maintain tissue homeostasis (as reviewed in ten Dijke and Arthur, Nature Reviews, *Molecular Cell Biology*, Vol. 8, Nov. 2007, p. 857-869). Levels of TFGbeta are controlled by regulation through TGFbeta expression as well as through binding to proteoglycan, *i.e.,* the extracellular matrix (ECM).

Dysregulated TGFbeta signaling, such as excess TGFbeta signaling and high levels of bioavailable TGFbeta, is implicated in a number of pathologies, including fibroses of various tissues, such as pulmonary fibrosis and cirrhosis, chronic hepatitis, rheumatoid arthritis, ocular disorders, vascular restenosis, keloid of skin, and the onset of nephrosclerosis.

Accordingly, there is a need to provide compounds that block or disrupt TGFbeta signaling in a specific manner, such as through binding to the TGFbeta receptor II. Such compounds can be used in therapeutics.

SUMMARY ~~OF THE INVENTION~~

In a first ~~aspect~~ embodiment, ~~the invention provides~~ there is provided an anti-TGFbetaRII immunoglobulin single variable domain. Suitably, an anti-TGFbetaRII immunoglobulin single variable domain in accordance with the first ~~aspect~~ embodiment is one which binds to TGFbetaRII with a dissociation constant (Kd) in the range of 10pM to 50nM, preferably 10pM to 10nM, preferably 250pM to 10nM. In one embodiment, the anti-TGFbetaRII immunoglobulin single variable domain is one which binds TGFbetaRII with high affinity (high potency) and has a dissociation constant of 10pM to 500pM. In another embodiment, the anti-TGFbetaRII immunoglobulin single variable domain is one which binds TGFbetaRII with moderate affinity (low potency) and has a dissociation constant of 500pM to 50nM, preferably 500pM to 10nM. In another ~~aspect~~ embodiment, ~~the invention provides~~ there is provided an isolated polypeptide comprising an anti-TGFbetaRII immunoglobulin single variable domain. Suitably, the isolated polypeptide comprises an amino acid sequence that is at least 70 % identical to at least one amino acid sequence selected from the group of any of the amino acid sequences set out in SEQ ID NOs: 1 to 23 and which binds to TGFbetaRII. Suitably, the isolated polypeptide binds to human TGFbetaRII. In another embodiment, the isolated polypeptide also binds to TGFbetaRII derived from a different species such as mouse, dog or monkeys, such as cynomolgus monkeys (cyno). Suitably, the isolated polypeptide binds to both mouse and human TGFbetaRII. Such cross reactivity between TGFbetaRII from humans and other species allows the same antibody construct to be used in an animal disease model, as well as in humans.

In one ~~aspect~~ embodiment, ~~the invention provides~~ there is provided an isolated polypeptide comprising an amino acid sequence selected from the group of the amino acid sequences set out in SEQ ID NOs: 1 to 23. In one embodiment, ~~the invention provides~~ there is provided an isolated polypeptide comprising an amino acid sequence as set out in any of SEQ ID NOs: 4 (DOM23h-271), 8 (DOM23h-437) or 10 (DOM23h-439).

In another ~~aspect~~ embodiment, ~~the invention provides~~ there is provided an isolated polypeptide encoded by a nucleotide sequence that is at least 60% identical to the nucleotide sequence selected from the group of any of the nucleic acid sequences set out in SEQ ID NOS: 24 to 46 and which binds to TGFbetaRII. Suitably, the isolated polypeptide binds to human TGFbetaRII

In another ~~aspect~~ embodiment, there is provided an anti-TGFbetaRII immunoglobulin single variable domain comprising an amino acid sequence that is at least 90% identical to the amino acid sequence of any one amino acid sequence selected from the group of any of the amino acid sequences set out in SEQ ID NOs: 1 to 23. In one embodiment, the anti-TGFbetaRII immunoglobulin single variable domain comprises an amino acid sequence that is identical to the amino acid sequence of any of the amino acid sequences set out in SEQ ID NOs: 1 to 23. Suitably, the anti-TGFbetaRII immunoglobulin single variable domain comprises an amino acid sequence as set out in any of SEQ ID NOs: 4 (DOM23h-271), 8 (DOM23h-437) or 10 (DOM23h-439). In another embodiment, the anti-TGFbetaRII immunoglobulin single variable domain comprises an amino acid sequence selected from the group of any of the amino acid sequences set out in SEQ ID NOs: 1 to 23. Suitably, the anti-TGFbetaRII immunoglobulin single variable domain binds TGFbetaRII, preferably human TGFbetaRII.

In another ~~aspect~~ embodiment, there is provided an anti-TGFbetaRII immunoglobulin single variable domain comprising an amino acid sequence of any one of the amino acid sequences set out in SEQ ID NOs: 1 to 23 that is modified at no more than 25 amino acid positions and comprises a CDR1 sequence that is at least 50% identical to the CDR1 sequence in any one of SEQ ID NOs: 1 to 23.

In a further ~~aspect~~ embodiment, there is provided an anti-TGFbetaRII immunoglobulin single variable domain comprising an amino acid sequence of any one of the amino acid sequences set out in SEQ ID NOs: 1 to 23 that is modified at no more than 25 amino acid positions and comprises a CDR2 sequence that is at least 50% identical to the CDR2 sequence in any one of SEQ ID NOs: 1 to 23.

In another ~~aspect~~ embodiment, there is provided an anti-TGFbetaRII immunoglobulin single variable domain comprising an amino acid sequence of any one of the amino acid sequences set out in SEQ ID NOs: 1 to 23 that is modified at no more than 25 amino acid positions and comprises a CDR3 sequence that is at least 50% identical to the CDR3 sequence in any one of SEQ ID NOs: 1 to 23.

In a further ~~aspect~~ embodiment, there is provided an anti-TGFbetaRII immunoglobulin single variable domain comprising an amino acid sequence of any one of the amino acid sequences set out in SEQ ID NOs: 1 to 23 that is modified at no more than 25 amino acid positions and comprises a CDR1 sequence that is at least 50% identical to a CDR1 sequence in any one of SEQ ID NOs: 1 to 23 and comprises a CDR2 sequence that is at least 50% identical to a CDR2 sequence in any one of SEQ ID NOs: 1 to 23.

In another ~~aspect~~ embodiment, there is provided an anti-TGFbetaRII immunoglobulin single variable domain comprising an amino acid sequence of any one of the amino acid sequences set out in SEQ ID NOs: 1 to 23 that is modified at no more than 25 amino acid positions and comprises a CDR1 sequence that is at least 50% identical to the CDR1 sequence in any one of SEQ ID NOs: 1 to 23 and comprises a CDR3 sequence that is at least 50% identical to the CDR3 sequence in any one of SEQ ID NOs: 1 to 23.

In yet another ~~aspect~~ embodiment, there is provided an anti-TGFbetaRII immunoglobulin single variable domain comprising an amino acid sequence of any one of the amino acid sequences set out in SEQ ID NOs: 1 to 23 that is modified at no more than 25 amino acid positions and comprises a CDR2 sequence that is at least 50% identical to the CDR2 sequence in any one of SEQ ID NOs: 1 to 23 and comprises a CDR3 sequence that is at least 50% identical to the CDR3 sequence in any one of SEQ ID NOs: 1 to 23.

In another ~~aspect,~~ embodiment, there is provided an anti-TGFbetaRII immunoglobulin single variable domain comprising an amino acid sequence of any one of the amino acid sequences set out in SEQ ID NOs: 1 to 23 that is modified at no more than 25 amino acid positions and comprises a CDR1 sequence that is at least 50% identical to the CDR1 sequence in any one of SEQ ID NOs: 1 to 23 and comprises a CDR2 sequence that is at least 50% identical to the CDR2 sequence in any one of SEQ ID NOs: 1 to 23 and comprises a CDR3 sequence that is at least 50% identical to the CDR3 sequence in any one of SEQ ID NOs: 1 to 23.

In a further ~~aspect~~ embodiment , there is provided an anti-TGFbetaRII immunoglobulin single variable domain comprising a CDR3 sequence that is at least 50% identical to a CDR3

sequence selected from the group consisting of: the CDR3 sequence in any one of SEQ ID NOs: 1 to 23.

In another ~~aspect~~ embodiment, there is provided an anti-TGFbetaRII immunoglobulin single variable domain comprising a CDR3 sequence selected from the group consisting of: the CDR3 sequence in any one of SEQ ID NOs: 1 to 23.

In yet another ~~aspect~~ embodiment, there is provided an anti-TGFbetaRII immunoglobulin single variable domain comprising at least one CDR selected from the group consisting of: CDR1, CDR2, and CDR3, wherein the CDR1, CDR2, or CDR3 is identical to a CDR1, CDR2, or CDR3 sequence in any one of SEQ ID NOs: 1 to 23.

Suitably, CDR sequences are determined using the method of Kabat described herein. In one embodiment, the CDR sequences of each sequence are those set out in Figure 7.

In one embodiment, there is provided an anti-TGFbetaRII immunoglobulin single variable domain or a polypeptide in accordance with any aspect of the invention which comprises comprising any of the following amino acids: N at position 61, R at position 64, H at position 102, R at position 39, D at position 53 or S at position 103 of the immunoglobulin single variable domain. In one embodiment, the immunoglobulin single variable domain or polypeptide comprises a combination of these amino acids. In another embodiment, the immunoglobulin single variable domain or polypeptide comprises amino acid N at 61 and R at 64. In these embodiments, the amino acid numbering is that of the immunoglobulin single variable domain, as exemplified, for example, by those sequences given in SEQ ID NOs: 1 to 23.

In another ~~aspect~~ embodiment, there is provided a ligand or binding moiety that has binding specificity for TGFbetaRII and inhibits the binding of an anti-TGFbetaRII immunoglobulin single variable domain comprising an amino acid sequence selected from the group of SEQ ID NOs: 1 to 23.

In a further ~~aspect of the invention~~ embodiment, there is provided a fusion protein comprising an immunoglobulin single variable domain, polypeptide or ligand in accordance with any ~~aspect of the invention~~ embodiment disclosed herein.

In one embodiment, the immunoglobulin single variable domain, polypeptide, ligand or fusion protein in accordance with the invention is one which neutralises TGFbeta activity. Suitably, the immunoglobulin single variable domain or polypeptide in accordance with the ~~invention~~ disclosure inhibits binding of TGFbeta to TGFbetaRII. In another embodiment, the immunoglobulin single variable domain or polypeptide in accordance

with the ~~invention~~ disclosure inhibits TGFbeta signalling activity through TGFbetaRII. In another embodiment, the immunoglobulin single variable domain or polypeptide in accordance with the ~~invention~~ disclosure suppresses TGFbeta activity, in particular, TGFbeta cell growth activity. Suitably, TGFbetaRII is human TGFbetaRII.

In one embodiment, the immunoglobulin single variable domain, polypeptide, ligand or fusion protein in accordance with the ~~invention~~ disclosure is devoid of TGFbetaRII agonist activity at 10 micromolar (μM).

In another ~~aspect~~ embodiment, there is provided an immunoglobulin single variable domain, polypeptide, ligand or fusion protein in accordance with ~~any aspect of the invention~~ embodiment disclosed herein further comprising a half-life extending moiety. Suitably, the half-life extending moiety is a polyethylene glycol moiety, serum albumin or a fragment thereof, transferrin receptor or a transferrin-binding portion thereof, or an antibody or antibody fragment comprising a binding site for a polypeptide that enhances half-life *in vivo*. In one embodiment, the half-life extending moiety is an antibody or antibody fragment comprising a binding site for serum albumin or neonatal Fc receptor. In another embodiment, the half-life extending moiety is a dAb, antibody or antibody fragment.

In another ~~aspect~~ embodiment, ~~the invention provides~~ there is provided an isolated or recombinant nucleic acid encoding a polypeptide comprising an anti-TGFbetaRII immunoglobulin single variable domain, polypeptide, ligand or fusion protein in accordance with ~~any aspect of the invention~~ any embodiment disclosed herein.

In one embodiment, the isolated or recombinant nucleic acid molecule comprises a nucleic acid molecule selected from the group of any of the nucleic acid molecules having the sequences set out in SEQ ID NOS: 24 to 46.

In one ~~aspect~~ embodiment, ~~the invention provides~~ there is provided an isolated or recombinant nucleic acid, wherein the nucleic acid comprises a nucleotide sequence that is at least 70% identical to the nucleotide sequence of any of the nucleic acid molecules having the sequences set out in SEQ ID NOS: 24 to 46, and wherein the nucleic acid encodes a polypeptide comprising an immunoglobulin single variable domain that specifically binds to TGFbetaRII.

In another ~~aspect~~ embodiment, there is provided a vector comprising a nucleic acid in accordance with the ~~invention~~ disclosure.

In a further ~~aspect~~ embodiment, there is provided a host cell comprising a nucleic acid or a vector in accordance with the invention. In yet ~~another aspect of the invention~~embodiment there is provided a method of producing a polypeptide comprising an anti-TGFbetaRII

immunoglobulin single variable domain or a polypeptide or ligand in accordance with the ~~invention~~ disclosure, the method comprising maintaining a host cell in accordance with the ~~invention~~ disclosure under conditions suitable for expression of said nucleic acid or vector, whereby a polypeptide comprising an immunoglobulin single variable domain, polypeptide or ligand is produced. Optionally, the method further comprises the step of isolating the polypeptide and optionally producing a variant, *e.g.*, a mutated variant, having an improved affinity (Kd); or EC_{50} for TGFbeta neutralization in a standard assay than the isolated polypeptide. Suitable assays for TGFbeta activity, such as a cell sensor assay, are described herein, for example, in the Examples section.

In one ~~aspect of the invention~~ embodiment, the anti-TGFbetaRII immunoglobulin single variable domain, polypeptide or ligand in accordance with the ~~invention~~ disclosure is for use as a medicament. Accordingly, there is provided a composition comprising anti-TGFbetaRII immunoglobulin single variable domain, polypeptide or ligand in accordance with the ~~invention~~ disclosure for use as a medicament.

Suitably, the anti-TGFbetaRII immunoglobulin single variable domain, polypeptide or ligand or composition in accordance with the ~~invention~~ disclosure is for treatment of a disease associated with TGFbeta signaling. Suitably, the disease is a tissue fibrosis, such as pulmonary fibrosis including idiopathic pulmonary fibrosis, liver fibrosis, including cirrhosis and chronic hepatitis, rheumatoid arthritis, ocular disorders, or fibrosis of the skin including keloid of skin, and kidney such as nephritis, kidney fibrosis and nephrosclerosis, or a vascular condition such as restenosis. Other diseases associated with TGFbeta signaling include vascular diseases such as hypertension, pre-eclampsia, hereditary haemorrhagic telangtiectasia type I (HHT1), HHT2, pulmonary arterial hypertension, aortic aneurysms, Marfan syndrome, familial aneurysm disorder, Loeys-Dietz syndrome, arterial tortuosity syndrome (ATS). Other diseases associated with TGFbeta signaling include diseases of the musculoskeletal system, such as Duchenne's muscular dystrophy and muscle fibrosis. Further diseases associated with TGFbeta signaling include cancer, such as colon, gastric, and pancreatic cancer, as well as glioma and NSCLC. In addition, the ~~invention~~ disclosure provides methods for targeting cancer by modulating TGFbeta signaling in tumour angiogenesis. Other diseases or conditions include those related to tissue scarring. Other diseases include pulmonary diseases such as COPD (Chronic obstructive pulmonary disease). In one ~~aspect~~ embodiment, there is provided ~~the invention provides~~ the anti-TGFbetaRII single variable domain or antagonist, composition or fusion protein for pulmonary delivery. In one ~~aspect~~ embodiment, ~~the invention provides~~ there is provided the anti-

TGFbetaRII single variable domain or antagonist or fusion protein for delivery to the lung of a patient. In one ~~aspect~~ embodiment, ~~the invention provides~~ there is provided the use of the anti-TGFbetaRII single variable domain or antagonist or fusion protein in the manufacture of a medicament for pulmonary delivery. In one ~~aspect~~ embodiment, ~~the invention provides~~ there is provided the use of the anti-TGFbetaRII single variable domain or antagonist or fusion protein in accordance with the ~~invention~~ disclosure in the manufacture of a medicament for delivery to the lung of a patient.

In one embodiment, the variable domain *per se,* or when part of the antagonist or fusion protein, is resistant to proteases such as leucozyme and/or trypsin.

In one embodiment, the composition is for pulmonary delivery. Suitably, the composition comprises a monomeric dAb for pulmonary delivery. In another embodiment, the composition consists of a monomeric dAb for pulmonary delivery.

Suitably, the composition is for therapy or prophylaxis of a TGFbeta-mediated condition in a human.

Accordingly, in one embodiment, there is provided an anti-TGFbetaRII dAb for treating idiopathic pulmonary fibrosis. Suitably, the anti-TGFbetaRII dAb is provided as a monomeric dAb for pulmonary delivery, preferably lacking any tag (*i.e.*, untagged) such as a myc or another purification tag.

In one ~~aspect~~ embodiment, the composition is a pharmaceutical composition and further comprises a pharmaceutically acceptable carrier, excipient or diluent.

In another ~~aspect~~ embodiment, there is provided a method of treating and/or preventing an TGFbeta-mediated condition in a human patient, the method comprising administering a composition comprising an anti-TGFbetaRII immunoglobulin single variable domain, polypeptide or ligand in accordance with the ~~invention~~ disclosure ~~the~~ to the patient.

In a further ~~aspect~~ embodiment, ~~the invention provides~~ there is provided a pulmonary delivery device containing a composition in accordance with the ~~invention~~ disclosure. ~~.~~Suitably, such a device is an inhaler or an intranasal administration device.

In one aspect, there is provided an anti-TGFbetaRII immunoglobulin single variable domain comprising an amino acid sequence that is at least 90% identical to amino acid sequence SEQ ID NO: 10 that binds to TGFbetaRII with a dissociation constant (Kd) in the range of 10pM to 50nM.

In another aspect, there is provided an isolated polypeptide comprising an anti-TGRbetaRII immunoglobulin single variable domain as disclosed herein wherein said isolated polypeptide binds to TGRbetaRII.

In another aspect, there is provided a ligand that has binding specificity for TGFbetaRII and inhibits the binding of an anti-TGFbetaRII immunoglobulin single variable domain, comprising amino acid sequence SEQ ID NO: 10.

In another aspect, there is provided a fusion protein comprising an immunoglobulin single variable domain, polypeptide or ligand as disclosed herein.

In another aspect, there is provided an immunoglobulin single variable domain, polypeptide, ligand or fusion protein as disclosed herein, wherein said anti-TGFbetaRII immunoglobulin single variable domain, polypeptide, ligand, or fusion protein neutralises TGFbeta activity.

In another aspect , there is provided an immunoglobulin single variable domain, polypeptide, ligand or fusion protein disclosed herein, wherein said immunoglobulin single variable domain, polypeptide, ligand or fusion protein inhibits binding of TGFbeta to TGFbetaRII.

In another aspect, there is provided an isolated nucleic acid encoding an anti-TGFbetaRII immunoglobulin single variable domain, polypeptide, ligand or fusion protein as disclosed herein.

In another aspect, there is provided a vector comprising a nucleic acid molecule as disclosed herein.

In another aspect, there is provided a host cell comprising a nucleic acid or a vector as disclosed herein

In another aspect, there is provided a method of producing a polypeptide comprising an anti-TGFbetaRII immunoglobulin single variable domain, a polypeptide, ligand or fusion protein as disclosed herein, the method comprising maintaining a host cell as disclosed herein under conditions suitable for expression of said nucleic acid or vector, whereby a polypeptide comprising an immunoglobulin single variable domain, polypeptide, ligand or fusion protein is produced.

In another aspect, there is provided an anti-TGFbetaRII immunoglobulin single variable domain, polypeptide, ligand or fusion protein as disclosed herein for use in the treatment of a disease associated with TGFbeta signalling, optionally wherein the disease associated with TGFbeta signalling is selected from the group consisting of:

tissue fibrosis, such as pulmonary fibrosis, including idiopathic pulmonary fibrosis, liver fibrosis, including cirrhosis and chronic hepatitis, rheumatoid arthritis, ocular disorders, or fibrosis of the skin, including keloid of skin, and kidney such as nephritis, kidney fibrosis and nephrosclerosis, and a vascular condition, such as restenosis.

In another aspect, there is provided a pharmaceutical composition comprising an anti-TGFbetaRII immunoglobulin single variable domain, polypeptide, ligand or fusion protein as disclosed herein.

In another aspect, there is provided use of a composition comprising an anti-TGFbetaRII immunoglobulin single variable domain, polypeptide, ligand or fusion protein as disclosed herein in the manufacture of a medicament for treating and/or preventing a TGFbeta-mediated condition in a human patient.

BRIEF DESCRIPTION OF THE FIGURES

FIGURE 1 shows amino acid (SEQ ID NOs: 1-23) and nucleic acid (SEQ ID NOs: 24-46) sequences of dAbs in accordance with the invention.

FIGURE 2 shows an alignment of DOM23h naive dAb amino acid sequences. Amino acids are numbered according to Kabat. CDR sequences are indicated in bold and underlined. Dots indicate sequence identity to DOM 23h-251 (SEQ ID NO: 2).