding agent in accordance with the present disclosure to a subject in need thereof. In such embodiments, the polypeptide or TGF β binding agent may reduce or inhibit a symptom or sequelae associated with SDS. Exemplary symptoms or sequelae associated with SDS are selected from the group consisting of neutropenia (e.g., exhibiting an absolute neutrophil count <1500/mL), anemia, thrombocytopenia (e.g., exhibiting a platelet count below 50,000/mm³), exocrine pancreatic dysfunction, growth retardation, chronic steatorrhea, metaphyseal dysplasia, myelodysplasia, megakaryocyte dysplasia, erythroid dysplasia, acute myeloid leukemia (AML), and generalized osteopenia. *See*, e.g., WO2016/138300 and WO2019/018662, for more discussion of bone marrow failure.

[00213] As used herein, the terms “effective amount” and “therapeutically effective amount” are used interchangeably to refer to the amount or dose of a compound or composition, upon single or multiple dose administration to a subject, which provides the desired effect (e.g., the desired biological or medicinal response, e.g., to ameliorate, lessen or prevent a disease, disorder or condition) in the subject being treated. In some embodiments, an effective amount is an amount or dose of a compound or composition that prevents or treats a TGF β -associated disease or condition in a subject, as described herein. In some embodiments, an effective amount is an amount or dose of a compound or composition that inhibits one or more activity of TGF β (e.g., TGF β 1 and/or TGF β 3) in a subject, as described herein.

[00214] The term “inhibition” or “inhibiting” is used herein to refer generally to reducing, slowing, restricting, delaying, suppressing, blocking, neutralizing, hindering, or preventing a process, such as without limitation reducing or slowing growth, spread or survival of a TGF β -associated disease or condition, such as without limitation, fibrosis, a cancer or tumor, or a bone marrow failure.

[00215] The term “treating” or “treatment” for purposes of this disclosure refers to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to ameliorate the targeted disease or condition. Those in need of treatment include those already with the disorder as well as those prone to have the disorder or those in whom the disorder is to be prevented. In certain embodiments “treating” or “treatment” refers to ameliorating at least one physical parameter, such as skin thickening, fibrotic scarring, or tumor size, growth, or migration. In certain embodiments, “treating” or “treatment” refers to inhibiting or improving a disease or condition, either physically (e.g., stabilization of a discernible symptom), physiologically (e.g., stabilization of a physical parameter), or both. In certain embodiments, “treating” or “treatment” refers to delaying the onset (or recurrence) of a disease or condition. The term “treating” or “treatment” may refer to any indicia of success in the treatment or amelioration of a disease or condition, including any objective or subjective parameter such as abatement; remission; diminishing of symptoms or making the disease or condition more tolerable to the subject; improving a subject's physical or mental well-being, such as reducing pain or discomfort experienced by the patient; and, in some situations additionally improving at least one clinical parameter of a disease or condition.

[00216] In some embodiments of the present disclosure, “treating” refers to neutralizing the biologic activity of excess TGF β . It may be determined by suitable clinical variables of improvement; by pathologic evaluation of the effects on e.g. fibrosis and/or immunosuppression or prevention of fibrosis; by a direct inhibition of TGF β signaling; or by another measure suitable for the disease or condition being treated.

[00217] As used herein, “preventing” or “prevention” is intended to refer at least to the reduction of the likelihood of, or the risk of, or susceptibility to acquiring a disease or disorder (i.e., causing at least one of the clinical symptoms of the disease not to develop in a patient that may be exposed to or predisposed to or at risk of the disease but does not yet experience or display

symptoms of the disease). The term "prevention" or "preventing" is also used to describe the administration of a compound or composition described herein to a subject who is at risk of (or susceptible to) such a disease or condition. Subjects amenable to treatment for prevention of a disease or condition include individuals at risk of the disease or condition but not showing symptoms, as well as patients presently showing symptoms. In some embodiments, "prevention" or "preventing" is used to describe the administration of a compound or composition described herein to a subject who has been diagnosed with or treated for a disease or condition and is at risk of recurrence of the disease or condition.

[00218] In some embodiments, treatment or prevention are within the context of the present invention if there is a measurable difference between the performances of subjects treated using the TGF β binding agents, compositions and methods provided herein as compared to members of a placebo group, historical control, or between subsequent tests given to the same subject.

[00219] The term "subject" includes living organisms with a TGF β -associated disease or condition, or who are susceptible to or at risk thereof. Examples of subjects include mammals, e.g., humans, monkeys, cows, rabbits, sheep, goats, pigs, dogs, cats, rats, mice, and transgenic species thereof. The term "subject" generally includes animals susceptible to states characterized by TGF β -associated diseases or conditions such as fibrosis or cancer, e.g., mammals, e.g. primates, e.g. humans. The animal can also be an animal model for a disorder, e.g., a mouse model, a xenograft recipient, and the like. In certain embodiments, the subject is a human.

[00220] There are no particular limitations on the dose of each of TGF β binding agents for use in compositions provided herein. Exemplary doses include milligram or microgram amounts of the compound per kilogram of subject or sample weight (e.g., about 50 micrograms per kilogram to about 500 milligrams per kilogram, about 1 milligram per kilogram to about 100 milligrams per kilogram, about 1 milligram per kilogram to about 50 milligram per kilogram, about 1 milligram per kilogram to about 10 milligrams per kilogram, or about 3 milligrams per kilogram to about 5 milligrams per kilogram). Additional exemplary doses include doses of about 5 to about 500 mg, about 25 to about 300 mg, about 25 to about 200 mg, about 50 to about 150 mg, or about 50, about 100, about 150 mg, about 200 mg or about 250 mg, and, for example, daily or twice daily, or lower or higher amounts.

[00221] In some embodiments, the dose range for adult humans is generally from 0.005 mg to 10 g/day. Polypeptides, TGF β binding agents, and compositions thereof may be provided in Unit dosage form, e.g., in a unit which is effective at such dosage or as a multiple of the same, for instance, units containing 5 mg to 500 mg, usually around 10 mg to 200 mg. A dosage unit can include from, for example, 1 to 30 mg, 1 to 40 mg, 1 to 100 mg, 1 to 300 mg, 1 to 500 mg, 2 to 500 mg, 3 to 100 mg, 5 to 20 mg, 5 to 100 mg (e.g. 1 mg, 2 mg, 3 mg, 4 mg, 5 mg, 6 mg, 7 mg, 8 mg, 9 mg, 10 mg, 11 mg, 12 mg, 13 mg, 14 mg, 15 mg, 16 mg, 17 mg, 18 mg, 19 mg, 20 mg, 25 mg, 30 mg, 35 mg, 40 mg, 45 mg, 50 mg, 55 mg, 60 mg, 65 mg, 70 mg, 75 mg, 80 mg, 85 mg, 90 mg, 95 mg, 100 mg, 150 mg, 200 mg, 250 mg, 300 mg, 350 mg, 400 mg, 450 mg, or 500 mg) of a polypeptide, TGF β binding agent, or composition described herein.

[00222] It should be understood that the effective amount of polypeptide or TGF β binding agent for therapeutic treatment of a disease or condition varies depending upon the manner of administration, the age, body weight, and general health of the subject. Ultimately, the attending physician or veterinarian decides the appropriate amount and dosage regimen. It should be understood that the dosage or amount of a polypeptide or TGF β binding agent used, alone or in combination with one or more active compounds to be administered, depends on the individual case and is, as is customary, to be adapted to the individual circumstances to achieve an optimum effect. Dosing and administration regimens are within the purview of the skilled artisan, and appropriate doses depend upon a number of factors within the knowledge of the ordinarily skilled physician, veterinarian, or researcher (e.g., see Wells et al. eds., *Pharmacotherapy Handbook*, 2nd Edition, Appleton and Lange, Stamford, Conn. (2000); *PDR Pharmacopoeia*, Tarascon Pocket Pharmacopoeia 2000, Deluxe Edition, Tarascon Publishing, Loma Linda, Calif. (2000)). For example, dosing and administration regimens depend on the nature and the severity of the disorder to be treated, and also on the sex, age, weight and individual responsiveness of the human or animal to be treated, on the efficacy and duration of action of the compounds used, on whether the therapy is acute or chronic or prophylactic, and/or on whether other active compounds are administered in addition to the therapeutic molecule(s).

[00223] Administration of compounds and compositions provided herein can be carried out using known procedures, at dosages and for periods of time effective to achieved the desired purpose. Dosage regimens can be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily or the dose may be proportionally

reduced as indicated by the exigencies of the therapeutic situation. In some embodiments, a compound or composition is administered at an effective dosage sufficient to prevent or treat fibrosis in a subject.

[00224] There are no particular limitations on the route of administration of each of TGF β binding agents for use in compositions provided herein. A polypeptide, TGF β binding agent or composition thereof may be administered using any suitable route or means, such as without limitation via oral, parenteral, intravenous, intraperitoneal, intramuscular, subcutaneous, sublingual, topical, or nasal administration, via inhalation, via injection, via infusion, or via such other routes as are known in the art. In a particular embodiment, the polypeptide, TGF β binding agent or composition thereof is administered by injection or infusion, for example and without limitation, intravenously, intraperitoneally, intramuscularly, or subcutaneously.

[00225] In some embodiments, in accordance with the methods of the present disclosure, one or more symptom of development or progression of a TGF β -associated disease or condition is reduced by at least 5%, e.g., at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or 100% in a subject.

[00226] In some embodiments, in accordance with the methods of the present disclosure, fibrotic symptoms are reduced in a subject. For example, the polypeptide, TGF β binding agent or composition may reduce fibrosis, fibrotic scarring, or skin thickening in a subject by at least 5%, e.g., at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or 100%.

[00227] In some embodiments, in accordance with the methods of the present disclosure, the differentiation of fibroblasts into myofibroblasts is inhibited in a subject, e.g., by at least 5%, e.g., at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or 100%.

[00228] In some embodiments, in accordance with the methods of the present disclosure, tumor growth and/or metastasis is inhibited in a subject, e.g., by at least 5%, e.g., at

least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or 100%.

[00229] In some embodiments, in accordance with the methods of the present disclosure, hematopoietic colony formation and/or hematopoiesis in bone marrow hematopoietic stem or progenitor cells (HSPCs) is increased in the bone marrow of a subject, e.g., by at least 5%, e.g., at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or 100%.

[00230] In some embodiments, in accordance with the methods of the present disclosure, a positive response in lung fibrosis is revealed as a consistent slowing in the rate of decline in lung function, as measured by forced vital capacity.

[00231] In some embodiments, in accordance with the methods of the present disclosure, a positive response for skin fibrosis associated with systemic sclerosis is determined by an improvement in the Modified Rodnan Skin Score (MRSS). For example, the MRSS may improve in a subject by at least 5%, e.g., at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or 100%.

[00232] In some embodiments, in bone marrow failure diseases including myelofibrosis, positive responses are revealed by improvements in anemia (for example, transfusion-independent patients exhibiting an increase in hemoglobin level, transfusion dependent patients become transfusion independent).

[00233] In some embodiments, the polypeptide or TGF β binding agent may be conjugated with a therapeutic moiety, as described herein. A desirable therapeutic moiety may be chosen for its ability to prevent or treat the same disease or condition being targeted by the polypeptide or TGF β binding agent.

[00234] In some embodiments, there are provided methods for prevention or treatment of a TGF β -associated disease or condition in a subject by administering an effective

amount of a polypeptide or TGF β binding agent described herein, such that the TGF β -associated disease or condition is prevented or treated in the subject.

[00235] In some embodiments, there are provided methods of inhibiting TGF β in a subject by administering an effective amount of a polypeptide or TGF β binding agent described herein, such that TGF β is inhibited in the subject. In some such embodiments, there are provided methods of inhibiting TGF β 3 in a subject by administering an effective amount of a polypeptide or TGF β binding agent described herein, such that TGF β 3 is inhibited in the subject. In some such embodiments, there are provided methods of inhibiting TGF β 3 and TGF β 1 in a subject by administering an effective amount of a polypeptide or TGF β binding agent described herein, such that TGF β 3 and TGF β 3 are inhibited in the subject.

[00236] In some embodiments, there are provided methods for inhibiting differentiation of fibroblasts into myofibroblasts either *in vitro*, *ex vivo*, or *in vivo*.

[00237] In some embodiments of therapeutic and prophylactic treatments provided herein, the polypeptide or TGF β binding agent is administered in combination with one or more additional therapy or therapeutic agent. The additional therapy or therapeutic agent can be administered before, after or simultaneously with the administration of the polypeptide, TGF β binding agent or composition described herein. In some embodiments, the additional therapy or therapeutic agent is formulated together with the polypeptide or TGF β binding agent in the same composition. In other embodiments, the additional therapy or therapeutic agent is administered separately. Examples of additional therapies and therapeutic agents include, without limitation, an anti-fibrotic agent; an anti-cancer agent; another TGF β -binding agent or inhibitor, such as an antibody, antibody fragment, antigen-binding fragment, soluble TGF β ligand trap, and the like. In one embodiment, the additional therapeutic agent is nintedanib (marketed under the brand names Ofev® and Vargatef®). In one embodiment, the additional therapeutic agent is pirfenidone. In one embodiment, the additional therapeutic agent is an immune checkpoint inhibitor.

[00238] Alternatively, in some embodiments, the polypeptide or TGF β binding agent may be conjugated with a detectable moiety or a diagnostic moiety that is useful for tracking the polypeptide or TGF β binding agent, or cells or tissues expressing TGF β . In some such

embodiments, there are provided methods of diagnosis of a TGF β -associated disease or condition comprising administering to a subject a polypeptide or TGF β binding agent of the present disclosure conjugated with a detectable moiety or a diagnostic moiety, and detecting the polypeptide or TGF β binding agent such that a disease or condition associated with TGF β (e.g., overexpression of TGF β 1 and/or TGF β 3) is diagnosed.

Kits

[00239] In accordance with the present disclosure, polypeptides, TGF β binding agents and pharmaceutical compositions described herein may be assembled into kits or pharmaceutical systems for use in treating or preventing TGF β -associated diseases or conditions. Kits or pharmaceutical systems may comprise a container (e.g. packaging, a box, a carton, a vial, etc.), having in close confinement therein one or more container, such as vials, tubes, ampoules, bottles, and the like, that contains the polypeptide, TGF β binding agent or pharmaceutical composition. Additional kit components may include acids, bases, buffering agents, inorganic salts, solvents, antioxidants, preservatives, or metal chelators. The additional kit components may be present as pure compositions, or as aqueous or organic solutions that incorporate one or more additional kit components. Any or all of the kit components optionally further comprise buffers. Kits may also include tools for administration, such as needles, syringes, and the like. The kit may be used according to the methods described herein and may include instructions for use in such methods. Kits may also include instructions for administration and use of the polypeptide, TGF β binding agent or pharmaceutical composition.

[00240] Sequences are given in the Listing of Sequences in Table 2. In Table 2, the N-terminal IDR in the human TGF β RII ectodomain and sequences derived therefrom are shown underlined; the C-terminal IDR in the human TGF β RII ectodomain and sequences derived therefrom are shown double underlined; Gly-Ser linker regions are shown in italics and underlined; and multimerization domains are shown in italics and boldface.

Table 2. Listing of Sequences.

SEQ ID NO:	Description	Sequence	#amino acids
1	hTGFβRII-ECD	<u>IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAAASPKCIMKEKKKPGETFFMCSCSSDECNDNII FSEEYNTSNPD</u>	136
2	TGFβR-LBD	<u>PQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAAASPKCIMKEKKKPGETFFMCSCSSDECNDNII F</u>	102
3	N-terminal IDR	<u>IPPHVQKSVNNDMIVTDNNGAVKF</u>	24
4	C-terminal IDR	<u>SEEYNTSNPD</u>	10
5	TGFβ-binding region from T22d35-Fc-IgG1-v1(CC)	<u>IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAAASPKCIMKEKKKPGETFFMCSCSSDECNDNII FSEEYNTSNPD</u> <u>IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAAASPKCIMKEKKKPGETFFMCSCSSDECNDNII FSEEYNTSNPD</u>	272
6	T22d35-Fc-IgG1-v1(CC) (Control)	<u>IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAAASPKCIMKEKKKPGETFFMCSCSSDECNDNII FSEEYNTSNPD</u> <u>THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVSCSVMHEALHNHYTQKSLSLSPG</u>	496
7	Linker	<u>SEEYNTSNPDIPPHVQKSVNNDMIVTDNNGAVKF</u>	34
8	Linker	<u>SEEYNTSNPDNDNNGAVKF</u>	18
9	Linker	<u>SEEYNTSNPDNGAVKF</u>	16
10	Linker	<u>SEEYNTSNPDNDMIVTDNNGAVKF</u>	24

SEQ ID NO:	Description	Sequence	#amino acids
11	Linker	<u>SEEYNTIPPHVQKSVNNDMIVTDNNGAVKF</u>	30
12	Linker	<u>SEEYNDMIVTDNNGAVKF</u>	18
13	Linker	<u>SEEYPHVQKSVNNDMIVTDNNGAVKF</u>	26
14	Linker	<u>SEEYNTSNPDVTDNNGAVKF</u>	20
15	Linker	<u>SEEMYIVTDNNGAVKF</u>	16
16	Linker	<u>SEEYNTSNPDPHVQKSVNNDMIVTDNNGAVKF</u>	32
17	Linker	<u>GGGGSGGGSGGGSGGGSGGGSGGGSGGGG</u>	30
18	Linker	<u>SEEYNTGGGGSGGGSGGGSGGGSGGGSGGGG</u>	30
19	Linker	<u>SEEYNTSNPDVQKSVNNDMIVTDNNGAVKF</u>	30
20	Linker	<u>GGGGSGGGSGGGSGG</u>	16
21	Linker	<u>GGGGSGGGSGGGSGGG</u>	18
22	Linker	<u>SEEDMIVTDNNGAVKF</u>	17
23	Linker	<u>SEYNNDMIVTDNNGAVKF</u>	19
24	Linker	<u>GGGGSGGGSGGGSGGGSGGGSGGGSGGGSGGGG</u>	34
25	Linker	<u>SEEYNTSNPDGGGGSGGGSGGGSGGGSGGGSGGGG</u>	34
26	Linker	<u>SEEYNTSNPDVNNDMIVTDNNGAVKF</u>	26
27	TGFβ-binding region - Protein 61	<u>IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAA</u> <u>SPKCIMKEKKKPGETFFMCSCSSDECNDNII</u> <u>FSEEYNTSNPD</u> <u>DNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAA</u> <u>SPKCIMKEKKKPGETFFMCSCSSDECNDNII</u> <u>FSEEYNTSNPDNGAVKF</u>	262

SEQ ID NO:	Description	Sequence	#amino acids
28	TGFβ-binding region - Protein 93	IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKS CMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYH DFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNII FSEE <u>YNTSNPDNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMS</u> NCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFI LEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNII FSEEYNT <u>SNPDNGAVKF</u>	268
29	TGFβ-binding region - Protein 96	IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKS CMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYH DFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNII FSEE <u>YNTSNPDNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITS</u> ICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAAS PKCIMKEKKKPGETFFMCSCSSDECNDNII FSEEYNTSNPDNG <u>AVKF</u>	262
30	TGFβ-binding region - Protein 97	IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKS CMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYH DFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNII FSEE <u>YNTSNPDNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMS</u> NCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFI LEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNII FSEEYNT <u>SNPD</u>	262
31	TGFβ-binding region - Protein 99	IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKS CMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYH DFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNII FSEE <u>YNTIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDN</u> QKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKL PYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNII F <u>SEEYNTIPPHVQKSVNNDMIVTDNNGAVKF</u>	288
32	TGFβ-binding region - Protein 101	IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKS CMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYH DFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNII FSEE <u>YNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITS</u> ICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAAS PKCIMKEKKKPGETFFMCSCSSDECNDNII FSEEYNTIPPHVQ <u>KSVNNDMIVTDNNGAVKF</u>	276
33	TGFβ-binding region - Protein 106	IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKS CMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYH DFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNII FSEE <u>YPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSC</u>	280

SEQ ID NO:	Description	Sequence	#amino acids
		MSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAAAPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYPHVQKSVNNDMIVTDNNGAVKF	
34	TGFβ-binding region - Protein 107	IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAAAPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEETYNTSNPDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAAAPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEETYNTSNPD	256
35	TGFβ-binding region - Protein 108	IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAAAPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEETYNTSNPDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAAAPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEETYNTSNPDNNGAVKF	260
36	TGFβ-binding region - Protein 109	IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAAAPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEETYNTSNPDVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAAAPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEETYNTSNPDNNGAVKF	264
37	TGFβ-binding region - Protein 112	IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAAAPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEETYNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAAAPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEETYNDMIVTDNNGAVKF	262
38	TGFβ-binding region - Protein 113	IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAAAPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEETYNTSNPDPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAAAPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEETYNTSNPDPHVQKSVNNDMIVTDNNGAVKF	292

SEQ ID NO:	Description	Sequence	#amino acids
39	TGFβ-binding region - Protein 115	<u>I P P H V Q K S V N N D M I V T D N N G A V K F P Q L C K F C D V R F S T C D N Q K S C M S N C S I T S I C E K P Q E V C V A V W R K N D E N I T L E T V C H D P K L P Y H D F I L E D A A S P K C I M K E K K K P G E T F F M C S C S S D E C N D N I I F S E E Y N T S N P D P H V Q K S V N N D M I V T D N N G A V K F P Q L C K F C D V R F S T C D N Q K S C M S N C S I T S I C E K P Q E V C V A V W R K N D E N I T L E T V C H D P K L P Y H D F I L E D A A S P K C I M K E K K K P G E T F F M C S C S S D E C N D N I I F S E E Y N T S N P D P H V Q K S V N N D M I V T D N N G A V K F</u>	292
40	TGFβ-binding region - Protein 116	<u>I P P H V Q K S V N N D M I V T D N N G A V K F P Q L C K F C D V R F S T C D N Q K S C M S N C S I T S I C E K P Q E V C V A V W R K N D E N I T L E T V C H D P K L P Y H D F I L E D A A S P K C I M K E K K K P G E T F F M C S C S S D E C N D N I I F S E E Y N T S N P D P H V Q K S V N N D M I V T D N N G A V K F P Q L C K F C D V R F S T C D N Q K S C M S N C S I T S I C E K P Q E V C V A V W R K N D E N I T L E T V C H D P K L P Y H D F I L E D A A S P K C I M K E K K K P G E T F F M C S C S S D E C N D N I I F S E E Y N T S N P D I P P H V Q K S V N N D M I V T D N N G A V K F</u>	294
41	TGFβ-binding region - Protein 128	<u>I P P H V Q K S V N N D M I V T D N N G A V K F P Q L C K F C D V R F S T C D N Q K S C M S N C S I T S I C E K P Q E V C V A V W R K N D E N I T L E T V C H D P K L P Y H D F I L E D A A S P K C I M K E K K K P G E T F F M C S C S S D E C N D N I I F S E E Y N D M I V T D N N G A V K F P Q L C K F C D V R F S T C D N Q K S C M S N C S I T S I C E K P Q E V C V A V W R K N D E N I T L E T V C H D P K L P Y H D F I L E D A A S P K C I M K E K K K P G E T F F M C S C S S D E C N D N I I F S E E Y N T I P P H V Q K S V N N D M I V T D N N G A V K F</u>	276
42	TGFβ-binding region - Protein 112	<u>I P P H V Q K S V N N D M I V T D N N G A V K F P Q L C K F C D V R F S T C D N Q K S C M S N C S I T S I C E K P Q E V C V A V W R K N D E N I T L E T V C H D P K L P Y H D F I L E D A A S P K C I M K E K K K P G E T F F M C S C S S D E C N D N I I F S E E Y N D M I V T D N N G A V K F P Q L C K F C D V R F S T C D N Q K S C M S N C S I T S I C E K P Q E V C V A V W R K N D E N I T L E T V C H D P K L P Y H D F I L E D A A S P K C I M K E K K K P G E T F F M C S C S S D E C N D N I I F G G G G S G G G G S G G G G S</u>	276
43	TGFβ-binding region - Protein 130	<u>I P P H V Q K S V N N D M I V T D N N G A V K F P Q L C K F C D V R F S T C D N Q K S C M S N C S I T S I C E K P Q E V C V A V W R K N D E N I T L E T V C H D P K L P Y H D F I L E D A A S P K C I M K E K K K P G E T F F M C S C S S D E C N D N I I F S E E Y N D M I V T D N N G A V K F P Q L C K F C D V R F S T C D N Q K S C M S N C S I T S I C E K P Q E V C V A V W R K N D E N I T L E T V C H D P K L P Y H D F I L E D A A S P K C I M K E K K K P G E T F F M C S C S S D E C N D N I I F S E E Y N T G G G G S G G G S G G G G S G G G G S</u>	276
44	TGFβ-binding region - Protein 131	<u>I P P H V Q K S V N N D M I V T D N N G A V K F P Q L C K F C D V R F S T C D N Q K S C M S N C S I T S I C E K P Q E V C V A V W R K N D E N I T L E T V C H D P K L P Y H D F I L E D A A S P K C I M K E K K K P G E T F F M C S C S S D E C N D N I I F S E E Y N D M I V T D N N G A V K F P Q L C K F C D V R F S T C D N Q K S C M S N C S I T S</u>	276

SEQ ID NO:	Description	Sequence	#amino acids
		ICEKPQEVCAVVRKNDENITLETVCHDPKLPYHDFILEDAAAS PKCIMKEKKKPGETFFMCSCSSDECNDNII <u>FSEEYNTSNPDVQ</u> <u>KSVNNDMIVTDNNGAVKF</u>	
45	TGFβ-binding region - Protein 132	IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKS CMSNCSITSICEKPQEVCAVVRKNDENITLETVCHDPKLPYH DFILEDAAASPKCIMKEKKKPGETFFMCSCSSDECNDNII <u>FSEE</u> <u>YNTSNPDDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITS</u> ICEKPQEVCAVVRKNDENITLETVCHDPKLPYHDFILEDAAAS PKCIMKEKKKPGETFFMCSCSSDECNDNII <u>FSEEYNTSNPDVQ</u> <u>KSVNNDMIVTDNNGAVKF</u>	276
46	TGFβ-binding region - Protein 133	IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKS CMSNCSITSICEKPQEVCAVVRKNDENITLETVCHDPKLPYH DFILEDAAASPKCIMKEKKKPGETFFMCSCSSDECNDNII <u>FSEE</u> <u>YNTSNPDDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITS</u> ICEKPQEVCAVVRKNDENITLETVCHDPKLPYHDFILEDAAAS PKCIMKEKKKPGETFFMCSCSSDECNDNII <u>FSEEYNTIIPPHVQ</u> <u>KSVNNDMIVTDNNGAVKF</u>	276
47	TGFβ-binding region - Protein 134	IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKS CMSNCSITSICEKPQEVCAVVRKNDENITLETVCHDPKLPYH DFILEDAAASPKCIMKEKKKPGETFFMCSCSSDECNDNII <u>FSEE</u> <u>YNTSNPDDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITS</u> ICEKPQEVCAVVRKNDENITLETVCHDPKLPYHDFILEDAAAS PKCIMKEKKKPGETFFMCSCSSDECNDNII <u>FGGGGSGGGSGG</u> <u>GGSG</u>	262
48	TGFβ-binding region - Protein 135	IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKS CMSNCSITSICEKPQEVCAVVRKNDENITLETVCHDPKLPYH DFILEDAAASPKCIMKEKKKPGETFFMCSCSSDECNDNII <u>FGGG</u> <u>GSGGGSGGGSGGGPQLCKFCDVRFSTCDNQKSCMSNCSITS</u> ICEKPQEVCAVVRKNDENITLETVCHDPKLPYHDFILEDAAAS PKCIMKEKKKPGETFFMCSCSSDECNDNII <u>FSEEYNTSNPDNG</u> <u>AVKF</u>	262
49	hIgG1Fc(C C) region	<i>THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVD</i> <i>VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTV</i> <i>LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL</i> <i>PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTT</i> <i>PPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVMEALHNHYT</i> <i>QKSLSLSPG</i>	224

SEQ ID NO:	Description	Sequence	#amino acids
50	hIgG4Fc region	<i>GPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTIISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSPG</i>	224
51	hIgG1Fc	<i>APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG</i>	217
52	hIgG1Fc variant	<i>APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG</i>	216
53	hIgG1Fc variant	<i>PPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDEPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG</i>	220
54	hIgG1Fc variant	<i>DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG</i>	226
55	hIgG1Fc variant	<i>EPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG</i>	231
56	hIgG1Fc variant	<i>EPKSSDKTHTSPPSPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP</i>	231

SEQ ID NO:	Description	Sequence	#amino acids
		<i>ENNYK'TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH EALHNHYTQKSLSLSPG</i>	
57	hIgG2Fc	<i>APPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQ FNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGK EYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTK NQVSLTCLVKGFYPSDISEWESNGQPENNYKTTPMLDSDGS FFLYSKLTVDKSRWQQGNVFSCSVMH EALHNHYTQKSLSLSPG K</i>	216
58	hIgG2Fc variant	<i>VECPPCAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVH QDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPP SREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP MLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH EALHNHYTQK SLSLSPGK</i>	223
59	hIgG2Fc variant	<i>ERKCCVECPPCAPPVAGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVS LTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQV YTLPPSREEMTKNQVSLTCLVKGFYPSDISEWESNGQPENNY KTTPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH EALHN HYTQKSLSLSPGK</i>	228
60	hIgG2Fc variant	<i>ERKSSVECPPCAPPVAGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVS LTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQV YTLPPSREEMTKNQVSLTCLVKGFYPSDISEWESNGQPENNY KTTPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH EALHN HYTQKSLSLSPG</i>	227
61	hIgG2Fc variant	<i>ERKSSVESPPPCAPPVAGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVS LTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQV YTLPPSREEMTKNQVSLTCLVKGFYPSDISEWESNGQPENNY KTTPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH EALHN HYTQKSLSLSPG</i>	227
62	hIgG2Fc variant	<i>ERKSSVESPPSPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVS LTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQV YTLPPSREEMTKNQVSLTCLVKGFYPSDISEWESNGQPENNY KTTPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH EALHN HYTQKSLSLSPG</i>	227
63	hIgG2Fc variant	<i>APPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQ FNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGK EYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTK</i>	215

SEQ ID NO:	Description	Sequence	#amino acids
		<i>NQVSLTCLVKGFYPSDISVEWESNGQPENNYKTTTPMLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPG</i>	
64	hIgG2Fc variant	<i>PPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDISVEWESNGQPENNYKTTTPMLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPG</i>	219
65	hIgG2Fc variant	<i>VECPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDISVEWESNGQPENNYKTTTPMLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPG</i>	222
66	hIgG3Fc	<i>APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFKWYVDGVEVHNAKTKPREEQYNSTFRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPMLDSDGSFFLYSKLTVDKSRWQQGNIFSCVMHEALHNRTQKSLSLSPG</i>	217
67	hIgG3Fc variant	<i>APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFKWYVDGVEVHNAKTKPREEQYNSTFRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPMLDSDGSFFLYSKLTVDKSRWQQGNIFSCVMHEALHNRTQKSLSLSPG</i>	216
68	hIgG3Fc variant	<i>PRCPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFKWYVDGVEVHNAKTKPREEQYNSTFRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPMLDSDGSFFLYSKLTVDKSRWQQGNIFSCVMHEALHNRTQKSLSLSPG</i>	220
69	hIgG3Fc variant	<i>DTPPCPRCPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFKWYVDGVEVHNAKTKPREEQYNSTFRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPMLDSDGSFFLYSKLTVDKSRWQQGNIFSCVMHEALHNRTQKSLSLSPG</i>	226
70	hIgG3Fc variant	<i>EPKSSDTPPPCPRCPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFKWYVDGVEVHNAKTKPREEQYNSTFRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKTKGQPR</i>	231

SEQ ID NO:	Description	Sequence	#amino acids
		<i>EPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESSGQP ENNYNTTPPMLDSDGSFFLYSKLTVDKSRWQQGNIFSCSVME ALHNRFTQKSLSLSPG</i>	
71	hIgG3Fc variant	<i>EPKSSDTPPPSPRCPAPELLGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVQFKWYVDGVEVHNAKTKPREEQYNSTFR VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKTKGQPR EPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESSGQP ENNYNTTPPMLDSDGSFFLYSKLTVDKSRWQQGNIFSCSVME ALHNRFTQKSLSLSPG</i>	231
72	hIgG3Fc variant	<i>EPKSSDTPPPSPRSPAPELLGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVQFKWYVDGVEVHNAKTKPREEQYNSTFR VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKTKGQPR EPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESSGQP ENNYNTTPPMLDSDGSFFLYSKLTVDKSRWQQGNIFSCSVME ALHNRFTQKSLSLSPG</i>	231
73	hIgG4Fc	<i>APEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEV QFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMT KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG SFFLYSRLTVDKSRWQEGNVFSCSVMEALHNHYTQKSLSLSL GK</i>	217
74	hIgG4Fc variant	<i>APEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEV QFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMT KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG SFFLYSRLTVDKSRWQEGNVFSCSVMEALHNHYTQKSLSLSL G</i>	216
75	hIgG4Fc variant	<i>PSCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQED PEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQ EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV LDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMEALHNHYTQKSL SLSLG</i>	220
76	hIgG4Fc variant	<i>ESKYGPPCPSCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTC VVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQ VYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMEALH NHYTQKSLSLSLG</i>	228
77	hIgG4Fc variant	<i>ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTC VVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVS</i>	228

SEQ ID NO:	Description	Sequence	#amino acids
		<i>VLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQ VYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMEALH NHYTQKSLSLSLG</i>	
78	hIgG4Fc variant	<i>ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTC VVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQ VYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTTPVLDSDGSFFLYSKLTVDKSRWQEGNVFSCSVMEALH NHYTQKSLSLSLG</i>	228
79	hIgG4Fc variant	<i>ESKYGPPSPSCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTC VVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQ VYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMEALH NHYTQKSLSLSLG</i>	228
80	hIgG4Fc variant	<i>ESKYGPPSPSSPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTC VVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQ VYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMEALH NHYTQKSLSLSLG</i>	228
81	Protein 61	<i>IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKS CMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYH DFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNII FSEE YNTSNPDNDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITS ICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAAS PKCIMKEKKKPGETFFMCSCSSDECNDNII FSEEYNTSNPDNG AVKF</i> <i>GPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTC VVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQ VYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMEALH NHYTQKSLSLSLG</i>	486
105	Protein 70	<i>IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKS CMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYH DFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNII FSEE YNTSNPDNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMS NCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFI LEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNII FSEEYNT SNPD</i> <i>GPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTC VVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVS</i>	486

SEQ ID NO:	Description	Sequence	#amino acids
		<i>VLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNMFSCSVMEALH</i> <i>NHYTQKSLSLSLG</i>	
82	Protein 93	<i>IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAAASPKCIMKEKKKPGETFFMCSCSSDECNDNII FSEEYNTSNPDNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAAASPKCIMKEKKKPGETFFMCSCSSDECNDNII FSEEYNTSNPDNGAVKF</i> <i>GPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISR</i> <i>TPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLT</i> <i>VLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNMFSCSV</i> <i>MHEALHNHYTQKSLSLSLG</i>	492
83	Protein 94	<i>IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAAASPKCIMKEKKKPGETFFMCSCSSDECNDNII FSEEYNTSNPDNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAAASPKCIMKEKKKPGETFFMCSCSSDECNDNII FSEEYNTSNPDNDMIVTDNNGAVKF</i> <i>GPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISR</i> <i>TPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLT</i> <i>VLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNMFSCSV</i> <i>MHEALHNHYTQKSLSLSLG</i>	500
84	Protein 96	<i>IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAAASPKCIMKEKKKPGETFFMCSCSSDECNDNII FSEEYNTSNPDNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAAASPKCIMKEKKKPGETFFMCSCSSDECNDNII FSEEYNTSNPDNGAVKF</i> <i>THTCPPELGGPSVFLFPPKPKDTLMISR</i> <i>TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLT</i> <i>VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNMFSCSV</i> <i>MHEALHNHYTQKSLSLSPG</i>	486
85	Protein 97	<i>IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAAASPKCIMKEKKKPGETFFMCSCSSDECNDNII FSEEYNTSNPDNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMS</i>	486

SEQ ID NO:	Description	Sequence	#amino acids
		NCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFI LEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNII FSEEYNT <u>SNPD</u> <u>THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC</u> <u>VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS</u> <u>VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ</u> <u>VYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN</u> <u>YKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALH</u> <u>NHYTQKSLSLSPG</u>	
86	Protein 99	IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKS CMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYH DFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNII FSEE YNTIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDN QKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKL PYHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNII F SEEYNTIPPHVQKSVNNDMIVTDNNGAVKF <u>GPPCPPCPAPEFL</u> <u>GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSDQEDPEVFQFNWY</u> <u>VDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKC</u> <u>KVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVS</u> <u>LTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLY</u> <u>SRLTVDKSRWQEGNVFSCFSVMHEALHNHYTQKSLSLSPG</u>	512
87	Protein 101	IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKS CMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYH DFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNII FSEE YNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITS ICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAAS PKCIMKEKKKPGETFFMCSCSSDECNDNII FSEEYNTIPPHVQ KSVNNDMIVTDNNGAVKF <u>THTCPPCPAPELLGGPSVFLFPPKPK</u> <u>KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK</u> <u>PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE</u> <u>KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSD</u> <u>IAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQ</u> <u>GNVFCFSVMHEALHNHYTQKSLSLSPG</u>	500
88	Protein 106	IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKS CMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYH DFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNII FSEE YPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSC MSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHD FILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNII FSEEY PHVQKSVNNDMIVTDNNGAVKF <u>THTCPPCPAPELLGGPSVFLF</u> <u>PPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN</u> <u>AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP</u> <u>APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGF</u> <u>YPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKS</u> <u>RWQQGNVFCFSVMHEALHNHYTQKSLSLSPG</u>	504

SEQ ID NO:	Description	Sequence	#amino acids
89	Protein 107	IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKS CMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYH DFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEE YNTSNPDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITS ICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAAS PKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPDTH TCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPP SRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP VLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVMHEALHNHYTQK SLSLSPG	480
90	Protein 108	IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKS CMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYH DFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEE YNTSNPDNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSIC EKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAASPK CIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPDNGAV KFTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVMHEALHNH YTQKSLSLSPG	484
91	Protein 109	IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKS CMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYH DFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEE YNTSNPDVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSI TSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDA ASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPD NGAVKFTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE NNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVMHEA LHNHYTQKSLSLSPG	488
92	Protein 112	IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKS CMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYH DFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEE YNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITS	486

SEQ ID NO:	Description	Sequence	#amino acids
		ICEKPQEVCAVVRKNDENITLETVCHDPKLPYHDFILEDAAASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYMIIVTDNNGAVKF <i>THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG</i>	
93	Protein 113	IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVVRKNDENITLETVCHDPKLPYHDFILEDAAASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVVRKNDENITLETVCHDPKLPYHDFILEDAAASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDPHVQKSVNNDMIVTDNNGAVKF <i>THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG</i>	516
94	Protein 115	IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVVRKNDENITLETVCHDPKLPYHDFILEDAAASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVVRKNDENITLETVCHDPKLPYHDFILEDAAASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDPHVQKSVNNDMIVTDNNGAVKF <i>GPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSPG</i>	516
95	Protein 116	IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVVRKNDENITLETVCHDPKLPYHDFILEDAAASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVVRKNDENITLETVCHDPKLPYHDFILEDAAASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEYNTSNPDIIPPHVQKSVNNDMIVTDNNGAVKF <i>THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG</i>	518

SEQ ID NO:	Description	Sequence	#amino acids
		<i>GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDS DGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSLSPG</i>	
96	Protein 128	<i>IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNII FSEEYNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNII FSEEYNTIPPHVQKSVNNDMIVTDNNGAVKF GPPCPPCPAPEFLGGPSVFLFPPKP KDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTK PREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDS DGSFFLYSRLTVDKSRWQEGNV FSCSV MHEALHNHYTQKSLSLSPG</i>	500
97	Protein 129	<i>IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNII FSEEYNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNII FGGGGSGGGSGGGGGGGGGGGSTHTCPPCPAPELLGGPSVFLFPPKP KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDS DGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSLSPG</i>	500
98	Protein 130	<i>IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNII FSEEYNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDNII FSEEYNTGGGGSGGGSGGGGGGGGGGGSTHTCPPCPAPELLGGPSVFLFPPKP KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDS DGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSLSPG</i>	500

SEQ ID NO:	Description	Sequence	#amino acids
99	Protein 131	IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKS CMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYH DFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEE <u>YNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITS</u> ICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAAS PKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPDVQ KSVNNDMIVTDNNGAVKF <i>THTCP</i> <i>CPAPELLGGPSVFLFPPKP</i> <i>KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK</i> <i>PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE</i> <i>KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSD</i> <i>IAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ</i> <i>GNVFS</i> <i>CSVMHEALHNHYTQKSLSLSPG</i>	500
100	Protein 132	IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKS CMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYH DFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEE <u>YNTSNPDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITS</u> ICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAAS PKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPDVQ KSVNNDMIVTDNNGAVKF <i>THTCP</i> <i>CPAPELLGGPSVFLFPPKP</i> <i>KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK</i> <i>PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE</i> <i>KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSD</i> <i>IAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ</i> <i>GNVFS</i> <i>CSVMHEALHNHYTQKSLSLSPG</i>	500
101	Protein 133	IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKS CMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYH DFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEE <u>YNTSNPDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITS</u> ICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAAS PKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTIPPHVQ KSVNNDMIVTDNNGAVKF <i>THTCP</i> <i>CPAPELLGGPSVFLFPPKP</i> <i>KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK</i> <i>PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE</i> <i>KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSD</i> <i>IAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ</i> <i>GNVFS</i> <i>CSVMHEALHNHYTQKSLSLSPG</i>	500
102	Protein 134	IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKS CMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYH DFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEE <u>YNTSNPDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITS</u> ICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAAS	486

SEQ ID NO:	Description	Sequence	#amino acids
		PKCIMKEKKKPGETFFMCSCSSDECNDNIIIFGGGGSGGGSGGGGGSGG GGSGTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ VYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVMEALH NHYTQKSLSLSPG	
103	Protein 135	IPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKS CMSNCSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYH DILEDAAASPKCIMKEKKKPGETFFMCSCSSDECNDNIIIFGGG GSGGGSGGGSGGGPQLCKFCDVRFSTCDNQKSCMSNCSITS ICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAAAS PKCIMKEKKKPGETFFMCSCSSDECNDNIIIFSEEYNTSNPDNG AVKE THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ VYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVMEALH NHYTQKSLSLSPG	486
104	Signal peptide	MDWTWRILFLVAAATGTHA	19
106	cDNA encoding Protein 61	ATTCCCTCCACACGTGCAGAAATCCGTGAACAACGACATGATCG TGACCGACAACAATGGCGCCGTGAAGTTCCTCAGCTGTGCAA GTTCTGCGACGTGCGGTTCTCTACCTGCGACAACAGAAATCC TGCATGTCCAACCTGCTCCATCACCTCCATCTGCGAGAAGCCCC AAGAAGTGTGCGTCGCCGTCTGGCGGAAGAACGACGAGAACAT CACCTTGGAACCGTGTGCCACGATCCTAAGCTGCCCTACCAC GACTTCATCCTGGAAGATGCCGCCTCTCCTAAGTGACATCATGA AGGAAAAGAAGAAGCCCGGCGAGACTTTCTTCATGTGCAGCTG CTCCTCCGACGAGTGCAACGACAACATCATCTTCTCCGAAGAG TACAACACCAGCAATCCCGACGATAACAACGGGGCTGTGAAAT TCCCACAGCTCTGTAAATTTTGTGATGTGCGGTTGAGCACCTG TGACAATCAAAGTCTTGATGAGCAACTGCAGCATCACCAGC ATCTGTGAAAAACCTCAAGAAGTCTGCGTTGAGTTTGGCGCA AAAATGATGAGAATATCACACTCGAGACAGTGTGTCATGACCC CAAGCTGCCTTATCATGATTTTCATCTTGAGGACGCTGCTAGC CCCAAGTGTATTATGAAGGAAAAAAGAAACCTGGGGAAACCT TCTTTATGTGCTCCTGCAGCAGCGACGAGTGTAAATGATAACAT TATTTTCAGTGAAGAGTATAATACCTCCAATCCTGACAACGGC GCTGTCAAGTTTGGGCCCCCTTGTCTCCTTGTCCAGCTCCTG AATTTCTCGGCGGACCCCTCCGTGTTCTGTTTCTCCAAAGCC TAAGGACACCCTGATGATCTCTCGGACCCCTGAAGTGACCTGC	n/a

SEQ ID NO:	Description	Sequence	#amino acids
		GTGGTGGTGGATGTGTCCCAAGAGGACCCTGAGGTGCAGTTCA ATTGGTACGTGGACGGCGTGGAAGTGCACAACGCCAAGACCAA GCCTAGAGAGGAACAGTTCAACTCCACCTACAGAGTGGTGTCC GTGCTGACCGTGCTGCACCAGGATTGGCTGAACGGCAAAGAAT ACAAGTGCAAGGTGTCCAACAAGGGCCTGCCTTCCAGCATCGA AAAGACCATCTCCAAGGCCAAGGGCCAGCCTAGGGAACCCAG GTGTACACACTGCCTCCAAGCCAAGAGGAAATGACCAAGAACC AGGTGTCCCTGACCTGCCTGGTCAAGGGCTTCTACCCTTCCGA TATCGCCGTGGAATGGGAGAGCAATGGCCAGCCTGAGAACAAC TACAAGACCACACCTCCTGTGCTGGACTCCGACGGCTCCTTCT TTCTGTACTCCCGCCTGACCGTGGACAAGTCCAGATGGCAAGA GGGCAACGTGTTCTCCTGCTCCGTGATGCACGAGGCCCTGCAC AATCACTACACCCAGAAGTCCCTGTCTCTAAGCTTAGGCTGA	
107	cDNA encoding Protein 96	ATCCCCCCTCATGTGCAGAAGTCCGTGAACAATGACATGATCG TGACCGATAACAATGGCGCCGTGAAGTTTCCACAGCTGTGCAA GTTCTGTGACGTGAGGTTTAGCACCTGCGATAATCAGAAGTCC TGCATGAGCAACTGTTCTATCACATCCATCTGCGAGAAGCCAC AGGAGGTGTGCGTGGCCGTGTGGCGGAAGAACGACGAGAATAT CACCTGGAGACAGTGTGCCACGATCCAAAGCTGCCCTACCAT GACTTCATCCTGGAGGATGCTGCCTCTCCTAAGTGTATCATGA AGGAGAAGAAGAAGCCAGGCGAGACCTTCTTTATGTGCTCCTG TTCCAGCGACGAGTGCAACGATAATATCATCTTCAGCGAGGAG TATAACACATCTAATCCCGACGATAACAATGGCGCTGTGAAGT TTCCTCAGCTGTGCAAATTTTGTGACGTGAGATTTTCTACCTG TGATAATCAGAAGAGCTGCATGTCTAACTGTTCCATCACATCT ATTTGTGAAAACCTCAGGAAGTGTGCGTGGCCGTGTGGAGAA AAAATGATGAAAACATCACCTGGAAACAGTGTGCCACGATCC TAAGCTGCCATATCACGACTTCATCCTGGAAGACGTGCCAGC CCAAAATGCATTATGAAAGAGAAGAAGAAGCCCGGTGAGACCT TCTTTATGTGCAGCTGTTCTTCTGATGAATGTAATGACAATAT CATCTTCTCCGAGGAGTATAACACAAGCAATCCTGACAACGGC GCTGTGAAGTTTACCCACACATGCCCACCATGTCCTGCTCCAG AGCTGCTGGGAGGACCATCCGTGTTCTGTTTCTCCAAAGCC CAAGGACACCCTGATGATCAGCAGGACCCCCGAGGTGACATGC GTGGTGGTGGACGTGTCTCACGAGGACCCCCGAGGTGAAGTTTA ATTGGTACGTGGATGGCGTGAGGTGCATAACGCTAAGACCAA GCCAAGGGAGGAGCAGTACAACCTCTACCTATCGGGTGGTGTCC GTGCTGACAGTGTGCATCAGGATTGGCTGAATGGCAAGGAGT ATAAGTGCAAGGTGTCCAACAAGGCTCTGCCCCCCCCTATCGA GAAGACCATCAGCAAGGCTAAGGGCCAGCCCAGAGAGCCTCAG GTGTACACACTGCCCCCTTCTCGCGACGAGCTGACCAAGAATC AGGTGTCCCTGACATGTCTGGTGAAGGGCTTCTATCCTTCCGA TATCGCTGTGGAGTGGGAGAGCAACGGACAGCCAGAGAACAAT	n/a

SEQ ID NO:	Description	Sequence	#amino acids
		TACAAGACCACACCACCCGTGCTGGACTCTGATGGCTCCTTCT TTCTGTATAGCAAGCTGACCGTGGACAAGTCTAGGTGGCAGCA GGGCAACGTGTTCTCCTGTTCTGTGATGCACGAGGCCCTGCAC AACCATTACACACAGAAGTCCCTGAGCCTGTCTCCTGGCTGA	
108	cDNA encoding Protein 101	ATCCCCCCTCATGTGCAGAAGTCCGTGAACAATGACATGATCG TGACAGATAACAATGGCGCCGTGAAGTTTCCCCAGCTGTGCAA GTTCTGTGACGTGAGGTTTAGCACCTGCGATAACCAGAAGTCC TGCATGAGCAATTGTTCTATCACATCCATCTGCGAGAAGCCTC AGGAGGTGTGCGTGGCCGTGTGGCGGAAGAACGACGAGAATAT CACCCCTGGAGACAGTGTGCCACGATCCCAAGCTGCCTTACCAT GACTTCATCCTGGAGGATGCTGCCTCTCCAAAGTGATCATGA AGGAGAAGAAGAAGCCCGGCGAGACCTTCTTTATGTGCTCCTG TTCCAGCGACGAGTGCAACGATAATATCATCTTCAGCGAGGAG TATAACGATATGATTGTACAGATAACAATGGCGCTGTGAAGT TTCCACAGCTGTGCAAATTTTGTGACGTGAGATTTTCCACCTG TGATAACCAGAAGAGCTGCATGTCTAATTGTTCCATCACAAGC ATCTGCGAGAAGCCACAGGAAGTGTGCGTGGCCGTGTGGAGAA AAAATGATGAAAACATCACCCCTGGAAACAGTGTGCCACGATCC AAAGCTGCCCTATCACGACTTCATCCTGGAAGACGCTGCCAGC CCTAAATGCATTATGAAAGAGAAGAAGAAGCCAGGTGAGACCT TCTTTATGTGCAGCTGTTCTTCTGATGAATGTAATGACAATAT CATCTTCTCCGAGGAGTATAACACCATCCCTCCACACGTGCAG AAGTCTGTGAATAACGATATGATTGTGACCGACAATAACGGTG CTGTGAAGTTTACCCATACATGCCACCTTGTCCAGCTCCAGA GCTGCTGGGAGGACCATCCGTGTTCTGTTTCCACCCAAGCCT AAGGACACCCTGATGATCAGCAGGACCCAGAGGTGACATGCG TGGTGGTGGACGTGTCTCACGAGGACCCGAGGTGAAGTTTAA CTGGTACGTGGATGGCGTGGAGGTGCATAATGCTAAGACCAAG CCTAGGGAGGAGCAGTACAACCTACCTATCGGGTGGTGTCCG TGCTGACAGTGCTGCACCAGGACTGGCTGAACGGCAAGGAGTA TAAGTGCAAGGTGTCCAATAAGGCTCTGCCTGCCCCAATTGAG AAGACCATCAGCAAGGCTAAGGGCCAGCCTAGAGAGCCACAGG TGACACACTGCCTCCATCTCGCGACGAGCTGACCAAGAACCA GGTGTCCCTGACATGTCTGGTGAAGGGCTTCTATCCCTCCGAT ATCGCTGTGGAGTGGGAGAGCAACGGACAGCCTGAGAACAAAT ACAAGACCACACCCCTGTGCTGGACTCTGATGGCTCCTTCTT TCTGTATAGCAAGCTGACCGTGGATAAGTCTAGGTGGCAGCAG GGCAACGTGTTTAGCTGTTCTGTGATGCATGAGGCCCTGCACA ATCATTACACACAGAAGTCCCTGAGCCTGTCTCCAGGCTGA	n/a
109	cDNA encoding Protein 128	ATCCCCCCTCATGTGCAGAAGAGCGTCAATAACGACATGATCG TGACCGATAACAATGGCGCCGTGAAGTTCCACAGCTGTGCAA GTTCTGTGACGTGAGGTTTAGCACCTGCGATAATCAGAAGTCT	n/a

SEQ ID NO:	Description	Sequence	#amino acids
		TGCATGTCCAACCTGTAGCATCACATCTATCTGCGAGAAGCCAC AGGAGGTGTGCGTGGCCGTGTGGCGGAAGAACGACGAGAATAT CACCCTGGAGACAGTGTGCCACGATCCAAAGCTGCCCTACCAT GACTTTATCCTGGAGGATGCCGCTAGCCCTAAGTGTATCATGA AGGAGAAGAAGAAGCCAGGCGAGACATTCTTTATGTGCTCTTG TTCCAGCGACGAGTGCAACGATAATATCATCTTCTCCGAGGAG TATAACGATATGATCGTGACAGACAATAACGGCGCCGTGAAGT TTCTCAGCTGTGCAAATTTTGTGACGTGAGATTTTCTACCTG TGATAATCAGAAGTCTGTCATGAGCAACTGTTCTATCACATCC ATTTGTGAAAAACCTCAGGAAGTGTGCGTGGCCGTGTGGAGAA AGAATGACGAGAACATCACCCTGGAGACAGTGTGCCATGATCC TAAGCTGCCATACCATGACTTCATCCTGGAAGACGCCGCTTCC CCCAAATGCATTATGAAAGAGAAGAAGAAGCCTGGCGAGACAT TCTTCATGTGCAGCTGTTCTTCTGATGAGTGCAACGATAACAT CATCTTTTCTGAGGAGTACAACACCATCCCCCTCACGTGCAG AAGTCTGTGAACAATGACATGATCGTGACAGATAACAATGGAG CTGTGAAGTTCGGGCCCCCATGCCCTCCATGTCCAGCTCCTGA GTTCTGGGCGGCCCATCCGTGTTCTGTTTCCCCCTAAGCCC AAGGACACCCTGATGATCTCCAGGACCCCTGAGGTGACATGCG TGGTGGTGGACGTGAGCCAGGAGGACCCCGAGGTGCAGTTCAA TTGGTACGTGGATGGCGTGGAGGTGCACAACGCCAAGACAAAG CCTAGAGAGGAGCAGTTTAATAGCACCTACCGCGTGGTGTCTG TGCTGACAGTGCTGCATCAGGATTGGCTGAATGGCAAGGAGTA TAAGTGCAAGGTGTCTAACAAGGGCCTGCCAAGCTCTATCGAG AAGACCATCTCCAAGGCTAAGGGACAGCCAAGGGAGCCTCAGG TGTACACACTGCCACCCAGCCAGGAGGAGATGACCAAGAATCA GGTGTCTCTGACATGTCTGGTGAAGGGCTTCTATCCATCTGAC ATCGCTGTGGAGTGGGAGTCCAACGGCCAGCCCGAGAACAATT ACAAGACCACACCTCCAGTGCTGGACAGCGATGGCTCTTTCTT TCTGTATTCCAGGCTGACCGTGGATAAGAGCCGGTGGCAGGAG GGCAACGTGTTTTCTGTAGCGTGATGCACGAAGCACTGCACA ACCACTACACTCAGAAATCACTGTCACTGTCCCTGGGCTAA	
110	DNA encoding Kozak sequence (italics) and signal peptide	<i>GCCACCATGGACTGGACCTGGAGAATCCTGTTCTGGTGGCTG</i> <i>CTGCTACCGGAACACACGCT</i>	n/a
111	DNA encoding Kozak sequence	<i>GCCACCATGGACTGGACTTGGAGAATCCTGTTCTGGTCGCCG</i> <i>CCGCAACTGGTACTCATGCT</i>	n/a

SEQ ID NO:	Description	Sequence	#amino acids
	(italics) and signal peptide		

n/a: not applicable.

EXAMPLES

[00241] The present invention will be more readily understood by referring to the following examples, which are provided to illustrate the invention and are not to be construed as limiting the scope thereof in any manner.

[00242] Unless defined otherwise or the context clearly dictates otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. It should be understood that any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the invention.

Example 1. Characterization and Structural analysis of known TGF β binding agents.

[00243] The TGF β RII ectodomain (SEQ ID NO: 1) includes a structured portion, which represents the ligand-binding domain (SEQ ID NO: 2), flanked at both the N-terminal and C-terminal ends by intrinsically disordered regions (IDRs; SEQ ID NOs: 3 and 4 respectively).

[00244] Previously, TGF β RII ectodomain fusion molecules were described (WO2017/037634, WO2018/158727). Such fusion molecules include two structured ligand-binding domains (SEQ ID NO: 2) linked together in tandem (head-to-tail) by linkers derived from the IDRs and variants thereof. As reported in WO2018/158727, such polypeptide constructs demonstrated at least 600-fold higher inhibition potency than similar constructs having only a single ligand-binding domain (see WO2018/158727, page 2, lines 27-31, where it is stated: “[a construct] wherein the first region comprises a (T β RII-ECD)-(T β RIIECD) doublet linked at its C-terminus with an antibody constant domain inhibits TGF β activity with at least 600-fold more potency than a counterpart construct having a single T β RII-ECD linked at its C-terminus with an antibody constant domain (i.e when a second ECD is absent, also referred to herein as a singlet)”). Further, such “doublet” polypeptide constructs (referred to as “T22d35-Fc”) may bind to and

neutralize, to varying extents, all three isoforms of TGF β (that is, TGF- β 1, β 2, and β 3), although TGF β 2 was generally neutralized to a much lesser extent than TGF β 1 and TGF β 3. For example, for the T22d35-Fc-IgG1-v1 (CC) variant, the neutralization potency for TGF β 1 and TGF β 3 was stated to be very similar, whereas this potency was much lower for TGF β 2 (IC₅₀=17.33 nM for TGF β 2 compared to 0.003327 nM and 0.003251 nM, respectively, for TGF β 1 and TGF β 3; see WO2018/158727, page 26, lines 24-27). Similar results were reported for the other variants.

[00245] To further study the binding and neutralization properties of such TGF β binding agents, we chose an exemplary fusion molecule, “T22d35-Fc-IgG1-v1 (CC)”, for further investigation. In this fusion molecule (SEQ ID NO: 6 herein; SEQ ID NO: 14 in WO2018/158727), the linker between the two structured ligand-binding domains is a fusion of the C-terminal and N-terminal IDRs, in that order (the entire linker sequence is shown in SEQ ID NO: 7), and the linker between the second ligand-binding domain and the multimerization domain is the C-terminal IDR (SEQ ID NO: 4) of the T β RII-ECD. In this fusion molecule the multimerization domain is the hIgG1Fc(CC) region (SEQ ID NO: 49).

[00246] First, we performed a more detailed characterization of the inhibition potency (IC₅₀) of the T22d35-Fc-IgG1-v1 (CC) fusion for the various isoforms of TGF β , particularly TGF β 1 and TGF β 3, using a A549 IL-11 release assay. The A549 IL-11 release assay was performed substantially as described (WO2018/158727) and is described in further detail in Example 2 below.

[00247] To analyze inhibition potency in greater detail and obtain IC₅₀ values with greater accuracy, we tested more concentrations of TGF β close to the IC₅₀ (more points on the curve) in the A549 IL-11 release assay. Representative results are shown in FIGs. 4A and 4B and results from the average of 6 experiments are given in Table 3. We determined an average IC₅₀ value of 2.89 pM $\pm$ 0.16 for TGF β 1, which is not inconsistent with previous reports (WO2018/158727). However, for TGF β 3, the average IC₅₀ was 8.64 pM $\pm$ 0.43, which is significantly higher than expected (i.e., lower inhibition potency than expected). The results indicate approximately 3-3.5 fold higher potency of inhibition for TGF β 1 compared to TGF β 3, indicating preferential inhibition or neutralization of the TGF β 1 ligand by the T22d35-Fc-IgG1-

v1 (CC) fusion. It is noted that the inhibition potency for TGF β 2 was significantly lower than for TGF β 1 and TGF β 3, as previously reported (WO2018/158727; FIG. 11).

Table 3. IC₅₀ values for T22d35-Fc-IgG1-v1 (CC) (SEQ ID NO: 6).

TGF β 1		TGF β 3		IC ₅₀ Ratio TGF β 3: TGF β 1		n (number of replicates)
Average IC ₅₀ (pM)	SEM	Average IC ₅₀ (pM)	SEM	Average ratio	SEM	25
2.89	0.16	8.64	0.43	3.20	0.24	

SEM: standard error of the mean

[00248] These results are consistent with statements in WO2018/158727 that suggest greater inhibition or neutralization of TGF β 1 compared to TGF β 3 (See, for example, page 3, lines 26-28 of WO2018/158727, where it is stated the “Fc-doublet (T22d35-Fc) exhibits a potency enhancement that is at least 970-fold greater for TGF β 1 and at least 240-fold greater for TGF β 3 when compared to a non-Fc fused ECD doublet”).

[00249] In order to understand better the potential mechanism underlying the preferential inhibition of TGF β 1 ligand versus TGF β 3 ligand by previous fusion constructs, we next conducted a structural comparison of the two ligand isoforms. It should be noted that the ligand-binding domains of the previous fusion constructs interact with essentially identical epitopes on the TGF β 1 and TGF β 3 ligands (Baardsnes, J. et al., 2009). Accordingly, differential affinity for the monomers within the TGF β dimers does not readily explain the preferential inhibition of TGF β 1 ligand. An overlay of the monomeric structures of TGF β 1 (blue) and TGF β 3 (green) is shown in FIG. 2A. An overlay of TGF β 1 and TGF β 3 dimers as observed in Protein Data Bank (PDB) IDs 3KFD and 1KTZ, respectively, is shown in FIG. 2B (Protein Data Bank (PDB) IDs for the structures are 3KFD and 1KTZ, respectively). It can be seen that, although TGF β 1 and TGF β 3 are very similar at the amino acid sequence level and both monomers adopt a similar extended-cysteine knot fold (FIG. 2A), the arrangement of the two monomers within the biologically active dimers is significantly different when comparing the structures of the two isoforms. It is evident that each ligand isoform has a specific range of dimerization angle. The range of the dimerization angle affects the overall shape, spatial extent and compactness of the

dimeric molecule. This difference in shape of the TGF β 1 and TGF β 3 dimers could lead to preferential neutralization of TGF β 1 over TGF β 3 by previous fusion constructs, i.e. the particular spacing of the ligand binding domains within the fusion construct may have led to preferential interactions (preferential avidity) with the TGF β 1 ligand due to that isoform dimer having a distinct shape.

[00250] FIG. 2C shows a representative model of a fusion construct (T22d35-Fc-IgG1-v1(CC), SEQ ID NO: 6) bound to TGF β ligand showing the second ligand-binding domain, second linker, and Fc regions. This model is shown here to illustrate the effects of a short (10 amino acid) second linker. The green line shows that the length of the linker/spacer is short by at least 25 angstroms to allow ligand binding between the attached binding domains. Specifically, the length of the 10 amino acid linker in T22d35-Fc-IgG1-v1(CC) is ~ 35 Å even in an extended conformation, which is shorter than the optimal linker length by about ~ 20 Å calculated using molecular modeling. Thus, in this fusion construct, the second ligand binding domains are sterically restricted from accommodating the TGF β dimer.

[00251] It should be noted that the structured ligand-binding domain is the portion of the fusion constructs that contributes to the interaction interface with TGF β ligands, e.g., TGF β 1 and TGF β 3, and that the linker regions do not directly interact with bound ligand. However, considering the differences in dimeric structure for TGF β 1 and TGF β 3 and the conformational constraints imposed by the linker regions, our analysis suggested that modifying the linker regions may influence the binding properties of the TGF β binding agent so as to alter its ligand binding specificity. In particular, shortening the first linker region between the ligand-binding domains, and lengthening the second linker region between the second ligand-binding domain and the multimerization domain, might alleviate steric and conformational constraints so as to alter the relative inhibition potencies for the TGF β 1 and TGF β 3 ligands.

Example 2. Design and Characterization of TGF β binding agents with altered isoform specificity.

[00252] We generated molecules with varying linker sequences and lengths to see if modifying the linkers could differentially affect isoform specificity and inhibition potency for the TGF β ligands, particularly TGF β 1 and TGF β 3. Based on the structural analysis, we focused on molecules

with shortened linker portions between the two ligand binding domains (first linker portion) and elongated linker portions between the second ligand binding domain and the multimerization domain (second linker portion). Our goal was to design TGF β binding agents with less preferential inhibition of TGF β 1 over TGF β 3 (i.e., lower TGF β 3: TGF β 1 IC₅₀ ratio), while maintaining good potency of inhibition overall, in order to provide binding agents with beneficial therapeutic properties for particular disease indications.

[00253] A series of TGF β -binding agents with linkers of varying length and sequence was designed. The structures of representative fusion proteins are summarized in Table 4. Sequences are given in Table 2.

[00254] The test binding agents were homodimers, each polypeptide in the homodimer comprising: an N-terminal region comprising the N-terminal IDR of the TGF β R2 ectodomain (SEQ ID NO: 3); two TGF β Receptor Type II (TGF β R2) ligand-binding domains (SEQ ID NO: 2); an 18 amino acid first linker portion between the two ligand-binding domains (SEQ ID NOs: 8 or 12); a 10, 16, or 30 amino acid second linker portion between the second ligand-binding domain and the multimerization domain (SEQ ID NOs: 4, 9, 11, or 15); and the hIgG1Fc(CC) multimerization domain (SEQ ID NO: 49). The T22d35-Fc-IgG1-v1 (CC) fusion (SEQ ID NO: 6; WO2018/158727) was used as a positive control (CTL). The complete sequences of Protein 61 (p61), Protein 96 (p96), Protein 101 (p101), Protein 107 (p107), and Protein 112 (p112) are given in SEQ ID NOs: 81, 84, 87, 89, and 92, respectively (Table 2).

Table 4. Structures of representative TGF β -binding agents in accordance with certain embodiments.

TGF β Binding Agent	Linker 1		Linker 2		Multimerization domain	
	SEQ ID NO	Length (amino acids)	SEQ ID NO	Length (amino acids)	SEQ ID NO	Name
T22d35-Fc-IgG1-v1 (CC) (positive control)	7	34	4	10	49	hIgG1Fc(CC)
Protein 61	8	18	9	16	49	hIgG1Fc(CC)
Protein 93	10	24	9	16	50	hIgG4Fc

Protein 94	10	24	10	24	50	hIgG4Fc
Protein 96	8	18	9	16	49	hIgG1Fc(CC)
Protein 97	10	24	4	10	49	hIgG1Fc(CC)
Protein 99	11	30	11	30	50	hIgG4Fc
Protein 101	12	18	11	30	49	hIgG1Fc(CC)
Protein 106	13	26	13	26	49	hIgG1Fc(CC)
Protein 107	8	18	4	10	49	hIgG1Fc(CC)
Protein 108	9	16	9	16	49	hIgG1Fc(CC)
Protein 109	14	20	9	16	49	hIgG1Fc(CC)
Protein 112	12	18	15	16	49	hIgG1Fc(CC)
Protein 113	16	32	16	32	49	hIgG1Fc(CC)
Protein 115	16	32	16	32	50	hIgG4Fc
Protein 116	16	32	7	34	49	hIgG1Fc(CC)
Protein 128	12	18	11	30	50	hIgG4Fc
Protein 129	12	18	17	30	49	hIgG1Fc(CC)
Protein 130	12	18	18	30	49	hIgG1Fc(CC)
Protein 131	12	18	19	30	49	hIgG1Fc(CC)
Protein 132	8	18	19	30	49	hIgG1Fc(CC)
Protein 133	8	18	11	30	49	hIgG1Fc(CC)
Protein 134	8	18	20	16	49	hIgG1Fc(CC)
Protein 135	21	18	9	16	49	hIgG1Fc(CC)

[00255] *Production and purification of recombinant fusion molecules.* All constructs included the secretion signal sequence MDWTWRILFLVAAATGTHA (SEQ ID NO: 104) at the N-terminus when expressed. The complementary (c) DNAs coding for constructs were prepared synthetically (GeneArt, ThermoFisher Scientific). The cDNAs were cloned into the EcoR1 (5' end) and BamH1 (3' end) of the pTT5 mammalian expression plasmid vector (Durocher et al., 2002). Representative cDNA sequences used for expression of fusion proteins are given in Table 2 (SEQ ID NOs: 106-109, used for expression of p61, p96, p101, and p128, respectively). The signal peptide is cleaved off in the cells during expression and is not included in the purified fusion proteins.

[00256] Fusion proteins were expressed by transient transfection of Chinese Hamster Ovary

(CHO). Briefly, expression plasmids encoding the fusion proteins were each transfected into a 100 mL culture of CHO-3E7 cells in Freestyle F17 medium (Invitrogen) containing 4 mM glutamine and 0.1 % Kolliphor p-1 88 (Sigma).

[00257] All cell culture was conducted at 37°C, 5% CO₂. Transfection conditions were as follows: the transfected DNA was comprised of plasmid DNA for expressing the fusion protein and 30% salmon sperm DNA, which was mixed with polyethylenimine-pro (Polyplus) at a ratio = 1:4. At 24 hours post-transfection, 1% Tryptone NI feed (TekniScience Inc.). 0.5 mM VPA (Sigma) was added and the incubator temperature was dropped to 32°C, 5% CO₂. This was done to promote the production and secretion of the fusion proteins and maintained for 4 days post-transfection (dpt) after which the cultures were harvested. On Day 4, the harvest supernatant was filtered (0.2 µm) and purified using an AKTA pure 25L (GE). Supernatant was loaded onto an MabSelect Prisma protein A column and purified by affinity chromatography. The column was then washed with 8 column volumes of PBS and the protein was eluted with 5 column volumes of 0.1 M sodium citrate, pH 3.2. Fractions were then buffer exchanged into formulation buffer (20 mM L-Histidine, 100 mM NaCl, pH 7) using a HiPrep 26/10 desalting column (GE).

[00258] FIGs. 3A and 3B show polyacrylamide gel electrophoresis analysis of samples from purified Proteins 61, 96, 101, 107, and 112 (see the lanes indicated as p61, p96, p101, p107, and p112, respectively) and T22d35-Fc-IgG1-v1 (CC) (Ctl), under both non-reducing (FIG. 3A) and reducing (FIG. 3B) conditions. Proteins (P) were electrophoresed on a 12% Bis-Tris acrylamide gel (NuPAGE™ 12% Bis-Tris Protein Gels, Cat# NP0341BOX, Life Technologies) under both non-reducing and reducing conditions. These fusion proteins are tetravalent, homodimeric TGFβ-binding agents, each comprising two polypeptide chains (i.e., they are homodimers of two polypeptide chains, the first and second polypeptides being the same, and each polypeptide including two ligand-binding domains). The two polypeptide chains are dimerized via disulfide bridges that involve one or more cysteine residues in their multimerization domains, as confirmed by the difference in size under reducing vs. non-reducing conditions.

[00259] *Inhibition of TGFβ1 and TGFβ3 activities by fusion proteins.* To determine the inhibition potencies of Proteins 61, 96, 101, 107, and 112, TGFβ neutralization was assessed, and the inhibition potency was compared to that of a positive control (T22d35-Fc-IgG1-v1 (CC), two TGFβRII-ECD doublets associated via an Fc portion (SEQ ID NO: 6)). It should be noted that a

single non-FC-fused TGF β RII ectodomain (SEQ ID NO: 1) does not neutralize any of TGF β 1, β 2, or β 3 (De Crescenzo et al, 2004). The terms “inhibition potency” and “neutralization potency” are used interchangeably herein.

[00260] TGF β neutralization potencies for the purified fusion proteins were determined using a cell-based signaling assay, specifically an A549 cell/IL-11 release assay using a colorimetric ELISA. Briefly, human A549 lung cancer cells (ATCC-CCL-185, Cedarlane Burlington ON) were seeded in 96-well plates (5×10^3 cells/well) and incubated at 37°C, 5% CO₂, in a humidified atmosphere. The following day, 10 pM TGF β in complete media in the absence or presence of increasing concentrations of fusion protein was incubated for 30 min at room temperature (RT) prior to adding to the cells. After 24 hours (h) of incubation, the conditioned medium was harvested and stored at 4°C. The next day, the IL-11 ELISA was performed according to the manufacturer’s instructions (Human IL-11 DuoSet ELISA Kit, Cat# DY218, R&D Systems, Inc.). This IL-11 release assay acts as a model of TGF β -mediated signaling: relative IL-11 release after TGF β treatment is a measure of TGF β activity. A decrease in IL-11 release after addition of test fusion protein indicates of TGF β activity. The data was plotted and analyzed using Prism8 (GraphPad, San Diego) to generate a dose response curve from the absorbance values using 4-parameter fit logistic model (absorbance versus concentration). Values were then normalized to a positive control (TGF β treatment in the absence of any inhibitor).

[00261] Results from a representative set of experiments are shown in FIGs. 4A and 4B, in which the inhibition potency of Proteins 61, 96, 101, 107, and 112 for TGF β 1 and TGF β 3 were compared to the positive control (SEQ ID NO: 6). The highest potency was seen with the positive control. However, the positive control also had the highest IC₅₀ ratio for TGF β 3: TGF β 1 (3.41 in this experiment). In contrast, the TGF β 3: TGF β 1 IC₅₀ ratios for Proteins 61, 96, 101, 107, and 112 were 1.66, 1.72, 1.51, 1.24, and 1.95, respectively, in this experiment (FIGs. 4A-4B).

Table 5. Inhibition potencies in A549/IL-11 release assay for representative TGF β binding agents in accordance with certain embodiments.

TGF β Binding Agent	TGF β 1		TGF β 3		IC ₅₀ Ratio TGF β 3: TGF β 1		n (number of replicates)
	Average IC ₅₀ (pM)	SEM	Average IC ₅₀ (pM)	SEM	Average ratio	SEM	

Protein 61	6.38	0.29	10.63	0.05	1.67	0.07	2
Protein 96	6.47	0.57	11.22	1.13	1.73	0.02	2
Protein 101	6.75	0.25	9.73	0.57	1.44	0.07	5
Protein 106	3.27	0.15	8.27	0.63	2.54	0.31	2
Protein 107	8.06	0.85	11.81	1.74	1.46	0.12	3
Protein 112	15.74	0.42	29.58	1.28	1.88	0.03	2
Protein 113	2.19	0.37	5.73	1.17	2.49	0.24	6
Protein 115	2.59	0.49	5.11	0.91	2.00	0.31	3
Protein 116	2.44	0.14	6.17	0.12	2.53	0.10	3
Protein 128	6.67	0.44	8.42	0.72	1.28	0.12	4
Protein 129	15.22	1.48	26.47	5.11	1.73	0.29	5
Protein 130	7.09	0.49	8.91	1.45	1.24	0.27	3
Protein 131	5.71	0.79	7.70	1.25	1.31	0.33	3
Protein 132	5.99	0.31	8.00	0.07	1.34	0.08	2
Protein 133	4.79	0.54	8.35	0.82	1.77	0.18	3
Protein 134	20.24	3.44	29.57	4.17	1.54	0.47	2
Protein 135	39.48	16.75	37.46	1.10	1.17	0.52	2

SEM: standard error of the mean

[00262] The results show that altering the linker regions, specifically shortening the first linker region and lengthening the second linker region, lowered the TGFβ3:TGFβ1 IC₅₀ ratio significantly, indicating reduced preferential inhibition of TGFβ1, while still maintaining inhibition potency in the picomolar range.

[00263] Another set of representative binding agents is shown in FIGs. 5A-5B, which show polyacrylamide gel electrophoresis analysis of samples from purified Proteins 112, 111, 106, 105, 104, 101, 99, and 71, respectively, under non-reducing (FIG. 5A) and reducing (FIG. 5B) conditions.

[00264] In the representative set of experiments shown in FIGs. 6A-6B, the neutralization potency of Proteins 113, 115 and 116 compared to positive control (SEQ ID NO: 6) is shown. Results are also shown in Table 5. The results show that the inhibition potency for these proteins was comparable to control for TGF β 1 but significantly higher for TGF β 3, resulting in a significantly lower TGF β 3:TGF β 1 IC₅₀ ratio.

[00265] In the representative set of experiments shown in FIGs. 7A-7B, the neutralization potency of Proteins 101, 129, and 130 compared to positive control (SEQ ID NO: 6) is shown. Results are also given in Table 5. The results show that inhibition potency for these proteins was lower for TGF β 1 compared to control, and about the same (P101, P130) or lower (P129) for TGF β 3, resulting in a significantly lower TGF β 3:TGF β 1 IC₅₀ ratio.

[00266] In the representative set of experiments shown in FIGs. 8A-8B, the neutralization potency of Proteins 101, 131, 132, and 133 compared to positive control (SEQ ID NO: 6) is shown. Results are also given in Table 5. The results show that inhibition potency for these proteins was lower for TGF β 1 compared to control, and about the same or higher for TGF β 3, resulting in a significantly lower TGF β 3:TGF β 1 IC₅₀ ratio.

[00267] In the representative set of experiments shown in FIGs. 9A-9B, the neutralization potency of Proteins 96, 134, and 135 compared to positive control (SEQ ID NO: 6) is shown. Results are also given in Table 5. The results show that inhibition potency for these proteins was reduced more for TGF β 1 than for TGF β 3 compared to control, so that the TGF β 3:TGF β 1 IC₅₀ ratio was significantly lower than for control. Comparing Proteins 134 and 135 to Protein 96, the results show that replacing either the first linker or the second linker with a Gly-Ser linker significantly reduced inhibition potency for both TGF β 1 and TGF β 3, while maintaining a low TGF β 3:TGF β 1 IC₅₀ ratio.

[00268] *Multimerization domain does not affect TGF β isoform specificity.* TGF β binding agents having the same TGF β binding region and differing only in the multimerization domain were tested to see what effect, if any, the multimerization domain has on relative inhibition potency for TGF β 1 and TGF β 3 isoforms. Results are shown in FIGs. 10A-10B, which show neutralization potency of Proteins 101 and 128 compared to positive control (SEQ ID NO: 6), and in Table 5. FIGs. 10A-10B show one representative assay; results averaged from several assays are given in

Table 5. The results show that changing the multimerization domain from IgG1 (Protein 101) to IgG4 (Protein 128) had no significant effect on inhibition potency for TGF β 1 and TGF β 3, as expected. The same result was obtained for Proteins 61 and 96, and for Proteins 113 and 115 (Table 5).

[00269] *Neutralization of the TGF β 2 isoform.* We also tested whether the relative inhibition of TGF β 2 was affected, compared to control, by TGF β binding agents provided herein. A representative set of experiments is shown in FIG. 11. As shown in FIG. 11 for Proteins 61, 96, and 101, the neutralization potency for the TGF β 2 isoform was more than 1000-fold lower than for the TGF β 1 and TGF β 3 isoforms (in other words, IC₅₀ was more than 1000-fold higher), same as for control. The results demonstrate that equalization or shifting of the relative inhibition potencies for TGF β 1 and TGF β 3 isoforms did not have a significant effect on the very low inhibition of the TGF β 2 isoform.

[00270] Taken together, the results reported herein show that shortening the first linker region to less than 34 amino acids, and/or lengthening the second linker region to more than 10 amino acids, was effective to lower the TGF β 3:TGF β 1 IC₅₀ ratio for these binding agents, in some cases almost equalizing the inhibition potencies for the two isoforms. It should be noted that in some cases, the TGF β 3:TGF β 1 IC₅₀ ratio was lowered by increasing the inhibition potency for TGF β 3 without adversely affecting the potency for TGF β 1 (e.g., Proteins 113, 115, 116). In other cases, the ratio was lowered primarily by lowering the inhibition potency for TGF β 1 without adversely affecting potency for TGF β 3 (e.g., Proteins 61, 96, 101, 107, 128), although in some cases a slight reduction in TGF β 3 potency was also observed. Nevertheless, all binding agents maintained significantly higher inhibition potency for both TGF β 1 and TGF β 3 than for TGF β 2, consistent with their potential use as therapeutics for the treatment of TGF β -associated disorders, particularly those mediated by TGF β 3.

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[00313] The contents of all documents and references cited herein are hereby incorporated by reference in their entirety.

[00314] Although this invention is described in detail with reference to embodiments thereof, these embodiments are offered to illustrate but not to limit the invention. It is possible to make other embodiments that employ the principles of the invention and that fall within its spirit and scope as defined by the claims appended hereto.