

Title: TGF-beta-RII BINDING PROTEINS

FIELD OF THE INVENTION

5

The present invention relates to the field of medicine. More specifically, the present invention relates to proteins that bind transforming growth factor-beta receptor II (TGF- β RII) and their use in the treatment of humans, in particular in the treatment of cancer.

10

BACKGROUND TO THE INVENTION

Transforming Growth Factor- β (TGF- β) is a signaling molecule part of the TGF- β superfamily. There are three TGF- β ligands (TGF- β 1, 2 and 3) that regulate a variety of cellular functions. TGF- β signaling has a tumor-suppressive role in normal cells but is capable of having a tumor-promoting role in malignant cells. They play a role in processes such as proliferation, migration, differentiation, apoptosis, angiogenesis and epithelial-mesenchymal transition (Bierie and Moses, 2006. Siegel and Massague, 2003). These signals are mediated through the binding to TGF- β receptor type-2 (TGF- β RII), which leads to the dimerization with and phosphorylation of TGF- β RI. This heterotetrameric complex composed of two TGF- β RII and two TGF- β RI then recruits and phosphorylates SMAD2 and SMAD3, which in turn recruits and binds to the co-SMAD molecule SMAD4 to form the SMAD/co-SMAD complex, and translocates to the nucleus where it regulates the transcription of TGF- β target genes (Hata and Chen, 2016. Vander Ark, Cao et al., 2018).

High levels of TGF- β expression have been shown to be associated with a poor prognosis in cancer. TGF- β may induce epithelial-mesenchymal transition which facilitates the migration and metastasis of cancer cells. Gene expression profiles have indicated that TGF- β signaling is a significant pathway in liver metastases of colorectal cancer (Jung, Staudacher et al., 2017). Furthermore, it has been reported that stromal-epithelial interactions play roles in cancer progression. An abundant stromal cellular component found in solid tumors, is the cancer associated fibroblast (CAF), which aside

from building and remodeling the extracellular matrix that may serve as a scaffold for the tumor, also contributes to immune evasion, as well as promotes tumor development and growth (Quail and Joyce, 2013). Tumor-derived TGF- β 1 induces the transdifferentiation of fibroblasts into the 'activated' CAFs by binding to TGF β RII, which is capable of promoting a pro-tumorigenic stromal environment.

Existing modes of targeting the TGF- β signaling pathway to inhibit aberrant TGF- β signaling characteristics of CAFs and tumors to date have had sub-optimal efficacy and/or development liabilities. One anti-TGF- β RII monoclonal antibody is LY3022859 disclosed in WO2010/053814, which was reported to block the ectodomain of TGF- β RII. However, dose escalation studies in phase I led to cytokine release syndrome and was considered unsafe in patients with advanced solid tumors. Another agent, disclosed in WO2012/093125, is an anti-TGF- β RII single variable domain with shorter half-life and wherein the precise mechanism of action or binding domain of this molecule has not been established. To date there has been no anti-TGF- β RII antibody which has successfully gone through clinical trials as the complexity and pleiotropic nature of TGF- β tumor regulation renders the drug development challenging (Hao, Baker et al., 2019). Herein, we describe new heavy chain variable regions, heavy chains, Fabs and full-length IgG monospecific antibodies capable of binding TGF- β RII. These antibodies, or antibodies comprising these heavy chains or heavy chain variable regions, target a novel position on TGF- β RII, and block the interaction between the receptor and its ligand, which is an improvement over existing antibodies.

SUMMARY OF THE INVENTION

The object of the present invention is to provide a new pharmaceutical agent for the treatment of human disease, in particular for the treatment of cancer. This object is met by the provision of an antibody or antibody fragment thereof, and particular binding domains, that specifically binds to the extracellular domain of human TGF- β RII, as described and claimed herein.

In a first aspect, the present invention relates to an antibody or antibody fragment thereof that specifically binds to the extracellular domain of human TGF- β RII, wherein the antibody or antibody fragment binds to an epitope of the extracellular

domain of human TGF- β RII of which phenylalanine (F) at position 25 of isoform A of human TGF- β RII, or at position 50 of isoform B of human TGF- β RII, is a critical residue for binding.

Preferably, such antibody or antibody fragment binds to TGF- β RII, blocks or
5 inhibits binding of a human TGF- β to a human TGF- β RII, thereby inhibiting the signal transduction into the cell, and blocking or inhibiting heterodimerization of TGF- β RII.

In a second aspect, the present invention relates to a vector comprising a polynucleotide encoding either or both the heavy chain and the light chain of the antibody or antibody fragment as described herein.

10 In a third aspect, the present invention relates to a cell producing the antibody or antibody fragment as described herein.

In a fourth aspect, the present invention relates to a pharmaceutical composition comprising the antibody or antibody fragment as described herein and a pharmaceutically acceptable carrier, diluent, or excipient.

15 In a fifth aspect, the present invention relates to a pharmaceutical agent for preventing, suppressing symptom progression or recurrence of, and/or treating cancer, wherein the pharmaceutical agent comprises the antibody or antibody fragment as described herein as an active ingredient.

In a sixth aspect, the present invention relates to a method of treating cancer in a
20 subject, the method comprising administering an effective amount of the antibody or antibody fragment as described herein, or the pharmaceutical composition as described herein, to the subject.

In a seventh aspect, the present invention relates to a method of blocking binding of human TGF- β to human TGF- β RII on a cell, the method comprising
25 providing the antibody or antibody fragment as described herein to the cell and allowing the antibody or antibody fragment to bind to the human TGF- β RII of the cell, to thereby block binding of human TGF- β to human TGF- β RII on the cell.

In an eighth aspect, the present invention relates to a method of inhibiting signal transduction into a cell induced by binding of a human TGF- β to a human TGF- β RII of
30 the cell, the method comprising providing the antibody or antibody fragment as described herein to the cell and allowing the antibody or antibody fragment to bind to the human TGF- β RII of the cell, to thereby inhibit the signal transduction into the cell.

In a ninth aspect, the present invention relates to a method of preventing or inhibiting metastasis, the method comprising administering an effective amount of the antibody or antibody fragment as described herein, or the pharmaceutical composition as described herein, to a subject.

5

DETAILED DESCRIPTION OF THE INVENTION

In general, the present invention relates to a protein, in particular an antibody or antibody fragment thereof, that specifically binds to the extracellular domain of human
10 TGF- β RII. Preferably, the antibody is isolated. Preferably, the antibody is a monoclonal antibody. More preferably, the antibody is an isolated monoclonal antibody.

An "isolated monoclonal antibody" refers to an antibody produced by a clonal cell. Examples of isolated antibodies include, but are not limited to, an antibody that has been affinity purified, an antibody that has been produced in a hybridoma or other cell
15 line *in vitro*, and a human antibody derived from a transgenic non-human animal.

The term "antibody" refers to an immunoglobulin molecule comprising four polypeptide chains: two heavy (H) chains and two light (L) chains. Each heavy chain comprises a heavy chain variable region (VH) and a heavy chain constant region (CH). The heavy chain variable region comprises three heavy chain complementarity
20 determining regions (HCDRs) and four framework regions (FRs), and is preferably human or humanized. The heavy chain constant region is comprised of three domains: CH1, CH2 and CH3; and may be derived from any organism, preferably a human. The CH1 and CH2 domains of the heavy chain constant region are connected through a flexible hinge region. Each light chain comprises a light chain variable region (VL) and
25 a light chain constant region (CL). The light chain variable region can be of two types: kappa (K) or lambda (λ) and, like the VH, comprises three light chain complementarity determining regions (LCDRs) and four framework regions. The light chain variable region is preferably human or humanized. The light chain constant region is comprised of one domain: CL; and may be derived from any organism, preferably a human. The
30 two heavy chains are linked to each other by disulfide bonds between the two hinge regions, and each heavy chain pairs with a light chain by a disulfide bond between the

CH1 and CL regions. In a conventional antibody, the two heavy chains and the two light chains are identical, providing the antibody with two identical antigen-binding sites.

Antibody binding has different qualities including specificity and affinity. The specificity determines which antigen or epitope thereof is specifically bound by an antibody binding domain. The affinity is a measure for the strength of binding to a particular antigen or epitope.

A “human” antibody refers to an antibody in which all antibody domains are derived from human germline immunoglobulin sequences. The human antibody to be used in the present invention can be produced by a method using a mouse transformed to produce a human antibody, e.g., Humab mouse, a KM mouse, a Xeno mouse, a Tc mouse, or a MeMo® mouse (WO2009/157771). The human antibody can also be prepared using SCID mice into which human immune cells have been reconstructed such that a human antibody response is made upon immunization.

A “humanized” antibody region refers to an antibody prepared by, e.g., grafting a complementarity determining region (CDR) sequence of an antibody derived from a germline of an animal other than human, such as mouse or chicken, into human framework sequences of a human antibody. Also, a humanized antibody can be produced by linking nucleic acid encoding CDR regions of an antibody isolated from antibody-producing hybridoma’s to a nucleic acid encoding a framework region of the human-derived antibody using a well-known method.

An antibody is capable of binding an antigen through its heavy chain and/or light chain variable regions, and in particular through its specific CDRs. The CDRs of the heavy and/or light chain variable regions of an antibody bind to an “epitope”, also referred to as “antigenic determinant”, of an antigen. Epitopes can be formed from contiguous amino acids or noncontiguous amino acids juxtaposed by tertiary folding of a protein, so-called linear and conformational epitopes, respectively. Epitopes formed from contiguous, linear amino acids are typically retained on exposure to denaturing solvents, whereas epitopes formed by tertiary folding, conformation is typically lost on treatment with denaturing solvents. An epitope may typically include 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 amino acids in a unique spatial conformation.

An “antigen” typically is a molecule capable of inducing an immune response in a host organism, thereby producing antibodies with specificity for the antigen. At the

molecular level, an antigen is characterized by its ability to be bound by the antigen-binding site of an antibody. As described above, the antigen-binding site of an antibody is formed by its heavy and/or light chain variable regions, and in particular by its CDRs. An antigen comprises at least one, but often more, epitopes.

5 Also mixtures of antigens can be regarded as an 'antigen', and the ordinary skilled person would appreciate that sometimes a lysate of tumor cells, or viral particles may be indicated as 'antigen' whereas such tumor cell lysate or viral particle preparation exists of many antigenic determinants.

10 In the present invention, the antigen is human TGF- β RII. Human TGF- β RII is a transmembrane protein of which there are different isoforms. . The amino acid sequence of human TGF- β RII isoform A is provided as SEQ ID NO: 101; the amino acid sequence of the extracellular domain of human TGF- β RII isoform A is provided as SEQ ID NO: 102. Human TGF- β RII isoform B is a splice variant encoding a longer isoform due to an insertion in the extracellular domain. The amino acid sequence of human
15 TGF- β RII isoform B is provided as SEQ ID NO: 103; the amino acid sequence of the extracellular domain of isoform B of human TGF- β RII is as set forth in SEQ ID NO: 104.

20 TGF- β RII is a member of the serine/threonine protein kinase family and the TGF β receptor subfamily. It is known under various synonyms, including TGFBR2, AAT3, FAA3, LDS1B, LDS2, LDS2B, MFS2, RIIC, TAAD2, TGFR-2, TGFbeta-RII, transforming growth factor beta receptor 2, and TBR-ii, TBRII. TGF- β RII forms a heterodimeric complex with another receptor protein, and binds TGF-beta. This receptor/ligand complex phosphorylates proteins, which then enter the nucleus and regulate the transcription of a subset of genes related to cell proliferation.

25 An antibody binds an antigen with a particular binding affinity. The "binding affinity" is determined by the dissociation constant (K_D), calculated by the formula: k_{off}/k_{on} , and refers to the strength of the antibody-antigen interaction. Depending on the desired biological activity, an antibody can be selected based on its high and/or low k_{on} and/or k_{off} rates.

30 An "antibody fragment" includes, but is not limited to, a functional fragment of a heavy chain and/or a light chain. Such functional fragment comprises at least one CDR derived from, or synthesized based on, a CDR from an antibody heavy chain or

light chain, and is capable of specifically recognizing an antigen. An antibody fragment can for instance be, but is not limited to, a Fab, F(ab')₂, scFv, minibody, or sdAb. An “antibody fragment” further refers to a proteinaceous moiety comprising a functional part of an antibody. In the present case at least the heavy chain variable region, or one
5 or more of the HCDRs, described herein. The antibody fragment can be any binding agent, including, but not limited to, single chain Fvs, single chain or Tandem diabodies (TandAb®), VHHs, Anticalins®, Nanobodies®, a BiTE®, a Fab, ankyrin repeat proteins or DARPINs®, Avimers®, a DART, a TCR-like antibody, Adnectins®, Affilins®, Trans-bodies®, Affibodies®, a TrimerX®, MicroProteins, Fynomers®,
10 Centyrins® or a KALBITOR®.

A “Fab” typically means a binding domain comprising a heavy chain variable region, a light chain variable region, a CH1 and a CL region.

A “F(ab')₂” typically means a binding domain comprising two Fab domains linked to each other by means of a hinge region.

15 A “single-chain variable fragment” (scFv) typically means a binding domain comprising a VH domain and a VL domain which are connected via a linker, for example a peptide linker of, for example, from about 10 to about 25 amino acids in length.

A “minibody” typically means a binding domain comprising two scFv's and a
20 CH3 domain.

A “single domain antibody” (sdAb) typically means a binding domain comprising only the VH or the VL domain of an antibody, usually fused or otherwise linked to, part of, an Fc region. Like a whole antibody, it is able to bind selectively to a specific antigen. Single-domain antibody fragments may be engineered from heavy-
25 chain antibodies found in camelids; these are at times called VHH fragments (Nanobodies®). Some fishes also have heavy-chain only antibodies (IgNAR, 'immunoglobulin new antigen receptor'), from which single-domain antibody fragments called VNAR fragments can be obtained. An alternative approach is to split the dimeric variable domains from common immunoglobulin G (IgG) from humans or mice into
30 monomers. Although most research into single domain antibodies is currently based on heavy chain variable domains, nanobodies derived from light chains have also been

shown to be capable of binding to target epitopes. Nanobodies are therefore also encompassed by the invention.

An “antibody fragment” also refers to at least one CDR, wherein the at least one CDR is part of a protein construct that exhibits binding specificity for an antigen.

- 5 Preferably, the at least one CDR is HCDR3. In one embodiment, the protein construct comprises HCDR1, HCDR2 and HCDR3. In another embodiment, the protein construct comprises HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3. The protein construct may further comprise, part of, an Fc region, including CH2 and/or CH3. The protein construct may also comprise a CH1 region. The CH1, CH2 and/or CH3 regions
10 may be engineered to obtain any desired properties with respect to antigen binding and/or effector function.

- The invention also encompasses variants of the antibody or antibody fragment of the invention. A “variant” of the antibody or antibody fragment of the invention comprises at least a functional part of the antibody or antibody fragment as described
15 herein, or a derivative or analogue thereof. Such functional part, derivative or analogue comprises at least the binding domain of the antibody or antibody fragment described herein, including the heavy chain variable region and/or light chain variable region comprising the CDRs as disclosed herein. A variant may comprise one to five amino acid substitutions in one or more of the CDRs. For instance, an amino acid residue may
20 be substituted with a conservative amino acid residue. A variant may also comprise one or more amino acid substitutions in the one or more framework regions. Preferably, the variant comprises a VH region that has at least 80%, 85%, 90%, 92%, 94%, 96%, 97%, 98%, or 99% sequence identity to the VH amino acid sequence of the antibody or antibody fragment of the invention. Preferably, the variant comprises a VL region that
25 has at least 80%, 85%, 90%, 92%, 94%, 96%, 97%, 98%, or 99% sequence identity to the VL amino acid sequence of the antibody or antibody fragment of the invention.

- “Percent (%) identity” as referring to nucleic acid or amino acid sequences herein is defined as the percentage of residues in a candidate sequence that are identical with the residues in a selected sequence, after aligning the sequences for optimal
30 comparison purposes. In order to optimize the alignment between the two sequences gaps may be introduced in any of the two sequences that are compared. Such alignment can be carried out over the full length of the sequences being compared. Alternatively,

the alignment may be carried out over a shorter length, for example over about 20, about 50, about 100 or more nucleic acids/based or amino acids. The sequence identity is the percentage of identical matches between the two sequences over the reported aligned region.

- 5 A comparison of sequences and determination of percentage of sequence identity between two sequences can be accomplished using a mathematical algorithm. The skilled person will be aware of the fact that several different computer programs are available to align two sequences and determine the identity between two sequences (Kruskal, J. B. (1983) An overview of sequence comparison In D. Sankoff and J. B.
- 10 Kruskal, (ed.), Time warps, string edits and macromolecules: the theory and practice of sequence comparison, pp. 1 -44 Addison Wesley).

- In particular, percent sequence identity according to the invention set out herein between two nucleic acid sequences may be determined using the AlignX application of the Vector NTI Program Advance 11.5.2 software using the default settings, which
- 15 employ a modified ClustalW algorithm (Thompson, J.D., Higgins, D.G., and Gibson T.J. (1994) Nuc. Acid Res. 22: 4673-4680), the swgapdnamt score matrix, a gap opening penalty of 15 and a gap extension penalty of 6.66. Amino acid sequences may be aligned with the AlignX application of the Vector NTI Program Advance 11.5.2 software using default settings, which employ a modified ClustalW algorithm
- 20 (Thompson, J.D., Higgins, D.G., and Gibson T.J., 1994), the blosum62mt2 score matrix, a gap opening penalty of 10 and a gap extension penalty of 0.1.

- A variant maintains the binding specificity of the antibody or antibody fragment of the invention, for example the antigen specificity. The binding affinity of the variant may, however, be different than that of the original antibody or antibody fragment of the
- 25 invention. The variant may, for instance, have a lower or higher K_{on} and/or K_{off} rate than the antibody or antibody fragment as described herein.

- A functional derivative of the antibody or antibody fragment of the invention can be an antibody mimetic, a polypeptide, an aptamer or a combination thereof. These proteins or aptamers typically bind to one target. It is to be understood that any
- 30 combination of these antibodies, antibody mimetics, polypeptides and aptamers can be linked together by methods known in the art. For example, in some embodiments the antibody or antibody fragment of the invention is a conjugate or part of a fusion protein.

An antibody mimetic is a polypeptide that, like antibodies, can specifically bind an antigen, but that is not structurally related to antibodies. Antibody mimetics are usually artificial peptides or proteins with a molar mass of about 3 to 20 kDa. Non-limiting examples of antibody mimetics are affibody molecules (typically based on the
5 Z domain of Protein A); affilins (typically based on Gamma-B crystalline or Ubiquitin); affimers (typically based on Cystatin); affitins (typically based on Sac7d from *Sulfolobus acidocaldarius*); alphabodies (typically based on Triple helix coiled coil); anticalins (typically based on Lipocalins); avimers (typically based on A domains of various membrane receptors); DARPins (typically based on ankyrin repeat motif);
10 fynomers (typically based on SH3 domain of Fyn 7); kunitz domain peptides (typically based on Kunitz domains of various protease inhibitors); and monobodies (typically based on type III domain of fibronectin).

Monobodies are synthetic binding proteins that are constructed using a fibronectin type III domain (FN3) as a molecular scaffold. Monobodies are an
15 alternative to antibodies for creating target-binding proteins. Monobodies and other antibody mimetics are typically generated from combinatorial libraries in which portions of the scaffold are diversified using molecular display and directed evolution technologies such as phage display, mRNA display and yeast surface display.

Aptamers are oligonucleotide or peptide molecules that bind to a specific target
20 molecule. Aptamers are usually created by selecting them from a large random sequence pool, but natural aptamers also exist in riboswitches. Aptamers can be used for both basic research and clinical purposes as macromolecules.

An antibody of the invention is preferably an IgG antibody, preferably an IgG1 or an IgG4 antibody. Most preferably, the antibody is an IgG1 antibody. Such full
25 length IgG antibody is preferred because of its favorable half-life and the desire to stay as close to fully autologous (human) molecules for reasons of immunogenicity. IgG1 is favored based on its long circulatory half-life in man.

The term 'full length IgG' or 'full length antibody' according to the invention is defined as comprising an essentially complete IgG, which however does not necessarily
30 have all functions of an intact IgG. For the avoidance of doubt, a full length IgG contains two heavy and two light chains. Each chain comprises constant (C) and variable (V) regions, which can be broken down into domains designated CH1, CH2,

CH3, VH, and CL, VL. An IgG antibody binds to antigen via the variable region domains contained in the Fab portion, and after binding can interact with molecules and cells of the immune system through the constant domains, mostly through the Fc portion. Full length antibodies according to the invention encompass IgG molecules
5 wherein mutations may be present that provide desired characteristics.

A full length IgG may contain variations in the constant regions to modulate effector function, both increasing and mitigating such functions, including antibody-dependent cellular cytotoxicity (ADCC), or cellular dependent cytotoxicity (CDC), to increase homo- or heterodimerization for generating monospecific or multispecific
10 antibodies from host cells comprising a nucleic acid encoding different heavy chains, and to facilitate separation of antibodies or antibody fragments produced by such host cells.

For instance, leucine at position 235 according to the EU numbering system may be substituted with glycine, and/or glycine at position 236 according to the EU
15 numbering system may be substituted with arginine. Such modification(s) ensure that binding to an Fc receptor and/or effector function is eliminated or decreased. Other variations of the CH2 and Fc region are known in the art to the same effect are also encompassed within the present invention.

Full length IgG should not have deletions of substantial portions of any of the
20 regions. However, IgG molecules wherein one or several amino acid residues are deleted, without essentially altering the binding characteristics of the resulting IgG molecule, are embraced within the term "full length IgG". For instance, such IgG molecules can have a deletion of between 1 and 10 amino acid residues, preferably in non-CDR regions, wherein the deleted amino acids are not essential for the binding
25 specificity of the IgG.

For instance, lysine at position 447 according to the EU numbering system may be deleted. Such deletion reduces the heterogeneity of the antibody. Furthermore, in order to suppress the swapping in an IgG4 antibody molecule, serine located in the hinge region at position 228 according to the EU numbering system may be substituted
30 with proline.

Suitable light chains for use in an antibody or antibody fragment of the invention include a light chain that is produced in response to immunization with an antigen,

referred to as a cognate light chain, or a synthetically produced light chain based thereon. A suitable light chain also includes a common light chain (cLC), such as those that can be identified by screening for the most commonly employed light chains in existing antibody libraries (wet libraries or in silico), where the light chains do not
5 substantially interfere with the affinity and/or selectivity of the epitope-binding domains of the heavy chains, but are also suitable to pair with an array of heavy chains. A common light chain is preferably encoded by germline sequences of V- and J gene segments which are rearranged but have not, or minimally, undergone somatic hypermutation. For example, a suitable light chain includes one from a transgenic non-
10 human animal, such as MeMo® having a common light chain integrated into its genome and which can be used to generate large panels of common light chain antibodies having diversity at the heavy chain and are capable of specifically binding an antigen upon exposure to said antigen.

The term "common light chain" thus refers to a light chain that can be associated
15 with two or more different heavy chains and exhibits binding ability to an antigen (see for instance WO2009/157771, WO2019/190327 and WO2014/051433). A preferred light chain V gene for such a common light chain is IGKV1-39. Preferred light chain J genes are jk1 and jk5. The joined sequences are indicated as IGKV1-39/jk1 and IGKV1-39/jk5; alternative names are IgV κ 1-39*01/IGJ κ 1*01 or IgV κ 1-
20 39*01/IGJ κ 5*01 (nomenclature according to the IMGT database worldwide web at imgt.org. Preferred examples of a common light chain include a light chain encoded by a human κ light chain IgV κ 1-39*01/IGJ κ 1*01 (nomenclatures of IMGT database) germline gene (hereinafter, abbreviated as the "IGVK1-39/JK1 common light chain"), as well as IgV κ 1-39*01/IGJ κ 5. A variety of MeMo® transgenic animals referred to
25 above comprises the IGVK1-39/JK1 common light chain.

A common light chain according to the invention also refers to light chains which may be identical or have some amino acid sequence differences while the binding specificity of the antibody or antibody fragment of the invention is not affected. Those of ordinary skill in the art will recognize that "common" also refers to functional
30 equivalents of the light chain of which the amino acid sequence is not identical. Variants of said light chain exist wherein changes are present in comparison to the parental common light chain that do not materially influence the formation of functional

binding regions. Such variants are thus also capable of binding different heavy chains and forming functional antigen binding domains. It is for instance possible within the scope of the definition of common light chains as used herein, to prepare or find variable light chains that are not identical but still functionally equivalent, e.g., by

5 introducing and testing conservative amino acid changes, changes of amino acids in regions that do not or only partly contribute to binding specificity when paired with a cognate chain, and the like. Such variants are thus also capable of binding different cognate chains and forming functional antigen binding domains. The term 'common light chain' as used herein thus refers to light chains which may be identical or have

10 some amino acid sequence differences while retaining the binding specificity of the resulting antibody after pairing with a heavy chain. A combination of a certain common light chain and such functionally equivalent variants is encompassed within the term "common light chain". Reference is made to WO 2004/009618, WO2019/190327 and WO2009/157771 for a detailed description of the use of common light chains.

15 Preferably, a common light chain is used in the present invention which is a germline-like light chain, more preferably a germline light chain, preferably a rearranged germline human kappa light chain, most preferably either the rearranged germline human kappa light chain IgV κ 1-39/J κ or IGV κ 3-20/J κ .

The antibody or antibody fragment can be produced, for example, from a

20 hybridoma using a hybridoma method. Alternatively, the antibody or antibody fragment can be produced by secretion from a mammalian cell co-expressing the heavy and/or light chains, or fragments thereof. Preferably, the antibody or antibody fragment is produced by immunizing a non-human animal. These, and other, methods are known in the art and can suitably be used for producing the antibody or antibody fragment of the

25 present invention.

Antibodies can be produced from various animal species. The MeMo® transgenic animals referred to above comprising the IGVK1-39/JK1 common light chain can suitably be used. Generally, transgenic mice are immunized with the human target DNA and/or protein followed by immunization boosts, thereby generating an

30 immune response including the production of antigen-specific antibodies. Serum titers from immunized mice can be determined by ELISA and FACS analysis. Spleen and draining lymphoid material of the immunized mice may then be collected and single

cell suspensions generated. RNA can be isolated and cDNA synthesized that encode the heavy chain and/or variable regions of such antibodies. VH and/or VL-family specific PCR can then be performed in order to generate phage display libraries. Human target protein binding Fabs can then be selected using for instance the Kingfisher selection robot. The nucleic acid encoding the heavy chain and/or light chain variable region of the Fabs that bind the target protein can be used for the production of antibodies in host cells.

There are instances where immunization of the transgenic organism generates antibodies, but where such antibodies are not cross-reactive for a human and transgenic animal, making the assaying and testing of such antibodies less efficient. Accordingly, it may be beneficial to obtain binding domains or antibodies, including chimeric or humanized binding domains or antibodies, that contain variable regions and/or complementary determining regions (CDR), and the nucleic acids that encode said variable regions and or CDRs, that are based on, derived or obtained from a nucleic acid, of species that are evolutionarily distant to humans. One potential source of such variable regions may be birds, for example, domesticated birds, such as chickens, ducks or ostriches. Antibody repertoires generated via immunization of birds, for example, chickens, ducks or ostriches may identify unique epitopes when compared to antibodies generated via the immunization of mice or other species evolutionarily close to humans (e.g., rodents and cynomolgus).

A primary DNA or protein immunization may be carried out followed by more booster immunizations. IgY antibodies can be isolated from yolk of the collected eggs or serum from the immunized birds. Spleen and/or bone marrow are removed from birds showing a significant humoral response against the target protein. RNA can subsequently be isolated and cDNA is synthesized. The generated cDNA samples are then used as a template to amplify and digest VH and/or VL genes using two primers. The PCR products are then cloned in a phagemid vector for the display of Fab fragments on phage essentially as described in de Haard et al. (J Biol Chem. (1999), Vol. 274(26), pp. 18218-18230) . The nucleic acids encoding the VH and/or VL regions are ligated into a vector and the resulting ligated vector transformed into cells to yield libraries. Panning selections using KingFisher Flex can then be performed to select the phages that bind the target protein. Screening can be carried out by FACS on cells

expressing the human or mouse target protein. The VH and/or VL genes of all clones that specifically bind the human and/or mouse target protein are sequenced. The nucleic acid encoding the heavy chain and/or light chain variable region of the binding domain that bind the target protein can be used for the production of antibodies in host
5 cells.

Antibody fragments can be produced by methods known in the art.

scFv antibodies can be produced by isolating mRNA from the hybridoma or mammalian cell, which is then reverse transcribed into cDNA for PCR amplification. This results in the production of large libraries containing a varied range of VH and VL
10 antibody genes (Marks and Hoogenboom, Journal of Molecular Biology (1991), Vol. 222, pp. 581-597). While constructing the scFv, the domains may be ordered as VH-linker-VL or as VL-linker-VH (Hu, O'Dwyer and Wall, Journal of Biotechnology (2005), Vol. 120, pp. 38-45). An example of a linker may be the classical (G4S)₃ linker (Huston et al., Proc Natl Acad Sci USA (1988), Vol. 85, pp. 5879-5883). For
15 minibodies, the DNA fragment encoding the hinge-CH3 domain can, for example, be based on the sequences of human IgG1 (Kim et al., PLOS One (2014), Vol. 9(12), e113442). scFv and hinge-CH3 regions may be assembled either by ligating cohesive ends generated by *XhoI* and *SAL1*, or by using PCR to bring the *XhoI* restriction site of the scFv product together with the hinge, to the NH2 terminus of the CH3 (Hu et al.,
20 Cancer Research (1996), Vol. 56, pp. 3055-3061). After PCR, the V domains of an antibody can then be recombined through *in vitro* recombination in plasmids or phagemids (Lilley et al., Journal of Immunological Methods (1994), Vol. 171, pp. 211-226. Hogrefe et al., Gene (1993), Vol. 128, pp. 119-126). This phage display technology relies on the fusion of the antibody fragments to the phage minor coat protein pIII or its
25 C-terminal domain (Smith, Science (1985), Vol. 228, pp. 1315-1317. Hoogenboom, Nature Biotechnology (2005), Vol. 23, pp. 1105-1116). Aside from displaying scFv fragments, phage display is now also widely used to display Fab fragments (Wieland et al., Veterinary Immunology and Immunopathology (2006), Vol. 110, pp. 129-140).

Fab fragments can be generated from monoclonal antibodies via enzymatic
30 digestion using papain or pepsin. Papain digestion of antibodies produces three distinct fragments: two antigen-binding fragments called Fab fragments or regions, each with a single antigen-binding site, and one Fc region by cleaving below the CH1 domain (Porter,

Biochem (1959), Vol. 73, pp. 119-126). Pepsin digestion of antibodies produces two fragments: a F(ab')₂ fragment containing two antigen binding regions connected via the disulfide bridge of the hinge region and an Fc fragment (Valedkarimi et al., Human Antibodies (2018), Vol. 26, pp. 171-176). These Fab and F(ab')₂ fragments may be
5 purified through a combination of the following techniques: ion exchange, protein A or G affinity, antigen affinity or gel filtration chromatographies (Mage and Lamoyi. Monoclonal Antibody Production Techniques and Applications, Marcel Dekker Inc, New York, 1987, pp. 79-97). F(ab')_n or (modified F(ab')_n) may be produced in connection with the invention described herein and can be obtained using any suitable enzymatic cleavage
10 and/or digestion techniques. In certain embodiments, the antibody fragment can be obtained by cleavage with the IdeS protease, an IgG-degrading enzyme of *Streptococcus pyogenes* that cleaves a human IgG1 at a specific site below the hinge leaving intact a F(ab')_n antibody fragment, wherein n is the number of antibody domains present on the IgG, wherein the heavy chain on one side of the F(ab')_n is paired to the heavy chain on
15 the other side at their respective C-terminus, wherein the pairing comprises two or more disulfide bridges. Methods for generation of bispecific and multispecific antibodies wherein n = 2 or more have been previously described. See PCT/NL2019/050199; PCT/NL2013/050294; PCT/NL2013/050293, which are incorporated by reference.

Alternatively, an antibody fragment lacking a Fc region can be obtained by use of
20 a cysteine protease from *Porphyromonas gingivalis*, that digests human IgG1 at a specific site above the hinge (KSCDK / THTCPPC), generating intact Fab and Fc fragments. An antibody fragment can be formed via this technique through the expression of a heavy chain comprising a variable domain and constant domain (e.g., CH1, CH2 and/or CH3), which is connected to an additional variable domain via a linker described
25 herein, or paired to a light chain, which is connected to an additional variable domain via a linker described herein, and wherein a proteolytic enzyme, such as from *Porphyromonas gingivalis* cleaves the constant domains of said heavy chain, leaving an intact truncated antibody binding fragment.

The generation of sdAbs has been achieved through phage display and by using
30 repertoires of naïve or synthetic VH or VL dAbs based on the incorporation of solubilizing residues from camelid sdAbs into human VHs (Tanha et al., J. Biol. Chem (2001), Vol. 276, pp. 24774-24780. Davies and Riechmann, Biotechnology (1995), Vol.

13, pp. 475-479). Human sdAbs have also been attained without the need of engineering, through a selection method based on reversible unfolding and affinity criteria which resulted in a number of VHs from synthetic human VH phage display libraries (Jespers et al., Nat. Biotechnol (2004), Vol. 337, pp. 893-903). Another phage
5 selection method may be used to obtain exclusively non-aggregating human VH domains from a naïve human VH display library (To et al., J Biol Chem (2005), Vol. 280, pp. 41395-41403). This same technique may be applied to obtained VLs (Kim et al., Landes Bioscience (2014), Vol. 6:1, pp. 219-235).

The antibody or antibody fragment of the present invention can be used for the
10 treatment of cancer, by administering an effective amount of the antibody or antibody fragment to a subject in need thereof.

As used herein, the terms "subject" and "patient" are used interchangeably and refer to a mammal such as a human, mouse, rat, hamster, guinea pig, rabbit, cat, dog, monkey, cow, horse, pig and the like (e.g., a patient, such as a human patient, having
15 cancer).

The terms "treat," "treating," and "treatment," as used herein, refer to any type of intervention or process performed on or administering an active agent or combination of active agents to a subject with the objective of curing or improving a disease or symptom thereof. This includes reversing, alleviating, ameliorating, inhibiting, or
20 slowing down a symptom, complication, condition or biochemical indicia associated with a disease, as well as preventing the onset, progression, development, severity or recurrence of a symptom, complication, condition or biochemical indicia associated with a disease.

As used herein, "effective treatment" or "positive therapeutic response" refers to
25 a treatment producing a beneficial effect, e.g., amelioration of at least one symptom of a disease or disorder, e.g., cancer. A beneficial effect can take the form of an improvement over baseline, including an improvement over a measurement or observation made prior to initiation of therapy according to the method. For example, a beneficial effect can take the form of slowing, stabilizing, stopping or reversing the
30 progression of a cancer in a subject at any clinical stage, as evidenced by a decrease or elimination of a clinical or diagnostic symptom of the disease, or of a marker of cancer. Effective treatment may, for example, decrease in tumor size, decrease the presence of

circulating tumor cells, reduce or prevent metastases of a tumor, slow or arrest tumor growth and/or prevent or delay tumor recurrence or relapse.

The term "therapeutic amount" or "effective amount" refers to an amount of an agent or combination of agents that provides the desired biological, therapeutic, and/or prophylactic result. That result can be reduction, amelioration, palliation, lessening, 5 delaying, and/or alleviation of one or more of the signs, symptoms, or causes of a disease, or any other desired alteration of a biological system. In some embodiments, a therapeutic amount is an amount sufficient to delay tumor development. In some embodiments, a therapeutic amount is an amount sufficient to prevent or delay tumor 10 recurrence.

The therapeutic amount of the drug or composition may: (i) reduce the number of cancer cells; (ii) reduce tumor size; (iii) inhibit, retard, slow to some extent and may stop cancer cell infiltration into peripheral organs; (iv) inhibit tumor metastasis; (v) inhibit tumor growth; (vi) prevent or delay occurrence and/or recurrence of tumor; 15 and/or (vii) relieve to some extent one or more of the symptoms associated with the cancer.

A therapeutic amount may vary according to factors such as the disease state, age, sex, and weight of the individual to be treated, and the ability of the agent or combination of agents to elicit a desired response in the individual.

20 A therapeutic amount can be administered in one or more administrations.

A therapeutic amount also includes an amount that balances any toxic or detrimental effects of the agent or combination of agents and the therapeutically beneficial effects.

In more specific terms, the present invention provides an antibody or antibody 25 fragment thereof that specifically binds to the extracellular domain of human TGF- β RII, wherein the antibody or antibody fragment binds to an epitope of the extracellular domain of human TGF- β RII of which phenylalanine (F) at position 25 is a critical residue for binding.

In the research leading up to the present invention, antibodies have been 30 identified that bind to a particular epitope of the extracellular domain of human TGF- β RII. This epitope comprises at least the amino acid residue at position 25 of the extracellular domain of isoform A of human TGF- β RII. The amino acid sequence of the

extracellular domain of isoform A of human TGF- β RII is as set forth in SEQ ID NO: 102. The amino acid at position 25 of this sequence is phenylalanine (F) (bold and underlined). The database accession number for the human TGF- β RII protein and the gene encoding isoform A is GenBank NM_001024847.2 (protein seq reference is NP_001020018.1; SEQ ID NO:102). A splice variant encoding a longer isoform due to an insertion in the extracellular domain is isoform B. The gene encoding isoform B is GenBank NM_003242.6 (protein seq reference is NP_003233.4; SEQ ID NO: 103 with the insertion underlined). The amino acid at position 25 of the extracellular domain of isoform A corresponds to the amino acid at position 50 of the extracellular domain of isoform B. The amino acid sequence of the extracellular domain of isoform B of human TGF- β RII is as set forth in SEQ ID NO: 104. The amino acid, phenylalanine (F), at position 50 of this sequence is in bold and underlined. These accession numbers are primarily given to provide a further method of identifying the TGF- β RII protein as a target, the actual sequence of the TGF- β RII protein bound by an antibody may vary, for instance because of a mutation in the encoding gene such as those occurring in some cancers or the like.

Anywhere this description refers to the amino acid at position 25 of human TGF- β RII, it refers to the amino acid at position 25 of the extracellular domain of isoform A of human TGF- β RII as well as to the corresponding position of another isoform of human TGF- β RII, in particular position 50 of isoform B. The same applies to other amino acid positions identified herein.

The amino acid residue at position 25 of the extracellular domain of human TGF- β RII was found to be a critical residue for binding of the antibodies of the invention, as determined by alanine scanning. In alanine scanning, the amino acids at each position of an antigen, in the present case TGF- β RII, are substituted one by one with alanine. If this diminishes or significantly reduces the binding of an antibody that binds the unmodified antigen, the amino acid that is substituted thus contributes to binding and is considered to be a critical residue. The antibody or antibody fragment thus specifically binds an epitope comprising such residue. In the present context, an amino acid residue is considered as a critical residue if its binding activity or reactivity is reduced by more than 50%, as compared to the unmodified amino acid sequence. "Significantly reduced" thus means a binding activity or reactivity which is reduced by

at least more than 50%, as compared to the unmodified amino acid sequence of the extracellular domain of human TGF β RII.

Accordingly, the antibody or antibody fragment of the present invention has a binding activity or reactivity of less than 50%, preferably less than 40%, 30%, 20%, 10%, 5%, or 2%, when the phenylalanine (F) at position 25 of the amino acid sequence of the extracellular domain of human TGF β RII, the wildtype sequence of which is as set forth in SEQ ID No.102, is replaced with alanine (A), as compared to the wildtype amino acid sequence of the extracellular domain of human TGF β RII.

Thus, an antibody or antibody fragment of the present invention specifically binds to an epitope of human TGF- β RII comprising phenylalanine (F) at position 25 of the amino acid sequence of the extracellular domain of human TGF- β RII. Preferably, the binding of an antibody or antibody fragment to this epitope is determined by alanine scanning, wherein an antibody or antibody fragment that binds to this epitope has a binding activity or reactivity of less than 50%, preferably less than 40%, 30%, 20%, 10%, 5%, or 2%, when alanine (A) is present at position 25 of the amino acid sequence of the extracellular domain of human TGF- β RII, as compared to when phenylalanine (F) is present at said position.

Another way of defining an antibody or antibody fragment of the present invention is that it binds to an epitope of the extracellular domain of human TGF β RII of which a substitution of a phenylalanine (F) residue that in isoform A of human TGF β RII, the wildtype sequence of which is as set forth in SEQ ID No.102, is at position 25, for an alanine (A), reduces the binding of the antibody or antibody fragment by at least 50%, preferably by at least 60%, 70%, 80%, 90%, 95%, or 98%.

An antibody or antibody fragment of the present invention may also be defined as an antibody or antibody fragment that binds to an epitope in the extracellular domain of human TGF- β RII, wherein the epitope comprises a phenylalanine (F) residue that in isoform A of human TGF- β RII, the wildtype sequence of which is as set forth in SEQ ID No.102, is at position 25. In addition to the critical amino acid residue at position 25, aspartate (D) at position 119 of the epitope of the extracellular domain of isoform A of human TGF- β RII was identified as a further critical residue for binding. The amino acid at position 119 of isoform A corresponds to the amino acid at position 144 of the extracellular domain of isoform B of human TGF- β RII (SEQ ID NO: 104). The

antibody or antibody fragment of the invention preferably has a binding reactivity of less than 10%, more preferably less than 5%, 4%, 3%, or 2%, when the aspartate (D) at position 119 of the wildtype amino acid sequence of the extracellular domain of human TGF- β RII is replaced with alanine (A), as compared to the wildtype amino acid
5 sequence of the extracellular domain of human TGF- β RII.

In a particular embodiment, the antibody or antibody fragment preferably has:

- a binding reactivity of less than 5%, preferably less than 3%, when the phenylalanine (F) at position 25 of the wildtype amino acid sequence of the extracellular domain of human TGF- β RII is replaced with alanine (A);
- 10 - a binding reactivity of less than 5%, preferably less than 3%, when the aspartate (D) at position 119 of the wildtype amino acid sequence of the extracellular domain of human TGF- β RII is replaced with alanine (A), as compared to the wildtype amino acid sequence of the extracellular domain of human TGF β RII.

15 Thus, the present invention encompasses an antibody or antibody fragment that specifically binds to an epitope of human TGF- β RII comprising phenylalanine (F) at position 25 and aspartate (D) at position 119 of the amino acid sequence of the extracellular domain of human TGF- β RII. Preferably, the binding of an antibody or antibody fragment to this epitope is determined by alanine scanning, wherein an
20 antibody or antibody fragment that binds to this epitope has a binding activity or reactivity of less than 50%, preferably less than 5%, or 3%, when alanine is present at position 25 of the amino acid sequence of the extracellular domain of human TGF- β RII, and a binding activity or reactivity of less than 10%, preferably less than 5%, or 3%, when alanine (A) is present at position 119 of the amino acid sequence of the
25 extracellular domain of human TGF- β RII, as compared to when phenylalanine (F) and aspartate (D) are present at said respective positions, wherein each change is tested in isolation and not as a combination of two changes as compared to the wildtype extracellular domain.

Another way of defining an antibody or antibody fragment of the present
30 invention is that it binds to an epitope of the extracellular domain of human TGF β RII of which

- a substitution of a phenylalanine (F) residue that in isoform A of human TGF β RII, the wildtype sequence of which is as set forth in SEQ ID No.102, is at position 25, for an alanine (A), reduces the binding of the antibody or antibody fragment by at least 50%, preferably by at least 95%, or 97%; and

- 5 - a substitution of an aspartate (D) residue that in isoform A of human TGF β RII, the wildtype sequence of which is as set forth in SEQ ID No.102, is at position 119, for an alanine (A), reduces the binding of the antibody or antibody fragment by at least 90%, preferably by at least 95%, or 97%.

10 An antibody or antibody fragment of the present invention may also be defined as an antibody or antibody fragment that binds to an epitope in the extracellular domain of human TGF- β RII, wherein the epitope comprises a phenylalanine (F) residue and an aspartate (D) residue that in isoform A of human TGF- β RII, the wildtype sequence of which is as set forth in SEQ ID No.102, are at positions 25 and 119 respectively.

15 In another embodiment, the antibody or antibody fragment binds to an epitope of the extracellular domain of human TGF- β RII of which threonine (T) at position 52 of the epitope of the extracellular domain of isoform A of human TGF- β RII is a further critical residue for binding. The amino acid at position 52 of isoform A corresponds to the amino acid at position 77 of isoform B of human TGF- β RII. In this embodiment, the antibody or antibody fragment preferably has:

- 20 - a binding reactivity of less than 5%, preferably less than 2%, when the phenylalanine (F) at position 25 of wildtype amino acid sequence of the extracellular domain of human TGF- β RII is replaced with alanine (A);
- a binding reactivity of less than 60%, preferably less than 40% or 30%, more preferably less than 20%, when the threonine (T) at position 52 of the
- 25 wildtype amino acid sequence of the extracellular domain of human TGF- β RII is replaced with alanine (A); and
- a binding reactivity of less than 3% when the aspartate (D) at position 119 of the wildtype amino acid sequence of the extracellular domain of human TGF- β RII is replaced with alanine (A),

30 as compared to the wildtype amino acid sequence of the extracellular domain of human TGF- β RII.

Thus, the present invention encompasses an antibody or antibody fragment that specifically binds to an epitope of human TGF- β RII comprising phenylalanine (F) at position 25, threonine (T) at position 52, and aspartate (D) at position 119, of the amino acid sequence of the extracellular domain of human TGF- β RII. Preferably, the binding of an antibody or antibody fragment to this epitope is determined by alanine scanning, wherein an antibody or antibody fragment that binds to this epitope has a binding activity or reactivity of less than 50%, preferably less than 5%, or 2%, when alanine (A) is present at position 25 of the amino acid sequence of the extracellular domain of human TGF- β RII, a binding activity or reactivity of less than 60%, preferably less than 40%, 30%, or 20%, when alanine (A) is present at position 52 of the amino acid sequence of the extracellular domain of human TGF- β RII, and a binding activity or reactivity of less than 10%, preferably less than 3%, when alanine (A) is present at position 119 of the amino acid sequence of the extracellular domain of human TGF- β RII, as compared to when phenylalanine (F), threonine (T) and aspartate (D) are present at said respective positions, wherein each change is tested in isolation and not as a combination of two changes as compared to the wildtype extracellular domain.

Another way of defining an antibody or antibody fragment of the present invention is that it binds to an epitope of the extracellular domain of human TGF β RII of which

- a substitution of a phenylalanine (F) residue that in isoform A of human TGF β RII, the wildtype sequence of which is as set forth in SEQ ID No.102, is at position 25, for an alanine (A), reduces the binding of the antibody or antibody fragment by at least 50%, preferably by at least 95%, or 98%;

- a substitution of a threonine (T) residue that in isoform A of human TGF β RII, the wildtype sequence of which is as set forth in SEQ ID No.102, is at position 52, for an alanine (A), reduces the binding of the antibody or antibody fragment by at least 60%, preferably by at least 70%, or 80%; and

- a substitution of an aspartate (D) residue that in isoform A of human TGF β RII, the wildtype sequence of which is as set forth in SEQ ID No.102, is at position 119, for an alanine (A), reduces the binding of the antibody or antibody fragment by at least 90%, preferably by at least 97%.

An antibody or antibody fragment of the present invention may also be defined as an antibody or antibody fragment that binds to an epitope in the extracellular domain of human TGF- β RII, wherein the epitope comprises a phenylalanine (F) residue, a threonine (T) residue, and an aspartate (D) residue that in isoform A of human TGF- β RII, the wildtype sequence of which is as set forth in SEQ ID No.102, are at positions 25, 52 and 119 respectively.

In another embodiment, the antibody or antibody fragment binds to an epitope of the extracellular domain of human TGF- β RII of which isoleucine (I) at position 54 and glutamate (E) at position 120 of the epitope of the extracellular domain of isoform A of human TGF- β RII are further critical residues for binding. The amino acids at positions 54 and 120 of isoform A correspond to the amino acids at positions 79 and 145, respectively, of isoform B of human TGF- β RII. In this embodiment, the antibody or antibody fragment preferably has:

- a binding reactivity of less than 10% when the phenylalanine (F) at position 25 of the wildtype amino acid sequence of the extracellular domain of human TGF- β RII is replaced with alanine (A);
- a binding reactivity of less than 20% when the isoleucine (I) at position 54 of the wildtype amino acid sequence of the extracellular domain of human TGF- β RII is replaced with alanine (A);
- a binding reactivity of less than 3%, preferably less than 2%, more preferably less than 1%, when the aspartate (D) at position 119 of the wildtype amino acid sequence of the extracellular domain of human TGF- β RII is replaced with alanine (A); and
- a binding reactivity of less than 80%, preferably less than 70%, 60%, 50%, 40%, more preferably less than 30%, most preferably less than 10 % or 5%, when the glutamate (E) at position 120 of the wildtype amino acid sequence of the extracellular domain of human TGF- β RII is replaced with alanine (A),

as compared to the wildtype amino acid sequence of the extracellular domain of human TGF- β RII.

Thus, the present invention encompasses an antibody or antibody fragment that specifically binds to an epitope of human TGF- β RII comprising phenylalanine (F) at position 25, isoleucine (I) at position 54, aspartate (D) at position 119, and glutamate

(E) at position 120, of the amino acid sequence of the extracellular domain of human TGF- β RII. Preferably, the binding of an antibody or antibody fragment to this epitope is determined by alanine scanning, wherein an antibody or antibody fragment that binds to this epitope has a binding activity or reactivity of less than 50%, preferably less than 10%, when alanine (A) is present at position 25 of the amino acid sequence of the extracellular domain of human TGF- β RII, a binding activity or reactivity of less than 20% when alanine (A) is present at position 54 of the amino acid sequence of the extracellular domain of human TGF- β RII, a binding activity or reactivity of less than 10%, preferably less than 3%, 2%, or 1%, when alanine (A) is present at position 119 of the amino acid sequence of the extracellular domain of human TGF- β RII, and a binding activity or reactivity of less than 80% preferably less than 70%, 60%, 50%, 40%, 30%, 10%, or 5%, when alanine (A) is present at position 120 of the amino acid sequence of the extracellular domain of human TGF- β RII, as compared to when phenylalanine (F), threonine (T), aspartate (D), and glutamate (E) are present at said respective positions, wherein each change is tested in isolation and not as a combination of two changes as compared to the wildtype extracellular domain.

Another way of defining an antibody or antibody fragment of the present invention is that it binds to an epitope of the extracellular domain of human TGF β RII of which

- a substitution of a phenylalanine (F) residue that in isoform A of human TGF β RII, the wildtype sequence of which is as set forth in SEQ ID No.102, is at position 25, for an alanine (A), reduces the binding of the antibody or antibody fragment by at least 50%, preferably by at least 90%;
- a substitution of an isoleucine (I) residue that in isoform A of human TGF β RII, the wildtype sequence of which is as set forth in SEQ ID No.102, is at position 54, for an alanine (A), reduces the binding of the antibody or antibody fragment by at least 80%;
- a substitution of an aspartate (D) residue that in isoform A of human TGF β RII, the wildtype sequence of which is as set forth in SEQ ID No.102, is at position 119, for an alanine (A), reduces the binding of the antibody or antibody fragment by at least 90%, preferably by at least 97%, 98%, or 99%; and

- a substitution of a glutamate (E) residue that in isoform A of human TGF β RII, the wildtype sequence of which is as set forth in SEQ ID No.102, is at position 120, for an alanine (A), reduces the binding of the antibody or antibody fragment by at least 30%, preferably by at least 40%, 50%, 60%, 70%, 90%, or 95%.

5 An antibody or antibody fragment of the present invention may also be defined as an antibody or antibody fragment that binds to an epitope in the extracellular domain of human TGF- β RII, wherein the epitope comprises a phenylalanine (F) residue, an isoleucine (I) residue, an aspartate (D) residue, and a glutamate (E) residue that in
10 ID No.102, are at positions 25, 54, 119, and 120 respectively.

The present inventors identified a number of antibodies that comprise a heavy chain variable region that specifically binds to an epitope of the extracellular TGF- β RII of which phenylalanine (F) at position 25 is critical for binding. One example of such antibody comprises a heavy chain variable region (VH) having:

15 (a) a VH-CDR1 comprising the amino acid sequence set forth in SEQ ID No. 1,
 (b) a VH-CDR2 comprising the amino acid sequence set forth in SEQ ID No. 2,
and
 (c) a VH-CDR3 comprising the amino acid sequence set forth in SEQ ID No. 3,
 wherein one to five amino acid residue(s) may be varied with conservative
20 amino acid(s) thereof in any one or more of the CDR(s) selected from VH-CDR1, VH-
CDR2 and VH-CDR3. This antibody is encompassed by the present invention.

Another example of such antibody comprises a heavy chain variable region (VH) having:

25 (a) a VH-CDR1 comprising the amino acid sequence set forth in SEQ ID No. 4,
 (b) a VH-CDR2 comprising the amino acid sequence set forth in SEQ ID No. 5,
and
 (c) a VH-CDR3 comprising the amino acid sequence set forth in SEQ ID No. 6,
 wherein one to five amino acid residue(s) may be varied with conservative
amino acid(s) thereof in any one or more of the CDR(s) selected from VH-CDR1, VH-
30 CDR2 and VH-CDR3. This antibody is also encompassed by the present invention.

Another example of such antibody comprises a heavy chain variable region (VH) having:

(a) a VH-CDR1 comprising the amino acid sequence set forth in SEQ ID No. 7,
 (b) a VH-CDR2 comprising the amino acid sequence set forth in SEQ ID No. 8,
 and

(c) a VH-CDR3 comprising the amino acid sequence set forth in SEQ ID No. 9,
 5 wherein one to five amino acid residue(s) may be varied with conservative
 amino acid(s) thereof in any one or more of the CDR(s) selected from VH-CDR1, VH-
 CDR2 and VH-CDR3. This antibody is also encompassed by the present invention.

The heavy chain variable region of the antibody or antibody fragment of the
 invention may thus comprise a CDR1 selected from the group consisting of the
 10 sequences as set forth in SEQ ID NOs: 1, 4, and 7; a CDR2 selected from the group
 consisting of the sequences as set forth in SEQ ID NOs: 2, 5, and 8; and/or a CDR3
 selected from the group consisting of the sequences as set forth in SEQ ID NOs: 3, 6,
 and 9; wherein one to five amino acid residue(s) may be varied with conservative amino
 acid(s) thereof in any one or more of the CDR(s) selected from CDR1, CDR2 and
 15 CDR3.

The framework regions of the heavy chain variable region may be selected from
 any suitable framework regions. Examples of suitable framework regions are the
 framework regions encoded by the human V genes IGHV3-15 and IGHV3-23. These
 germline V genes may comprise one or more somatic mutations.

20 In one embodiment of the present invention, the antibody or an antibody
 fragment thereof comprises a VH having amino acid sequence:
 EVQLVESGGGLVQPGGSLRLSCAASGFTFDIYAMTWVRQAPGKGLEWVSVISG
 SGGTTYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARRGQYRDIV
 GATDYWGQGT LTVSS (SEQ ID NO: 10), or a VH amino acid sequence having at
 25 least 80%, preferably at least 90%, more preferably at least 95%, identity thereto.

In another embodiment, the antibody or an antibody fragment thereof comprises
 a VH having amino acid sequence:
 QVQLVESGGGLVEPGGSLRLSCAASGFTFSNAWMSWVRQAPGKGLEWVGRIK
 TTISGGATDFAAPVKGRFTISRDDSKNTLYLQMNSLKTEDTAVYYCTDLRDY
 30 WGQGT LTVSS (SEQ ID NO: 11), or a VH amino acid sequence having at least
 80%, preferably at least 90%, more preferably at least 95%, identity thereto.

In another embodiment, the antibody or an antibody fragment thereof comprises a VH having amino acid sequence:

QVQLVESGGGLVQPGGSLRLSCAVSGFTFRRYAMSWVRQAPGKGLEWVSAIS
ASGDRTHNTDSVKGRFSISRDN SKNTLYLQMNSLRAEDTAVYFCAKGIAASGK

- 5 NYFDPWGQGTLVTVSS (SEQ ID NO: 12), or a VH amino acid sequence having at least 80%, preferably at least 90%, more preferably at least 95%, identity thereto.

- The heavy chain variable region can have 0-10, preferably 0-5 amino acid variations with respect to the indicated amino acid sequence. In a preferred embodiment the heavy chain variable region comprises 0-9, 0-8, 0-7, 0-6, 0-5, 0-4, preferably 0-3,
10 preferably 0-2, preferably 0-1 and more preferably 0 amino acid variations, insertions, deletions, substitutions, additions with respect to the indicated amino acid sequence, or a combination thereof at positions other than the CDRs. A combination of an insertion, addition, deletion or substitution is a combination if aligned sequences do not differ at more than 10, preferably no more than 5 positions. A gap in one of the aligned
15 sequences counts for as many amino acids as skipped in the other sequence. An amino acid substitution, if any, is preferably a conservative amino acid substitution. The substitutions in the heavy chain variable region may be in addition to the substitutions in the one or more CDRs as described above. Conservative amino acids are known in the art based on similarities of the respective functional groups based on characteristics
20 such as charge, hydrophobicity, hydrophilicity, acidity, basic, and size.

An amino acid variation, insertion, deletion, substitution, addition or combination thereof is preferably not made in the binding interface of the heavy and light chain.

- If an amino acid is changed in the interface of the H/L chain interaction, it is
25 preferred that the corresponding amino acids in the other chain are changed to accommodate the change. An insertion or addition of an amino acid preferably does not entail the insertion or addition of a proline.

- The invention encompasses antibodies or antibody fragments comprising a variant of the antibody or antibody fragment as described herein. In particular an
30 antibody or antibody fragment comprising a VH having any one of SEQ ID NO: 22-91 and 93, i.e. any one of the VH sequences as shown in Figure 4. Also encompassed are antibodies or antibody fragments comprising at least HCDR3, or all CDRs, of any one

of SEQ ID NO: 22-91 and 93. Preferred antibodies or antibody fragments comprise a heavy chain variable region having, or which is derived from, an amino acid sequence set forth in SEQ ID NO: 40; 43; 46; 47; 48 or 54, which are variants of antibodies comprising a heavy chain variable region comprising an amino acid sequence set forth

5 in SEQ ID NO: 11. Other preferred antibodies or antibody fragments comprise a heavy chain variable region having, or which is derived from, an amino acid sequence set forth in SEQ ID NO: 67; 70; 75; 76; 77; 78; 83; 84; or 88, which are variants of antibodies comprising a heavy chain variable region comprising an amino acid sequence set forth

10 in SEQ ID NO: 12. Other preferred antibodies or antibody fragments comprise a heavy chain variable region having, or which is derived from, an amino acid sequence set forth in SEQ ID NO: 24 or 26, which are variants of antibodies comprising a heavy chain variable region comprising an amino acid sequence set forth in SEQ ID NO: 10. The most preferred variants are those comprising a heavy chain variable region having an amino acid sequence set forth in SEQ ID NO: 40; 43; 46; 47; 48; 54; 67; 70; 75; 76; 77;

15 78; 83; 84; or 88.

The heavy chain variable region of such variant can have 0-10, preferably 0-5 amino acid variations with respect to the indicated amino acid sequence. In a preferred embodiment the heavy chain variable region comprises 0-9, 0-8, 0-7, 0-6, 0-5, 0-4, preferably 0-3, preferably 0-2, preferably 0-1 and more preferably 0 amino acid

20 variations, insertions, deletions, substitutions, additions with respect to the indicated amino acid sequence, or a combination thereof at positions other than the CDRs.

The antibody or antibody fragment, or the binding domain, of the present invention may comprise any suitable light chain, such as for instance a cognate light chain or a common light chain as defined herein.

25 In one embodiment, the light chain variable region comprises the amino acid sequence of an IgV κ 1-39*01 gene segment as depicted in Figure 1B with 0-10, preferably 0-5 amino acid variations, insertions, deletions, substitutions, additions or a combination thereof. IgV κ 1-39 is short for Immunoglobulin Variable Kappa 1-39 Gene. The gene is also known as Immunoglobulin Kappa Variable 1-39; IGKV139; IGKV1-

30 39. External Ids for the gene are HGNC: 5740; Entrez Gene: 28930; Ensembl: ENSG00000242371. A preferred amino acid sequence for IgV κ 1-39 is given in Figure 1A. This lists the sequence of the V-region. The V-region can be combined with one of

five J-regions. Figures 1B and 1C describe two preferred sequences for IgV κ 1-39 in combination with a J-region. The joined sequences are indicated as IGKV1-39/jk1 and IGKV1-39/jk5; alternative names are IgV κ 1-39*01/IGJ κ 1*01 or IgV κ 1-39*01/IGJ κ 5*01 (nomenclature according to the IMGT database worldwide web at imgt.org).

It is preferred that the IgV κ 1-39*01 comprising light chain variable region is a germline sequence. It is further preferred that the IGJ κ 1*01 or IGJ κ 5*01 comprising light chain variable region is a germline sequence. It is further preferred that the IGKV1-39/jk1 or IGKV1-39/jk5 light chain variable regions are germline sequences.

Mature B-cells that produce an antibody with a light chain often produce a light chain that has undergone one or more mutations with respect to the germline sequence, meaning the normal sequence in non-lymphoid cells of the organism. The process that is responsible for these mutations is referred to as somatic hypermutation. The resulting light chain is referred to as an affinity matured light chain. Such light chains, when derived from a germline IgV κ 1-39*01 sequence are IgV κ 1-39*01 derived light chains. In this specification, the phrase "IgV κ 1-39*01" will include IgV κ 1-39*01-derived light chains. The mutations that are introduced into nucleic acid encoding the light chain by somatic hypermutation can also be introduced artificially in the lab. In the lab also other variations to a light chain can be introduced without affecting the properties of the light chain in kind, not necessarily in amount. A light chain is at least an IgV κ 1-39*01 light chain if it comprises a sequence as depicted in Figure 1A, Figure 1B or Figure 1C with 0-10, preferably 0-5 amino acid variations, insertions, deletions, substitutions, additions or a combination thereof. Preferably, the IgV κ 1-39*01 light chain is a light chain comprising a sequence as depicted in Figure 1A, Figure 1B or Figure 1C with 0-9, 0-8, 0-7, 0-6, 0-5, 0-4 amino acid variations, insertions, deletions, substitutions, additions or a combination thereof, more preferably a sequence as depicted in Figure 1A, Figure 1B or Figure 1C with 0-5, preferably 0-4, 0-3, 0-2, 0-1 amino acid variations, insertions, deletions, substitutions, additions or a combination thereof, even more preferably a sequence as depicted in Figure 1A, Figure 1B or Figure 1C with 0-3 amino acid variations, insertions, deletions, substitutions, additions or a combination thereof, even more preferably a sequence as depicted in Figure 1A Figure 1B or Figure 1C with 0-2 amino acid variations, insertions, deletions, substitutions, additions or a combination

thereof, even more preferably a sequence as depicted in Figure 1A, Figure 1B or Figure 1C with 0-1 amino acid variations, insertions, deletions, substitutions, additions or a combination thereof, and most preferably 0 amino acid variations, insertions, deletions, substitutions, additions or a combination thereof.

5 In one embodiment, the antibody or an antibody fragment thereof, or the binding domain, comprises a light chain variable region (VL) having:

(a) a VL-CDR1 comprising the amino acid sequence set forth in SEQ ID No. 19,

(b) a VL-CDR2 comprising the amino acid sequence set forth in SEQ ID No. 20,

and

10 (c) a VL-CDR3 comprising the amino acid sequence set forth in SEQ ID No. 21, wherein one to five amino acid residue(s) may be substituted with conservative amino acid(s) thereof in any one or more of the CDR(s) selected from VL-CDR1, VL-CDR2 and VL-CDR3.

The light chain variable region of the antibody or antibody fragment, or binding domain, of the invention preferably comprises a CDR1, CDR2, and CDR3 region comprising the amino acid sequence CDR1 – QSISSY (SEQ ID NO: 13), CDR2 – AAS (SEQ ID NO: 14), CDR3 – QQSYSTPPT (SEQ ID NO: 15), i.e. the CDRs of IGKV1-39 (according to IMGT), allowing for 0-5 amino acid substitutions with respect to the indicated amino acid sequence. According to Kabat numbering, the amino acid sequences are: CDR1 – RASQSISSYLN (SEQ ID NO: 19), CDR2 – AASSLQS (SEQ ID NO: 20), CDR3 – QQSYSTPPT (SEQ ID NO: 21). Any amino acid variations, insertions, deletions, substitutions, additions or combination thereof are preferably not in the CDR3 region of the light chain variable region, preferably not in the CDR1 or CDR2 region of the light chain variable region. An amino acid substitution is preferably a conservative amino acid substitution.

In particular, the light chain variable region of the antibody or antibody fragment, or binding domain, of the invention preferably comprises the amino acid sequence
 DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQ
 30 SGVPSRFRSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPPTFGQGTKVEIK (SEQ ID NO: 16), or a VL amino acid sequence having at least 80% identity thereto. This thus, allows for 0-5 amino acid variations, insertions, deletions, substitutions, additions

or a combination thereof. An amino acid substitution is preferably a conservative amino acid substitution.

The antibody of the present invention may be of any isotype: IgA, IgM, IgG, IgD or IgE. Preferably, the antibody is an IgG, in particular an IgG1 or IgG4. Most
5 preferably, the antibody is an IgG1.

The constant region of an antibody of the present invention is preferably a human constant region, but may be of any animal. It can be a mouse or rat constant region. A mouse constant region or rat constant region may advantageously be used, depending on the host organism immunized or screening methodology employed to
10 select the recombinant antibodies generated in response to an antigen.

The antibody or antibody fragment thereof may comprise a heavy chain constant region comprising the amino acid sequence set forth in SEQ ID No. 17. The antibody or antibody fragment thereof may comprise a light chain constant region comprising the amino acid sequence set forth in SEQ ID No. 18. The constant region may contain one
15 or more, preferably not more than 10, preferably not more than 5 amino acid differences with the constant region of a conventional human antibody.

Some antibodies are modified in the CH2/lower hinge region, for instance to reduce Fc-receptor interaction or to reduce ADCC, C1q binding or other effector activity. The antibody of the invention may be an IgG antibody with a variant CH2
20 and/or lower hinge domain such that interaction of the antibody to an Fc-gamma receptor is reduced. Such a mutant CH2 and/or lower hinge domain preferably comprises an amino variation at position 235 and/or 236 (EU numbering), preferably comprising residues at the following positions 235G and/or 236R.

The isolated monoclonal antibody may comprise two heavy chain constant
25 regions of an IgG1 antibody, wherein lysine at position 447 according to the EU numbering system is deleted.

A preferred antibody or antibody fragment of the present invention comprises heavy chain variable region CDR and light chain variable region CDR combinations as listed in Table 1.

Antibody	HCDR1	HCDR2	HCDR3	LCDR1	LCDR2	LCDR3
1	SEQ ID NO: 1	SEQ ID NO: 2	SEQ ID NO: 3	SEQ ID NO: 19	SEQ ID NO: 20	SEQ ID NO: 21
2	SEQ ID NO: 4	SEQ ID NO: 5	SEQ ID NO: 6	SEQ ID NO: 19	SEQ ID NO: 20	SEQ ID NO: 21
3	SEQ ID NO: 7	SEQ ID NO: 8	SEQ ID NO: 9	SEQ ID NO: 19	SEQ ID NO: 20	SEQ ID NO: 21
4	SEQ ID NO: 105	SEQ ID NO: 106	SEQ ID NO: 107	SEQ ID NO: 19	SEQ ID NO: 20	SEQ ID NO: 21
5	SEQ ID NO: 108	SEQ ID NO: 109	SEQ ID NO: 110	SEQ ID NO: 19	SEQ ID NO: 20	SEQ ID NO: 21
6	SEQ ID NO: 111	SEQ ID NO: 112	SEQ ID NO: 113	SEQ ID NO: 19	SEQ ID NO: 20	SEQ ID NO: 21
7	SEQ ID NO: 114	SEQ ID NO: 115	SEQ ID NO: 116	SEQ ID NO: 19	SEQ ID NO: 20	SEQ ID NO: 21
8	SEQ ID NO: 117	SEQ ID NO: 118	SEQ ID NO: 119	SEQ ID NO: 19	SEQ ID NO: 20	SEQ ID NO: 21
9	SEQ ID NO: 120	SEQ ID NO: 121	SEQ ID NO: 122	SEQ ID NO: 19	SEQ ID NO: 20	SEQ ID NO: 21
10	SEQ ID NO: 123	SEQ ID NO: 124	SEQ ID NO: 125	SEQ ID NO: 19	SEQ ID NO: 20	SEQ ID NO: 21
11	SEQ ID NO: 126	SEQ ID NO: 127	SEQ ID NO: 128	SEQ ID NO: 19	SEQ ID NO: 20	SEQ ID NO: 21
12	SEQ ID NO: 129	SEQ ID NO: 130	SEQ ID NO: 131	SEQ ID NO: 19	SEQ ID NO: 20	SEQ ID NO: 21
13	SEQ ID NO: 132	SEQ ID NO: 133	SEQ ID NO: 134	SEQ ID NO: 19	SEQ ID NO: 20	SEQ ID NO: 21

Table 1. Possible combinations of heavy chain variable region CDRs and light chain variable region CDRs.

A preferred antibody or antibody fragment of the invention comprises a heavy chain having a VH comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 10, 11, 12, 22-91 and 93, in particular a preferred VH as described herein, and a light chain having a VL comprising the amino acid sequence set forth in SEQ ID NO: 16. This antibody or antibody fragment can comprise any constant domain as described herein.

In one embodiment, the antibody or antibody fragment thereof as described herein comprises a heavy chain having a VH comprising the amino acid sequence set forth in SEQ ID No. 10, and a light chain having a VL comprising the amino acid sequence set forth in SEQ ID No. 16.

In another embodiment, the antibody or antibody fragment thereof as described herein comprises a heavy chain having a VH comprising the amino acid sequence set forth in SEQ ID No. 11, and a light chain having a VL comprising the amino acid sequence set forth in SEQ ID No. 16.

In another embodiment, the antibody or antibody fragment thereof as described herein comprises a heavy chain having a VH comprising the amino acid sequence set forth in SEQ ID No. 12, and a light chain having a VL comprising the amino acid sequence set forth in SEQ ID No. 16.

In another embodiment, the antibody or antibody fragment thereof as described herein comprises a heavy chain having a VH comprising the amino acid sequence set forth in SEQ ID No. 43, and a light chain having a VL comprising the amino acid sequence set forth in SEQ ID No. 16.

In another embodiment, the antibody or antibody fragment thereof as described herein comprises a heavy chain having a VH comprising the amino acid sequence set forth in SEQ ID No. 75, and a light chain having a VL comprising the amino acid sequence set forth in SEQ ID No. 16.

In another embodiment, the antibody or antibody fragment thereof as described herein comprises a heavy chain having a VH comprising the amino acid sequence set forth in SEQ ID No. 70, and a light chain having a VL comprising the amino acid sequence set forth in SEQ ID No. 16.

In another embodiment, the antibody or antibody fragment thereof as described herein comprises a heavy chain having a VH comprising the amino acid sequence set

forth in SEQ ID No. 84, and a light chain having a VL comprising the amino acid sequence set forth in SEQ ID No. 16.

In another embodiment, the antibody or antibody fragment thereof as described herein comprises a heavy chain having a VH comprising the amino acid sequence set forth in SEQ ID No. 88, and a light chain having a VL comprising the amino acid sequence set forth in SEQ ID No. 16.

In another embodiment, the antibody or antibody fragment thereof as described herein comprises a heavy chain having a VH comprising the amino acid sequence set forth in SEQ ID No. 40, and a light chain having a VL comprising the amino acid sequence set forth in SEQ ID No. 16.

In another embodiment, the antibody or antibody fragment thereof as described herein comprises a heavy chain having a VH comprising the amino acid sequence set forth in SEQ ID No. 83, and a light chain having a VL comprising the amino acid sequence set forth in SEQ ID No. 16.

In another embodiment, the antibody or antibody fragment thereof as described herein comprises a heavy chain having a VH comprising the amino acid sequence set forth in SEQ ID No. 78, and a light chain having a VL comprising the amino acid sequence set forth in SEQ ID No. 16.

In another embodiment, the antibody or antibody fragment thereof as described herein comprises a heavy chain having a VH comprising the amino acid sequence set forth in SEQ ID No. 47, and a light chain having a VL comprising the amino acid sequence set forth in SEQ ID No. 16.

In another embodiment, the antibody or antibody fragment thereof as described herein comprises a heavy chain having a VH comprising the amino acid sequence set forth in SEQ ID No. 76, and a light chain having a VL comprising the amino acid sequence set forth in SEQ ID No. 16.

In one embodiment, the antibody or antibody fragment thereof as described herein comprises two heavy chain variable regions of SEQ ID NO: 10 and two light chain variable regions of SEQ ID NO: 16.

In another embodiment, the antibody or antibody fragment thereof as described herein comprises two heavy chain variable regions of SEQ ID NO: 11 and two light chain variable regions of SEQ ID NO: 16,

In another embodiment, the antibody or antibody fragment thereof as described herein comprises two heavy chain variable regions of SEQ ID NO: 12 and two light chain variable regions of SEQ ID NO: 16.

5 In one embodiment, the antibody or antibody fragment thereof as described herein comprises two heavy chain variable regions of SEQ ID NO: 43 and two light chain variable regions of SEQ ID NO: 16.

In one embodiment, the antibody or antibody fragment thereof as described herein comprises two heavy chain variable regions of SEQ ID NO: 75 and two light chain variable regions of SEQ ID NO: 16.

10 In one embodiment, the antibody or antibody fragment thereof as described herein comprises two heavy chain variable regions of SEQ ID NO: 70 and two light chain variable regions of SEQ ID NO: 16.

In one embodiment, the antibody or antibody fragment thereof as described herein comprises two heavy chain variable regions of SEQ ID NO: 84 and two light chain variable regions of SEQ ID NO: 16.

In one embodiment, the antibody or antibody fragment thereof as described herein comprises two heavy chain variable regions of SEQ ID NO: 88 and two light chain variable regions of SEQ ID NO: 16.

20 In one embodiment, the antibody or antibody fragment thereof as described herein comprises two heavy chain variable regions of SEQ ID NO: 40 and two light chain variable regions of SEQ ID NO: 16.

In one embodiment, the antibody or antibody fragment thereof as described herein comprises two heavy chain variable regions of SEQ ID NO: 83 and two light chain variable regions of SEQ ID NO: 16.

25 In one embodiment, the antibody or antibody fragment thereof as described herein comprises two heavy chain variable regions of SEQ ID NO: 78 and two light chain variable regions of SEQ ID NO: 16.

In one embodiment, the antibody or antibody fragment thereof as described herein comprises two heavy chain variable regions of SEQ ID NO: 47 and two light chain variable regions of SEQ ID NO: 16.

30

In one embodiment, the antibody or antibody fragment thereof as described herein comprises two heavy chain variable regions of SEQ ID NO: 76 and two light chain variable regions of SEQ ID NO: 16.

In one embodiment, the antibody of the invention comprises:

5 (A) a heavy chain having a VH comprising the amino acid sequence set forth in SEQ ID No. 10, and a heavy chain constant region comprising the amino acid sequence set forth in SEQ ID No. 17; and

(B) a light chain having a VL comprising the amino acid sequence set forth in SEQ ID No. 16, and a light chain constant region comprising the amino acid sequence
10 set forth in SEQ ID No. 18.

In another embodiment, the antibody of the invention comprises:

(A) a heavy chain having a VH comprising the amino acid sequence set forth in SEQ ID No. 11, and a heavy chain constant region comprising the amino acid sequence set forth in SEQ ID No. 17; and

15 (B) a light chain having a VL comprising the amino acid sequence set forth in SEQ ID No. 16, and a light chain constant region comprising the amino acid sequence set forth in SEQ ID No. 18.

In another embodiment, the antibody of the invention comprises:

20 (A) a heavy chain having a VH comprising the amino acid sequence set forth in SEQ ID No. 12, and a heavy chain constant region comprising the amino acid sequence set forth in SEQ ID No. 17; and

(B) a light chain having a VL comprising the amino acid sequence set forth in SEQ ID No. 16, and a light chain constant region comprising the amino acid sequence set forth in SEQ ID No. 18.

25 In one embodiment, the antibody of the invention comprises:

(A) a heavy chain having a VH comprising the amino acid sequence set forth in SEQ ID No. 43, and a heavy chain constant region comprising the amino acid sequence set forth in SEQ ID No. 17; and

30 (B) a light chain having a VL comprising the amino acid sequence set forth in SEQ ID No. 16, and a light chain constant region comprising the amino acid sequence set forth in SEQ ID No. 18.

In one embodiment, the antibody of the invention comprises:

(A) a heavy chain having a VH comprising the amino acid sequence set forth in SEQ ID No. 75, and a heavy chain constant region comprising the amino acid sequence set forth in SEQ ID No. 17; and

5 (B) a light chain having a VL comprising the amino acid sequence set forth in SEQ ID No. 16, and a light chain constant region comprising the amino acid sequence set forth in SEQ ID No. 18.

In one embodiment, the antibody of the invention comprises:

10 (A) a heavy chain having a VH comprising the amino acid sequence set forth in SEQ ID No. 70, and a heavy chain constant region comprising the amino acid sequence set forth in SEQ ID No. 17; and

(B) a light chain having a VL comprising the amino acid sequence set forth in SEQ ID No. 16, and a light chain constant region comprising the amino acid sequence set forth in SEQ ID No. 18.

In one embodiment, the antibody of the invention comprises:

15 (A) a heavy chain having a VH comprising the amino acid sequence set forth in SEQ ID No. 84, and a heavy chain constant region comprising the amino acid sequence set forth in SEQ ID No. 17; and

20 (B) a light chain having a VL comprising the amino acid sequence set forth in SEQ ID No. 16, and a light chain constant region comprising the amino acid sequence set forth in SEQ ID No. 18.

In one embodiment, the antibody of the invention comprises:

(A) a heavy chain having a VH comprising the amino acid sequence set forth in SEQ ID No. 88, and a heavy chain constant region comprising the amino acid sequence set forth in SEQ ID No. 17; and

25 (B) a light chain having a VL comprising the amino acid sequence set forth in SEQ ID No. 16, and a light chain constant region comprising the amino acid sequence set forth in SEQ ID No. 18.

In one embodiment, the antibody of the invention comprises:

30 (A) a heavy chain having a VH comprising the amino acid sequence set forth in SEQ ID No. 40, and a heavy chain constant region comprising the amino acid sequence set forth in SEQ ID No. 17; and

(B) a light chain having a VL comprising the amino acid sequence set forth in SEQ ID No. 16, and a light chain constant region comprising the amino acid sequence set forth in SEQ ID No. 18.

In one embodiment, the antibody of the invention comprises:

5 (A) a heavy chain having a VH comprising the amino acid sequence set forth in SEQ ID No. 83, and a heavy chain constant region comprising the amino acid sequence set forth in SEQ ID No. 17; and

(B) a light chain having a VL comprising the amino acid sequence set forth in SEQ ID No. 16, and a light chain constant region comprising the amino acid sequence
10 set forth in SEQ ID No. 18.

In one embodiment, the antibody of the invention comprises:

(A) a heavy chain having a VH comprising the amino acid sequence set forth in SEQ ID No. 78, and a heavy chain constant region comprising the amino acid sequence set forth in SEQ ID No. 17; and

15 (B) a light chain having a VL comprising the amino acid sequence set forth in SEQ ID No. 16, and a light chain constant region comprising the amino acid sequence set forth in SEQ ID No. 18.

In one embodiment, the antibody of the invention comprises:

(A) a heavy chain having a VH comprising the amino acid sequence set forth in
20 SEQ ID No. 47, and a heavy chain constant region comprising the amino acid sequence set forth in SEQ ID No. 17; and

(B) a light chain having a VL comprising the amino acid sequence set forth in SEQ ID No. 16, and a light chain constant region comprising the amino acid sequence set forth in SEQ ID No. 18.

25 In one embodiment, the antibody of the invention comprises:

(A) a heavy chain having a VH comprising the amino acid sequence set forth in SEQ ID No. 76, and a heavy chain constant region comprising the amino acid sequence set forth in SEQ ID No. 17; and

(B) a light chain having a VL comprising the amino acid sequence set forth in
30 SEQ ID No. 16, and a light chain constant region comprising the amino acid sequence set forth in SEQ ID No. 18.

The invention further provides a binding domain that specifically binds to human TGF- β RII, wherein the binding domain comprises:

any one of the heavy chain variable regions (VH) selected from:

- (A) a VH having a VH-CDR1, VH-CDR2, and VH-CDR3 of the VH having an amino acid sequence set forth in SEQ ID No. 12;
- (B) a VH having a VH-CDR1, VH-CDR2, and VH-CDR3 of the VH having an amino acid sequence set forth in SEQ ID No. 26;
- (C) a VH having a VH-CDR1, VH-CDR2, and VH-CDR3 of the VH having an amino acid sequence set forth in SEQ ID No. 30;
- (D) a VH having a VH-CDR1, VH-CDR2, and VH-CDR3 of the VH having an amino acid sequence set forth in SEQ ID No. 40;
- (E) a VH having a VH-CDR1, VH-CDR2, and VH-CDR3 of the VH having an amino acid sequence set forth in SEQ ID No. 61;
- (F) a VH having a VH-CDR1, VH-CDR2, and VH-CDR3 of the VH having an amino acid sequence set forth in SEQ ID No. 65;
- (G) a VH having a VH-CDR1, VH-CDR2, and VH-CDR3 of the VH having an amino acid sequence set forth in SEQ ID No. 70;
- (H) a VH having a VH-CDR1, VH-CDR2, and VH-CDR3 of the VH having an amino acid sequence set forth in SEQ ID No. 76;
- (I) a VH having a VH-CDR1, VH-CDR2, and VH-CDR3 of the VH having an amino acid sequence set forth in SEQ ID No. 85; and
- (J) a VH having a VH-CDR1, VH-CDR2, and VH-CDR3 of the VH having an amino acid sequence set forth in SEQ ID No. 86;

wherein one to five amino acid residue(s) may be substituted with conservative amino acid(s) thereof in any one or more of the CDR(s) selected from VH-CDR1, VH-CDR2 and VH-CDR3.

The VH-CDR1, VH-CDR2, and VH-CDR3 sequences are indicated in bold and underlined in the listing of sequences provided herein.

In one embodiment, the binding domain of the present invention comprises a VH amino acid sequence selected from SEQ ID NO: 12, 26, 30, 40, 61, 65, 70, 76, 85 and 86, or a VH amino acid sequence having at least 80% identity thereto.

The binding domains of the present invention have shown to be useful in monovalent and multivalent form, providing for a variety of applications in monovalent molecules or bivalent molecules, or as one or more binding domains incorporated into a multispecific molecule. Said binding domains in monovalent form have superior TGF-
5 β RII blocking capabilities as compared to a monovalent control antibody, rendering them suitable for the above applications.

In certain embodiments, the present invention provides an antibody, preferably a multispecific antibody, comprising the binding domain of the present invention in monovalent form, which antibody has comparable receptor blocking activity at
10 equimolar concentrations when compared to a bivalent monospecific control antibody, preferably as measured in the same assay. In certain embodiments, the control antibody comprises two heavy chain variable regions having an amino acid sequence as set forth in SEQ ID NO: 97 and two light chain variable regions having an amino acid sequence as set forth in SEQ ID NO: 135. In certain embodiments, the assay is a TGF β -reporter
15 assay, preferably a TGF β -reporter assay as described in Example 5. In certain embodiments, the comparable receptor blocking activity includes a 5 to 2 fold, preferably a 3 fold, deviation, from the receptor blocking activity of the control antibody.

The antibodies and binding domains described and claimed herein, such as for
20 instance those described in the embodiments above, are preferably isolated antibodies or binding domains. Preferably, they are monoclonal antibodies. More preferably, they are isolated monoclonal antibodies.

The antibody or antibody fragment, or binding domain, of the present invention interferes with binding of ligands TGF- β 1, TGF- β 2, and/or TGF- β 3 to TGF- β RII. The
25 term "interferes with binding" means that the antibody or antibody fragment is directed to an epitope on TGF- β RII and competes with TGF- β 1, TGF- β 2, and/or TGF- β 3 for binding to TGF- β RII. The antibody or antibody fragment, or binding domain, may diminish ligand binding, displace ligand binding when this is already bound to TGF- β RII, or may, for instance through steric hindrance, at least partially prevent ligand from
30 binding to TGF- β RII, and/or interfere or prevent TGF- β RI-TGF- β RII complex formation. The antibody or antibody fragment, or binding domain, may diminish the formation of a TGF- β RI and TGF- β RII complex, displace such complex, when a

complex is already formed, or may, for instance through steric hindrance, at least partially prevent TGF- β RI from complexing with TGF- β RII. The term “interferes with binding” also means that the antibody or antibody fragment, or binding domain, of the present invention blocks binding of ligands TGF- β 1, TGF- β 2, and/or TGF- β 3 to TGF-
5 β RII.

The present invention further provides an expression vector comprising a polynucleotide encoding either or both the heavy chain and the light chain of the antibody or antibody fragment, or binding domain, as described herein. Examples of vectors include plasmids, phagemids, cosmids, viruses and phage nucleic acids or other
10 nucleic acid molecules that are capable of replication in a prokaryotic or eukaryotic host cell, e.g. a mammalian cell. The vector may be an expression vector wherein the polynucleotide encoding either or both the heavy chain and the light chain of the antibody or antibody fragment of the invention is operably linked to expression control elements. Typical expression vectors contain transcription and translation terminators,
15 initiation sequences, and promoters useful for regulation of the expression of the polynucleotides.

In the art various methods exist to produce antibodies. Antibodies are typically produced by a cell that expresses nucleic acid encoding the antibody. The invention therefore also provides an isolated cell, or a cell in a tissue culture, that produces and/or
20 comprises the antibody or antibody fragment of the invention. Typically this is an in vitro, isolated or recombinant cell. Such cell comprises a nucleic acid encoding the antibody or antibody fragment of the invention. The cell is preferably an animal cell, more preferably a mammalian cell, more preferably a primate cell, most preferably a human cell. For the purposes of the invention a suitable cell is any cell capable of
25 comprising and preferably of producing an antibody according to the invention and/or comprising a nucleic acid according to the invention. Preferably, the cell is a hybridoma cell, a Chinese hamster ovary (CHO) cell, an NS0 cell or a PER.C6 cell. It is particularly preferred that the cell is a CHO cell.

Further provided is a cell culture, or cell line, comprising a cell according to the
30 invention. Cell lines developed for industrial scale production of proteins and antibodies are herein further referred to as industrial cell lines.

The invention further provides a method for producing the antibody or antibody fragment, or binding domain, of the invention, the method comprising culturing a cell of the invention and harvesting the antibody or antibody fragment, or binding domain, from said culture. Said cell may be cultured in a serum free medium. Preferably said
5 cell is adapted for suspension growth. The antibody or antibody fragment, or binding domain, may be purified from the medium of the culture. Preferably said antibody or antibody fragment, or binding domain, is affinity purified.

The present invention further provides a pharmaceutical composition comprising an antibody or antibody fragment, or binding domain, as described herein, and a
10 pharmaceutically acceptable carrier, diluent, or excipient.

When the antibody or antibody fragment of the present invention is formulated to be used as an injection or infusion solution for drip infusion, the injection or infusion solution may be in any form of an aqueous solution, suspension, or emulsion, or may be formulated as a solid agent together with pharmaceutically acceptable carrier such that
15 that agent will be dissolved, suspended, or emulsified in a solvent at the time of use. Examples of the solvent that is used in the injection or the infusion solution for drip infusion include distilled water for injection, physiological saline, glucose solutions, and isotonic solutions (e.g., in which sodium chloride, potassium chloride, glycerin, mannitol, sorbitol, boric acid, borax, propylene glycol or the like is soluble).

20 Examples of pharmaceutically acceptable carriers include stabilizers, solubilizers, suspending agents, emulsifiers, soothing agents, buffering agents, preservatives, antiseptic agents, pH adjusters, and antioxidants. As stabilizers, various amino acids, albumin, globulin, gelatin, mannitol, glucose, dextran, ethylene glycol, propylene glycol, polyethylene glycol, ascorbic acid, sodium bisulfite, sodium
25 thiosulfate, sodium edetate, sodium citrate, dibutylhydroxytoluene, or the like, can be used. As solubilizers, alcohols (e.g., ethanol), polyols (e.g., propylene glycol and polyethylene glycol), nonionic surfactants (e.g., Polysorbate 20 (registered trademark), Polysorbate 80 (registered trademark) and HCO-50), or the like, can be used. As
30 suspending agents, glyceryl monostearate, aluminum monostearate, methyl cellulose, carboxymethyl cellulose, hydroxymethyl cellulose, sodium lauryl sulfate, or the like, can be used. As emulsifiers, gum arabic, sodium alginate, tragacanth, or the like, can be used. As soothing agents, benzyl alcohol, chlorobutanol, sorbitol, or the like, can be

used. As buffering agents, phosphate buffer, acetate buffer, borate buffer, carbonate buffer, citrate buffer, Tris buffer, glutamic acid buffer, epsilon aminocaproic acid buffer, or the like, can be used. As preservatives, methyl parahydroxybenzoate, ethyl parahydroxybenzoate, propyl parahydroxybenzoate, butyl parahydroxybenzoate, chlorobutanol, benzyl alcohol, benzalkonium chloride, sodium dehydroacetate, sodium edeate, boric acid, borax, or the like, can be used. As antiseptic agents, benzalkonium chloride, parahydroxybenzoic acid, chlorobutanol, or the like, can be used. As the pH adjusters, hydrochloric acid, sodium hydroxide, phosphoric acid, acetic acid, or the like, can be used. As antioxidants, (1) aqueous antioxidants such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, and sodium sulfite, (2) oil-soluble antioxidants such as ascorbyl palmitate, butylated hydroxy anisole, butylated hydroxy toluene, lecithin, propyl gallate, and α -tocopherol, or (3) metal chelating agents such as citric acid, ethylenediaminetetraacetic acid, sorbitol, tartaric acid, and phosphoric acid, can be used.

The injection or infusion solution for drip infusion can be produced by performing sterilization in the final process, or aseptic manipulation, e.g., sterilization by filtration with a filter and subsequently filling an aseptic container. The injection or infusion solution for drip infusion may be used by dissolving the vacuum dried or lyophilized aseptic powder (which may include a pharmaceutically acceptable carrier powder) in an appropriate solvent at the time of use.

The invention further provides a method of treating cancer in a subject comprising administering an effective amount of an antibody or antibody fragment, binding domain, or a pharmaceutical composition, as described herein to the subject in need thereof. As such, the invention provides an antibody or antibody fragment, or binding domain, as described herein for use in the treatment of cancer in a subject. The invention further provides a pharmaceutical agent for use in for preventing, suppressing symptom progression or recurrence of, and/or treating cancer, wherein the pharmaceutical agent comprises the antibody or antibody fragment thereof, or binding domain, as described herein as an active ingredient.

Cancer patients usually have aberrant cells that are to be removed from the body. The term 'aberrant cells' as used herein includes tumor cells, more specifically tumor cells present on any type of cancerous tissues associated with cancer—types correlated

with higher than normal TGF- β RII expression. Aberrant cells herein further refers to those having increased signaling as a consequence of increased expression of TGF- β and/or release of TGF- β , and those forming a suppressive environment reducing effectiveness of tumor immunity due to abnormal TGF- β signaling and/or expression or
5 higher than normal TGF- β , TGF- β RI and/or TGF- β RII expression, and/or higher than normal release of latent TGF- β .

The antibody or antibody fragment, or binding domain, of the invention will be effective in the treatment of several types of cancer, including for instance cancer-types correlated with higher than normal TGF- β signaling, in particular with higher than
10 normal TGF- β RII expression. Examples include, but are not limited to, breast cancer, colon cancer, colorectal cancer, gastric cancer, glioblastoma, neck cancer, hepatocellular carcinoma, non-small cell lung cancer, small cell lung cancer, melanoma, myelodysplastic syndrome, pancreatic cancer, prostate cancer and renal cancer.

The antibody or antibody fragment, or binding domain, of the present invention
15 blocks binding of human TGF- β to human TGF- β RII on the cell, thereby inhibiting TGF- β RII signaling. This leads to decreased proliferation of deleterious or aberrant cells and/or enhanced tumor immunity and other beneficial effect.

The invention therefore also provides a method of blocking binding of human TGF- β to human TGF- β RII on a cell, the method comprising providing the antibody or
20 antibody fragment, or binding domain, as described herein to the cell and allowing the antibody or antibody fragment to bind to the human TGF- β RII of the cell, to thereby block binding of human TGF- β to human TGF- β RII on the cell. This may be an in vitro method.

Depending on the desired activity, the antibody of the present invention may
25 have modulated effector function. Antibody-dependent cellular cytotoxicity (ADCC), also referred to as antibody-dependent cell-mediated cytotoxicity, is a mechanism of cell-mediated immune defence whereby an effector cell of the immune system actively lyses a target cell, whose membrane-surface antigens have been bound by specific antibodies. ADCC effector function is typically mediated by Fc receptors (FcRs). The
30 receptors are key immune regulatory receptors connecting the antibody mediated (humoral) immune response to cellular effector functions. Receptors for all classes of immunoglobulins have been identified, including Fc γ R (IgG), Fc ϵ RI (IgE), Fc α RI

- (IgA), FcμR (IgM) and FcδR (IgD). There are three classes of receptors for human IgG found on leukocytes: CD64 (FcγRI), CD32 (FcγRIIa, FcγRIIb and FcγRIIc) and CD16 (FcγRIIIa and FcγRIIIb). FcγRI is classed as a high affinity receptor (nanomolar range KD) while FcγRII and FcγRIII are low to intermediate affinity (micromolar range KD).
- 5 In antibody dependent cellular cytotoxicity (ADCC), FcγRs on the surface of effector cells (natural killer cells, macrophages, monocytes and eosinophils) bind to the Fc region of an IgG which itself is bound to a target cell. Upon binding, a signalling pathway is triggered which results in the secretion of various substances, such as lytic enzymes, perforin, granzymes and tumour necrosis factor, which mediate in the
- 10 destruction of the target cell. The level of ADCC effector function varies for human IgG subtypes. Although this is dependent on the allotype and specific FcγR in simple terms ADCC effector function is high for human IgG1 and IgG3, and low for IgG2 and IgG4. Knowledge of the binding site of the FcγRs on the antibody has resulted in engineered antibodies that do not have ADCC effector functions.
- 15 Another type of effector function is independent on effector cells and is referred to as complement-dependent cytotoxicity (CDC). This is an effector function of IgG and IgM antibodies. It is another mechanism of action by which therapeutic antibodies or antibody fragments can achieve an antitumor effect. CDC is initiated when C1q, the initiating component of the classical complement pathway, is fixed to the Fc portion of
- 20 target-bound antibodies. This is the first step of a complex complement activation cascade that can ultimately result in the lysis of the antibody marked cell.
- In an antibody of the invention, ADCC activity can be enhanced by different techniques by slightly modifying the constant region, one of them being the removal of fucose. Removal of fucose has resulted in increased anti-tumour activity in several in
- 25 vivo models (Junttila et al., Cancer Research (2010), Vol. 70(22), pp. 4481-4489). Afucosylation technology may be applied, thereby preventing fucosylation of the N-linked carbohydrate structure in the Fc region.
- In an antibody of the invention, effector function can also be reduced or eliminated. For instance, leucine at position 235 according to the EU numbering system
- 30 may be substituted with glycine, and/or glycine at position 236 according to the EU numbering system may be substituted with arginine. Such modification(s) ensure that binding to an Fc receptor and/or effector function is eliminated or decreased. Other

substitutions, deletions, or insertions known in the art to the same effect are also encompassed within the present invention.

The invention further provides a method of inhibiting signal transduction into a cell induced by binding of a human TGF- β to a human TGF- β RII of the cell, the method
5 comprising providing the antibody or antibody fragment, or binding domain, as described herein to the cell and allowing the antibody or antibody fragment to bind to the human TGF- β RII of the cell, to thereby inhibit the signal transduction into the cell. This may be an in vitro method.

The invention also provides a method of preventing or inhibiting metastasis, the
10 method comprising administering an effective amount of the antibody or antibody fragment, binding domain, or the pharmaceutical composition, as described herein, to a subject.

The antibody or antibody fragment, or binding domain, of the present invention may be used in the treatment of cancer as a single therapy but may also be combined
15 with other anti-cancer agents. Other anti-cancer agents include, but are not limited to, therapeutic antibodies that target the same or different tumor antigens or modulate elements of the immune system, agents used in chemotherapy (e.g. cyclophosphamide), and agents used in hormone therapy, or in applications related to localized administration including an oncolytic virus. Treatment with the antibody or antibody
20 fragment, or binding domain, of the present invention may also be combined with other anti-cancer treatments, such as for instance surgery or radiotherapy. Combinations of treatment can be simultaneous, separate, or sequential.

Throughout the present specification and the accompanying claims, the words
25 “comprise”, “include” and “having” and variations such as “comprises”, “comprising”, “includes” and “including” are to be interpreted inclusively. That is, these words are intended to convey the possible inclusion of other elements or integers not specifically recited, where the context allows.

The articles “a” and “an” are used herein to refer to one or to more than one (i.e.
30 to one or at least one) of the grammatical object of the article. By way of example, “an element” may mean one element or more than one element.

“Plurality” means two or more.

Note that in the present specification, unless stated otherwise, amino acid positions assigned to CDRs and frameworks in a variable region of an antibody or antibody fragment are specified according to Kabat's numbering (see Sequences of Proteins of Immunological Interest (National Institute of Health, Bethesda, Md., 1987 and 1991)). Amino acids in the constant regions are indicated according to the EU numbering system based on Kabat's amino acid positions (see Sequences of proteins of immunological interest, NIH Publication No. 91-3242).

Accession numbers are primarily given to provide a further method of identification of a target, the actual sequence of the protein bound may vary, for instance because of a mutation in the encoding gene such as those occurring in some cancers or the like. The antigen binding site binds the antigen and a variety of variants thereof, such as those expressed by some antigen positive immune or tumor cells.

When herein reference is made to a gene, a protein, the reference is preferably to the human form of the gene or protein. When herein reference is made to a gene or protein reference is made to the natural gene or protein and to variant forms of the gene or protein as can be detected in tumors, cancers and the like, preferably as can be detected in human tumors, cancers and the like.

HGNC stands for the HUGO Gene nomenclature committee. The number following the abbreviation is the accession number with which information on the gene and protein encoded by the gene can be retrieved from the HGNC database. Entrez Gene provides the accession number or gene ID with which information on the gene or protein encoded by the gene can be retrieved from the NCBI (National Center for Biotechnology Information) database. Ensemble provides the accession number with which information on the gene or protein encoded by the gene can be obtained from the Ensemble database. Ensembl is a joint project between EMBL-EBI and the Wellcome Trust Sanger Institute to develop a software system which produces and maintains automatic annotation on selected eukaryotic genomes.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 presents the amino acid sequences of the IGKV1-39 light chain variable V region (Figure 1A); IGKV1-39/jk1 light chain variable region (Figure 1B);
5 and IGKV1-39/jk5 light chain variable region (Figure 1C).

Figure 2 presents the FACS data of antibody binding to endogenously expressed TGF- β RII on CCD18Co cells. Figure 2A shows the MFI PE of antibodies comprising heavy chain variable regions having SEQ ID NO: 92 (negative control); SEQ ID NO: 93; and SEQ ID NO: 94. Figure 2B shows the MFI PE of antibodies
10 comprising heavy chain variable regions having SEQ ID NO: 92 (negative control); SEQ ID NO: 10; SEQ ID NO: 11; SEQ ID NO: 12; SEQ ID NO: 95; and SEQ ID NO: 96.

Figure 3 presents the ELISA data of ligand blocking activity of antibodies comprising heavy chain variable regions having SEQ ID NO: 92 (negative control);
15 SEQ ID NO: 93; and SEQ ID NO: 94 (Figure 3A), and of antibodies comprising heavy chain variable regions having SEQ ID NO: 92 (negative control); SEQ ID NO: 10; SEQ ID NO: 11; SEQ ID NO: 12; SEQ ID NO: 95; and SEQ ID NO: 96 (Figure 3B).

Figure 4 presents an amino acid sequence alignment generated using the AlignX application of the Vector NTI Program Advance 11.5.2 software of the heavy
20 chains comprising heavy chain variable regions having SEQ ID NO: 10 (middle alignment); SEQ ID NO: 11 (bottom alignment); and SEQ ID NO: 12 (top alignment) with their respective affinity matured variants. Identical amino acids are indicated with black letters against a white background; weakly similar amino acids are indicated with white letters against a grey background; conservative changes are indicated with black
25 letters against a grey background; and non-similar amino acids are indicated with white letters against a dark grey background.

Figure 5 shows the binding affinity of the parental antibodies (SEQ ID NO: 10; SEQ ID NO: 11; SEQ ID NO: 12) and the affinity matured variants (SEQ ID NO: 10 variants; SEQ ID NO: 11 variants; SEQ ID NO: 12 variants). k_a = on-rate in 1/Ms. k_d
30 = off-rate in 1/s.

Figure 6 shows the data from the luciferase reporter assay of the affinity matured variants. The X-axis shows the concentration of antibody in μ g/ml. The Y-axis

shows the fold induction of the Smad complex as a measure of TGF- β signaling inhibition. Graphs A-K each compare the activity of affinity matured variants with a control antibody (SEQ ID NO: 97). Basal TGF- β is included as a control of TGF- β stimulation of the cells.

5 **Figure 7** shows the data from the alanine scan. Figure 7A presents an overview of how critical residues are identified and mapped. Figure 7B shows the mean fluorescence of SEQ ID NO: 10; SEQ ID NO: 12; and SEQ ID NO: 93, as a percentage compared to wildtype. Figure 7C is a table showing the binding reactivity of SEQ ID NO: 10; SEQ ID NO: 12; SEQ ID NO: 93; and SEQ ID NO: 98 (control), as a
10 percentage compared to wildtype. Residues critical for binding are indicated with rectangles. Figure 7D shows the mapping of the critical residues on TGF β RII.

Figure 8 shows the results from screening a variety of bispecific antibodies comprising a TGF- β RII binding domain of the invention in a TGF- β reporter assay. The X-axis shows the concentration of antibody in μ g/ml. The Y-axis shows the fold
15 induction of Smad signaling. Graphs A-E each compare the activity of the bispecific antibodies comprising a single TGF- β RII binding domain with the positive control antibody known to block ligand binding to TGF- β RII (+) as described in Example 1. Basal TGF- β is included as a control of TGF- β stimulation of the cells.

A) Δ TGF- β RII binding domain comprising a heavy chain variable region
20 having amino acid sequence as set forth in SEQ ID NO: 76; $\square$ TGF- β RII binding domain comprising a heavy chain variable region having amino acid sequence as set forth in SEQ ID NO: 61; $\bullet$ TGF- β RII binding domain comprising a heavy chain variable region having amino acid sequence as set forth in SEQ ID NO: 26.

B) $\circ$ TGF- β RII binding domain comprising a heavy chain variable region
25 having amino acid sequence as set forth in SEQ ID NO: 70; $\ast$ TGF- β RII binding domain comprising a heavy chain variable region having amino acid sequence as set forth in SEQ ID NO: 70; $\square$ TGF- β RII binding domain comprising a heavy chain variable region having amino acid sequence as set forth in SEQ ID NO: 61; $\bullet$ TGF- β RII binding domain comprising a heavy chain variable region having amino acid sequence as set
30 forth in SEQ ID NO: 86; Δ TGF- β RII binding domain comprising a heavy chain variable region having amino acid sequence as set forth in SEQ ID NO: 65.

C) * TGF- β RII binding domain comprising a heavy chain variable region having amino acid sequence as set forth in SEQ ID NO: 65; $\circ$ TGF- β RII binding domain comprising a heavy chain variable region having amino acid sequence as set forth in SEQ ID NO: 40; $\bullet$ TGF- β RII binding domain comprising a heavy chain variable region having amino acid sequence as set forth in SEQ ID NO: 12; $\square$ TGF- β RII binding domain comprising a heavy chain variable region having amino acid sequence as set forth in SEQ ID NO: 76; Δ TGF- β RII binding domain comprising a heavy chain variable region having amino acid sequence as set forth in SEQ ID NO: 26.

D) $\bullet$ TGF- β RII binding domain comprising a heavy chain variable region having amino acid sequence as set forth in SEQ ID NO: 12; $\square$ TGF- β RII binding domain comprising a heavy chain variable region having amino acid sequence as set forth in SEQ ID NO: 85.

E) $\bullet$ TGF- β RII binding domain comprising a heavy chain variable region having amino acid sequence as set forth in SEQ ID NO: 86.

15

The following Examples illustrate the invention but are not intended to limit the invention in any way.

EXAMPLES

20

Example 1 – Antibody production

Transgenic mice comprising a common IGKV1-39 light chain (MeMo® mice) were immunized with human TGF- β RII (isoform A), thereby generating an immune response including the production of human anti-TGF- β RII specific antibodies. Lymphoid material of the immunized mice was collected, from which nucleic acids were extracted and used for the synthesis of cDNA encoding the heavy chain variable regions of such antibodies. The cDNA was used to generate phage display libraries, from which human TGF- β RII binding Fabs were selected using the Kingfisher selection robot.

30

Two rounds of affinity-driven selections with different concentrations of biotinylated recombinant protein were performed using the Kingfisher robot. Human

- TGF- β RII-Fc was biotinylated with the EZ-Link™ Sulfo-NHS-Biotin kit (ThermoFisher; cat.no.21217) according to the protocol of the manufacturer, aliquoted and stored at -20°C until further use. Two subsequent rounds of in-solution selections were performed with the biotinylated human TGF- β RII-Fc using the Kingfisher robot.
- 5 In the first round of selection, three different concentrations of protein were used. As a negative control, selections without antigen were included. 50 μ g/ml of total human IgG (Sigma; cat.no.1456) was added in solution during all phage library incubations to minimize the selection of Fc binders. After washing, bound phage was eluted with trypsin. Phage output was determined according to the spot method, wherein TG1 cells
- 10 were infected and plated on LB agar amp/glu plates for picking single colonies for screening. A second round of selection was performed with the outputs from the first round. In the second round of selection, TGF- β RII-Fc-biotin was used at decreasing amounts of protein starting at the concentration used for the respective first selection round output.
- 15 Colonies were picked into 96-wells plates for preparation of periplasmic extracts containing soluble Fab. The obtained Fab-containing fractions were used for identification of TGF- β RII specific clones using FACS.
- Human TGF- β RII transiently transfected HEK293T cells were used for FACS screening. The Fab end-concentration was between 0.5 and 5 μ g/ml. Fabs binding TGF-
- 20 β RII were detected with goat anti-kappa light chain antibodies (Ab0646; 5 μ g/ml) followed by rabbit anti-goat PE (Ab0330; 1/100 dilution).
- Two rounds of immunization were performed resulting in a large panel of Fabs for further characterization. In the second round of immunization, a different vector was
- 25 used to increase expression levels and the mice were co-immunized with human TGF- β RI. This may have contributed to a higher immune response generated in the mice observed during the second round of immunization.
- A positive control antibody was produced based on information obtained from
- 30 US 2010/0119516. This positive control antibody comprises two heavy chain variable regions and two light chain variable regions having the amino acid sequences of mAb TGF1 (SEQ ID NO: 97 and SEQ ID NO: 135 respectively) described therein. An

antibody comprising these heavy and light chain variable regions is reported to block ligand binding to TGF- β RII.

5 A negative control antibody against RSV was produced. This negative control antibody comprises two heavy chain variable regions having an amino acid sequence as set forth in SEQ ID NO: 136 and two light chain variable regions as set forth in SEQ ID NO: 16.

10 Example 2 – Antibody characterization

The VH fragments of the TGF- β RII binding Fabs identified in Example 1 were recloned into an IgG expression format to express and purify IgG from 293T freestyle cells.

15 *Antigen binding*

Antibodies were screened for binding to endogenously expressed human TGF- β RII on CCD18Co cells using FACS. Results are shown in **Figure 2**. All antibodies tested showed similar binding to the CCD18Co cells.

20

The Fab comprising heavy chain variable regions having an amino acid set forth in SEQ ID NO: 11, obtained from the first round of immunization, comprises the same HCDR3 sequence as Fabs obtained from the second round of immunization, for example the Fab comprising heavy chain variable regions having an amino acid
25 sequence set forth in SEQ ID NO: 93. This indicates that the recombination of VDJ gene segments in the mice is to a great extent the same in response to this antigen.

Binding affinity

30 The binding affinity of antibodies comprising heavy chain variable regions having an amino acid sequence set forth in SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID

NO: 93 and SEQ ID NO: 12 was determined by SPR. For this, the antibodies were reformatted as bivalent IgG's monospecific for TGF β RII.

Anti-TGF- β RII IgG antibodies were captured on a CM5 sensor chip surface by an immobilized anti-CH1 antibody (Ab0669; BD cat.no.BD555784), followed by the addition of human recombinant TGF- β RII (R&D systems cat.no.241-R2/CF; region Ile24-Aps195). Measurements were carried out at 25°C. Coupling was performed at pH 4.5. Results are shown in **Table 2**.

IgG	k_{on} (1/Ms)	k_{off} (1/s)	K_d (nM)
SEQ ID NO: 10	9.698*10E+5	4.313*10E-3	4.45
SEQ ID NO: 11	1.232*10E+6	1.021*10-2	8.29
SEQ ID NO: 93	1.074*10E+6	7.104*10E-3	6.61
SEQ ID NO: 12	1.028*10E+5	7.519*10E-4	7.31

Table 2. Binding affinity of selected antibodies to TGF- β RII.

10

Ligand blocking

Ligand blocking activity of the IgG samples was determined using an ELISA assay.

15 An ELISA plate was coated with TGF- β 1 at 0.4 μ g/ml. Human TGF- β RII-Fc (R&D; cat.no.341-BR) was added with an end concentration of 0.01 μ g/ml. Antibodies were incubated in a three-fold concentration range starting at an end concentration of 10 μ g/ml. An antibody that specifically binds tetanus toxoid comprising a heavy chain variable region having an amino acid sequence as set forth in SEQ ID NO: 92, was included as a control. Antibodies that bind human TGF- β RII-Fc were detected with a
20 biotin-conjugated anti-human Fc antibody (1:10.000) and streptavidin-HRP (1:2000).

As shown in **Figure 3**, all antibodies tested show good ligand blocking activity when compared to the control antibody. The IC₅₀ values of selected antibodies are presented in **Table 3**.

25

IgG	IC ₅₀ (ug/ml)
SEQ ID NO: 93	0.1551

SEQ ID NO: 10	0.138
SEQ ID NO: 11	0.5264
SEQ ID NO: 12	0.1252

Table 3. IC50 values of selected antibodies.

Example 3 – Affinity maturation

5 Fab phage display libraries of variant heavy chains comprising variable heavy chain regions having an amino acid sequence set forth in SEQ ID NO: 10, SEQ ID NO: 11, and SEQ ID NO: 12, that specifically bind TGF- β RII and block ligand interaction and TGF- β RII and TGF- β RI heterodimerization, were generated to evaluate whether antibodies could be produced with higher affinity. The library was designed to generate
10 heavy chain variable regions with increased affinity and having typically three to four variations: one in CDR1, one in CDR2, and one or two in CDR3, with certain number of variants having more than 4 variations overall. An overview of the variants is provided as a sequence alignment in **Figure 4**.

15 Variants with higher affinity were selected using two different selection methods, both with biotinylated human TGF- β RII-Fc as antigen, and both using the Kingfisher selection robot. Several selection rounds were performed. The first selection round comprised an affinity based selection as described in Example 1, using different concentrations of biotinylated TGF- β RII-Fc. Only the output from the selection with the lowest antigen concentration showing clear enrichment was subsequently further used
20 in two separate selection methods. The first selection method entails two additional rounds of affinity based selections, however, this time with further decreasing concentrations of antigen as compared to the optimal antigen concentration used in the preceding selection round. The second selection method also entails two additional rounds of selections which are both affinity based, via decreasing concentrations of
25 antigen, as well as off-rate based, via including a wash step in the presence of an excess of non-biotinylated antigen.

50 μ g/ml of total human IgG (Sigma; cat.no.I4506) was added in solution during all phage library incubations to minimize the selection of Fc binders. After washing, bound phage was eluted with trypsin. Phage output was determined according to the

spot method, wherein TG1 cells were infected with serial dilutions of phage outputs and plated on LB agar amp/glu plates for counting colonies the next day.

Phage outputs were rescued via bacterial infection to generate sufficient phages for subsequent selections, as well as to generate plates with bacterial colonies. Colonies
5 were picked into 96-wells plates for antibody production. The generated antibodies were used for the preparation of periplasmic extracts containing soluble Fabs.

Affinity matured variants were reformatted as bivalent IgG's monospecific for TGF β RII. SPR analysis was performed to determine the affinity of the variants for TGF- β RII. Results are shown in **Table 4** and **Figure 5**.

IgG	k_{on} (1/Ms)	k_{off} (1/s)	K_d (M)
SEQ ID NO: 44	1.37E+06	3.99E-03	2.90E-09
SEQ ID NO: 49	1.39E+06	5.24E-03	3.76E-09
SEQ ID NO: 53	1.43E+06	1.15E-02	8.07E-09
SEQ ID NO: 43	1.57E+06	6.20E-03	3.96E-09
SEQ ID NO: 40	1.46E+06	2.82E-03	1.93E-09
SEQ ID NO: 47	1.99E+06	7.83E-03	3.94E-09
SEQ ID NO: 48	1.31E+06	7.71E-03	5.88E-09
SEQ ID NO: 45	1.41E+06	6.68E-03	4.74E-09
SEQ ID NO: 41	1.36E+06	7.28E-03	5.34E-09
SEQ ID NO: 54	1.16E+06	1.33E-02	1.15E-08
SEQ ID NO: 55	1.58E+06	2.25E-02	1.42E-08
SEQ ID NO: 46	2.39E+06	8.94E-03	3.74E-09
SEQ ID NO: 42	2.16E+06	8.80E-03	4.08E-09
SEQ ID NO: 39	1.71E+06	5.26E-03	3.08E-09
SEQ ID NO: 50	1.17E+06	9.49E-03	8.12E-09
SEQ ID NO: 51	1.58E+06	8.23E-03	5.21E-09
SEQ ID NO: 52	1.92E+06	9.56E-03	4.98E-09
SEQ ID NO: 38	2.07E+06	7.97E-03	3.85E-09
SEQ ID NO: 56	8.74E+05	2.28E-02	2.61E-08
SEQ ID NO: 11	1.07E+06	1.29E-02	1.21E-08
SEQ ID NO: 84	2.32E+05	2.49E-03	1.07E-08
SEQ ID NO: 76	2.91E+05	1.90E-03	6.54E-09
SEQ ID NO: 71	1.95E+05	1.85E-03	9.53E-09
SEQ ID NO: 80	2.28E+05	1.93E-03	8.46E-09
SEQ ID NO: 88	1.73E+05	2.38E-03	1.38E-08
SEQ ID NO: 73	1.43E+05	2.46E-03	1.72E-08
SEQ ID NO: 67	2.40E+05	2.07E-03	8.63E-09
SEQ ID NO: 77	2.34E+05	2.61E-03	1.11E-08
SEQ ID NO: 75	2.21E+05	1.91E-03	8.63E-09

SEQ ID NO: 85	2.21E+05	3.88E-03	1.76E-08
SEQ ID NO: 58	8.87E+04	1.79E-03	2.02E-08
SEQ ID NO: 65	8.11E+04	1.58E-03	1.95E-08
SEQ ID NO: 59	9.00E+04	1.66E-03	1.85E-08
SEQ ID NO: 79	2.14E+05	2.27E-03	1.06E-08
SEQ ID NO: 60	1.18E+05	1.98E-03	1.68E-08
SEQ ID NO: 83	2.73E+05	1.94E-03	7.12E-09
SEQ ID NO: 70	3.24E+05	1.97E-03	6.08E-09
SEQ ID NO: 74	3.02E+05	1.57E-03	5.18E-09
SEQ ID NO: 69	2.37E+05	2.20E-03	9.27E-09
SEQ ID NO: 68	2.33E+05	2.20E-03	9.41E-09
SEQ ID NO: 63	2.57E+05	2.00E-03	7.80E-09
SEQ ID NO: 89	1.73E+05	3.13E-03	1.81E-08
SEQ ID NO: 82	2.47E+05	2.26E-03	9.15E-09
SEQ ID NO: 91	1.00E+05	3.60E-03	3.59E-08
SEQ ID NO: 72	2.23E+05	2.63E-03	1.18E-08
SEQ ID NO: 66	9.70E+04	2.18E-03	2.25E-08
SEQ ID NO: 86	1.13E+05	3.21E-03	2.85E-08
SEQ ID NO: 61	6.58E+04	1.68E-03	2.56E-08
SEQ ID NO: 78	1.28E+05	1.96E-03	1.53E-08
SEQ ID NO: 81	1.87E+05	2.61E-03	1.40E-08
SEQ ID NO: 90	1.05E+05	2.91E-03	2.77E-08
SEQ ID NO: 57	9.07E+04	2.86E-03	3.15E-08
SEQ ID NO: 87	1.49E+05	2.31E-03	1.55E-08
SEQ ID NO: 12	1.17E+05	2.51E-03	2.14E-08
SEQ ID NO: 22	6.10E+05	2.07E-03	3.39E-09
SEQ ID NO: 23	6.11E+05	3.65E-03	5.97E-09
SEQ ID NO: 36	9.15E+05	9.81E-03	1.07E-08
SEQ ID NO: 27	1.13E+06	6.18E-03	5.49E-09
SEQ ID NO: 29	1.43E+06	9.27E-03	6.47E-09
SEQ ID NO: 30	3.35E+05	1.11E-02	3.32E-08
SEQ ID NO: 33	1.02E+06	1.05E-02	1.02E-08

SEQ ID NO: 28	1.54E+06	8.85E-03	5.75E-09
SEQ ID NO: 34	1.13E+06	9.88E-03	8.73E-09
SEQ ID NO: 32	1.35E+06	9.04E-03	6.68E-09
SEQ ID NO: 37	9.53E+05	1.29E-02	1.36E-08
SEQ ID NO: 35	1.47E+06	1.23E-02	8.38E-09
SEQ ID NO: 26	1.93E+06	7.95E-03	4.11E-09
SEQ ID NO: 24	1.26E+06	4.96E-03	3.95E-09
SEQ ID NO: 10	1.08E+06	8.41E-03	7.81E-09

Table 4. Binding affinity of affinity matured variants and parental antibodies.

Reporter assay of variants

5 Affinity matured variants were reformatted to bivalent IgG format as described in Example 2. The obtained antibodies were used in a TGF- β RII luciferase based reporter assay to test the capacity of the antibodies to inhibit ligand induced activation of the receptor.

293F Freestyle cells were transiently transfected with the reporter CAGA₁₂ luciferase. The next day, transfected cells were plated and stimulated with 1 ng/ml of hTGF- β 1 in the presence/absence of a 6-point, semi-log serial dilution of anti-TGF- β RII antibody. The anti-TGF- β RII antibody was added simultaneously. The starting and highest concentration of the antibody was 10 μ g/ml, and the lowest concentration was 0.03 μ g/ml. The 293F Freestyle cells, hTGF- β 1 and anti-TGF- β RII antibody mixture was incubated for 3 hours in a 37°C, 5% CO₂ incubator. Each plate a titration of a negative competition control comprising a heavy chain variable region having SEQ ID NO: 97 and one well which does not contain TGF- β . As a read out, Steady-Glo Luciferase Assay System detection reagent was added and luciferase was measured after a 5 minute incubation on the EnVision. The fold response was calculated as follows:

$$\frac{(\text{sample with IgG X} + \text{TGF}\beta) - (\text{negative control IgG} + \text{TGF}\beta)}{(\text{positive control IgG} + \text{TGF}\beta) - (\text{negative control IgG} + \text{TGF}\beta)} \times 100$$

Results are shown in **Figure 6**. This data shows that several of the variants exhibit improved ligand blocking activity compared to their parental antibodies. The IC50 values of selected antibodies are presented in **Table 5**.

IgG	IC50 (ug/ml)
SEQ ID NO: 10	1.53
SEQ ID NO: 12	0.86
SEQ ID NO: 24	0.04
SEQ ID NO: 26	0.59
SEQ ID NO: 40	0.08
SEQ ID NO: 43	0.04
SEQ ID NO: 46	n.a.
SEQ ID NO: 47	0.19
SEQ ID NO: 48	0.25
SEQ ID NO: 54	16.40
SEQ ID NO: 67	0.25
SEQ ID NO: 70	0.36
SEQ ID NO: 75	0.18
SEQ ID NO: 76	0.20
SEQ ID NO: 77	0.36
SEQ ID NO: 78	1.50
SEQ ID NO: 83	0.08
SEQ ID NO: 84	1.54
SEQ ID NO: 88	1.16

5 ***Table 5 . IC50 values of selected affinity matured variants and parental antibodies.***

Example 4 – Epitope mapping

10 To identify the residues of TGF- β RII that are part of the epitope bound by the antibodies generated herein, shotgun mutagenesis experiments were performed using standard techniques (Davidson and Doranz , 2014). As a control in the shotgun

mutagenesis approach, an antibody comprising heavy chain variable regions having SEQ ID NO: 98 was used. This antibody was unable to block ligand binding to the receptor and could bind the receptor in the presence of all functional TGF- β RII antibodies. Results are shown in **Figure 7**.

5

Example 5 – TGF- β RII blocking activity of antibodies comprising a TGF- β RII binding domain

RSV $\times$ TGF- β RII and Antigen A $\times$ TGF- β RII antibodies were screened in a TGF β reporter assay.

Bispecific antibodies were produced comprising a first binding domain that binds TGF- β RII comprising a VH region of a selection of antibodies as described herein and a second binding domain that binds RSV comprising a VH region having an amino acid sequence as set forth in SEQ ID NO: 136, or a second binding domain that binds an antigen expressed on the same cell as TGF- β RII (Antigen A). Antigen A is an arbitrarily selected antigen not reactive with TGF β or impacting the signaling cascade tested in the TGF β reporter assay. Also included were the positive control antibody and negative control antibodies as described in Example 1.

The TGF β reporter assay uses a CAGA₁₂ luciferase vector containing 12 copies of the CAGA box which are the SMAD3 and SMAD4 binding sequences [Dennler et al, 1998]. Binding of TGF β to TGF- β RII leads to phosphorylation of TGF- β RI. Phosphorylation of TGF- β RI initiates a signaling cascade that leads to phosphorylation and activation of SMAD2 and SMAD3, which then form a complex with SMAD4. The SMAD complex then translocates to the nucleus and binds to the SMAD binding element (SBE) in the nucleus, leading to transcription and expression of TGF β / SMAD responsive genes. The CAGA₁₂ luciferase reporter can be used to monitor activation by TGF β and to screen TGF- β RII blocking antibodies after transfecting the reporter in mammalian cells.

Cryopreserved 293FF cells stably transfected to express Antigen A were transiently transfected with the TGF β reporter. IgGs were tested at a final concentration of 10 μ g/ml to 100 μ g/ml. IgGs were added in a final volume of 25 μ l (4x concentrated). Subsequently, 25 μ l of hTGF β 1 (4x concentrated) was added with a final concentration

of 1 ng/ml. 50 μ L transfected cell suspension (5×10^4 cells) was added. 100 μ L Steady-Glo™ substrate was added to each well and allowed to incubate for 5 minutes. Luminescence was measured on EnVision and results analyzed using Graphpad Prism.

Results are shown in Figure 8. The bispecific antibodies comprising a
5 monovalent binding domain for TGF- β RII and a control binding domain against RSV, as well as the bispecific antibodies comprising a monovalent binding domain for TGF- β RII and Antigen A, block the interaction of TGF- β with TGF- β RII. The bispecific antibody targeting TGF- β RII and RSV, which include the TGF- β RII binding domains comprising a VH region having an amino acid sequence as set out in SEQ ID NO: 76
10 and 70 are nearly as potent as the bivalent monospecific positive control antibody. Bispecific antibodies targeting TGF- β RII and Antigen A, which include the TGF- β RII binding domain comprising a VH region having an amino acid sequence as set out in SEQ ID NO: 70, 61, 86, 65, 12, and 76, are more potent than the bivalent monospecific positive control antibody. Accordingly, anti- TGF- β RII binding domains of the present
15 disclosure demonstrate comparable, equal and superior TGF- β RII blocking capability in monovalent form, providing for a variety of applications as monovalent molecules, bivalent molecules, or as a one or more valencenes as incorporated into a multispecific molecule.

Sequencing of the heavy chain variable regions

The nucleic acids encoding VH regions of a selection of antibodies that were identified to bind human TGF- β RII and block interaction with its ligand were
5 sequenced. Sequence information is provided below.

SEQUENCES

10 **SEQ ID NO: 1: HCDR1 according to Kabat**
 IYAMT

SEQ ID NO: 2: HCDR2 according to Kabat
 VISGSGGTTYADSVKG

15 **SEQ ID NO: 3: HCDR3 according to Kabat**
 RGQYRDIVGATDY

SEQ ID NO: 4: HCDR1 according to Kabat
 NAWMS
20

SEQ ID NO: 5: HCDR2 according to Kabat
 RIKTTISGGATDFAAPVKG

SEQ ID NO: 6: HCDR3 according to Kabat
25 DLRDY

SEQ ID NO: 7: HCDR1 according to Kabat
 RYAMS

30 **SEQ ID NO: 8: HCDR2 according to Kabat**
 AISASGDRTHNTDSVKG

SEQ ID NO: 9: HCDR3 according to Kabat
GIAASGKNYFDP

5 **SEQ ID NO: 10: Heavy chain variable region**

EVQLVESGGGLVQPGGSLRLSCAASGFTFDIYAMTWVRQAPGKGLEWV
SVISGSGGTTYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCA
RRGQYRDIVGATDYWGQGTLVTVSS

10 **SEQ ID NO: 11: Heavy chain variable region**

QVQLVESGGGLVEPGGSLRLSCAASGFTFSNAWMSWVRQAPGKGLEW
VGRIKTTISGGATDFAAPVKGRFTISRDDSKNTLYLQMNSLKTEDTAVY
YCTDLRDYWGQGTLVTVSS

15

SEQ ID NO: 12: Heavy chain variable region

QVQLVESGGGLVQPGGSLRLSCAVSGFTFRRYAMSWVRQAPGKGLEW
VSAIASGDRTHNTDSVKGRFSISRDN SKNTLYLQMNSLRAEDTAVYFC
20 AKGIAASGKNYFDPWGQGTLVTVSS

SEQ ID NO: 13: LCDR1 according to IMGT
QSISSY

25 **SEQ ID NO: 14: LCDR2 according to IMGT**
AAS

SEQ ID NO: 15: LCDR3 according to IMGT
QQSYSTPPT

30

SEQ ID NO: 16: Light chain variable region

DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYA
ASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPPTFGQG
TKVEIK

5 **SEQ ID NO: 17: Heavy chain constant region**

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSG
VHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVE
PKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDV
SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDW
10 LNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ
VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTV
DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG

SEQ ID NO: 18: Light chain constant region

15 RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQS
GNSQESVTEQDSKSTYLSSTLTLSKADYEEKHKVYACEVTHQGLSSPV
TKSFNRGEC

SEQ ID NO: 19: LCDR1 according to Kabat

20 RASQSISSYLN

SEQ ID NO: 20: LCDR2 according to Kabat

AASSLQS

25 **SEQ ID NO: 21: LCDR3 according to Kabat**

QQSYSTPPT

SEQ ID NO: 22: Heavy chain variable region

EVQLVESGGGLVQPGGSLRLSCAASGFTFDINAMTWVRQAPGKGLEWV
30 SVISGSGGTYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCA
RRGQYRDIVGATDYWGQGT LTVSS

SEQ ID NO: 23: Heavy chain variable region

EVQLVESGGGLVQPGGSLRLSCAASGFTFDIQAMTWVRQAPGKGLEWV
SVISGSGGTTYADSVQGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCA
RRGQYRDIVGATDYWGQGTLVTVSS

5

SEQ ID NO: 24: Heavy chain variable region

EVQLVESGGGLVQPGGSLRLSCAASGFTFDIYRMTWVRQAPGKGLEWV
SVISGSGGTTYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCA
RQGQYREIVGATDYWGQGTLVTVSS

10

SEQ ID NO: 25: Heavy chain variable region

EVQLVESGGGLVQPGGSLRLSCAASGFYFDIYAMTWVRQAPGKGLEWV
SVISGSGGTTYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCA
RRSQYRDKVGATDYWGQGTLVTVSS

15

SEQ ID NO: 26: Heavy chain variable region

EVQLVESGGGLVQPGGSLRLSCAASGFTFDINAMTWVRQAPGKGLEW
VSVISGSGGTTYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYY
CARRGQYREIAGATDYWGQGTLVTVSS

20

SEQ ID NO: 27: Heavy chain variable region

EVQLVESGGGLVQPGGSLRLSCAASGFAFDIYAMTWVRQAPGKGLEWV
SVISGSGGTIYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCA
RRGQYRDIVGATDYWGQGTLVTVSS

25

SEQ ID NO: 28: Heavy chain variable region

EVQLVESGGGLVQPGGSLRLSCAASGFTFDIYAMTWVRQAPGKGLEWV
SVISGSGGTVYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCA
RRGQYRDIAGGTDYWGQGTLVTVSS

30

SEQ ID NO: 29: Heavy chain variable region

EVQLVESGGGLVQPGGSLRLSCAASGFDIYAMTWVRQAPGKGLEWV
SVISGSGGTTYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCA
RRSQYRDKVGATDYWGQGT LVT VSS

5 **SEQ ID NO: 30: Heavy chain variable region**

EVQLVESGGGLVQPGGSLRLSCAASGFRFDIYAMTWVRQAPGKGLEW
VS VISGSGGTTYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYY
CAR RGQYRRIVGATDYWGQGT LVT VSS

10 **SEQ ID NO: 31: Heavy chain variable region**

EVQLVESGGGLVQPGGSLRLSCAASGFTFDINAMTWVRQAPGKGLEWV
SVISGSGGTTAYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCA
RRGQYRYIAGATDYWGQGT LVT VSS

15 **SEQ ID NO: 32: Heavy chain variable region**

EVQLVESGGGLVQPGGSLRLSCAASGFTFDITAMTWVRQAPGKGLEWV
SVISGSGGTTYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCA
RRGQYRDIAGATDYWGQGT LVT VSS

20 **SEQ ID NO: 33: Heavy chain variable region**

EVQLVESGGGLVQPGGSLRLSCAASGFSFDIYAMTWVRQAPGKGLEWV
SVISGSGGTTYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCA
RRAQYRDKVGATDYWGQGT LVT VSS

25 **SEQ ID NO: 34: Heavy chain variable region**

EVQLVESGGGLVQPGGSLRLSCAASGFTFDIYAMTWVRQAPGKGLEWV
SVISGSGGTTYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCA
RRGQYRYVVGATDYWGQGT LVT VSS

30 **SEQ ID NO: 35: Heavy chain variable region**

EVQLVESGGGLVQPGGSLRLSCAASGFYFDIYAMTWVRQAPGKGLEWV
SVISGSGGTTYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCA
RRGQYRHIAGATDYWGQGTLVTVSS

5 **SEQ ID NO: 36: Heavy chain variable region**

EVQLVESGGGLVQPGGSLRLSCAASGFRFDIYAMTWVRQAPGKGLEWV
SVISGSGGTTYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCA
RRGQYRDIVGATDYWGQGTLVTVSS

10 **SEQ ID NO: 37: Heavy chain variable region**

EVQLVESGGGLVQPGGSLRLSCAASGFTFDINAMTWVRQAPGKGLEWV
SVISGSGATTTYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCA
RRGQYREIQGANDYWGQGTLVTVSS

15 **SEQ ID NO: 38: Heavy chain variable region**

QVQLVESGGGLVEPGGSLRLSCAASGFTFSRAWMSWVRQAPGKGLEW
VGRIKTTISGGATQFAAPVKGRFTISRDDSKNTLYLQMNSLKTEDTAVY
YCTDLRNYWGQGTLVTVSS

20 **SEQ ID NO: 39: Heavy chain variable region**

QVQLVESGGGLVEPGGSLRLSCAASGFTFSRAWMSWVRQAPGKGLEW
VGRIKTTVSGGATAFAAPVKGRFTISRDDSKNTLYLQMNSLKTEDTAVY
YCTDLRAYWGQGTLVTVSS

25 **SEQ ID NO: 40: Heavy chain variable region**

QVQLVESGGGLVEPGGSLRLSCAASGFTFSNYWMSWVRQAPGKGLEW
VGRIKTTYSGGATDFAAPVKGRFTISRDDSKNTLYLQMNSLKTEDTAV
YYCTLDLRDYWGQGTLVTVSS

30 **SEQ ID NO: 41: Heavy chain variable region**

QVQLVESGGGLVEPGGSLRLSCAASGFTFSRAWMSWVRQAPGKGLEW
VGRIKTTYSGGATDFAAPVKGRFTISRDDSKNTLYLQMNSLKTEDTAVY
YCTDLRKYWGQGTLVTVSS

5 SEQ ID NO: 42: Heavy chain variable region

QVQLVESGGGLVEPGGSLRLSCAASGFTFSRAWMSWVRQAPGKGLEW
VGRIKTTISGGATDFAAPVKGRFTISRDDSKNTLYLQMNSLKTEDTAVY
YCTDLRAYWGQGTLVTVSS

10 SEQ ID NO: 43: Heavy chain variable region

QVQLVESGGGLVEPGGSLRLSCAASGFKFSNAWMSWVRQAPGKGLEW
VGRIKTTISGGATQFAAPVKGRFTISRDDSKNTLYLQMNSLKTEDTAVY
YCTDLRDYWGQGTLVTVSS

15 SEQ ID NO: 44: Heavy chain variable region

QVQLVESGGGLVEPGGSLRLSCAASGFTFSNAWMSWVRQAPGKGLEW
VGRIKTTYSGGATDFAAPVKGRFTISRDDSKNTLYLQMNSLKTEDTAVY
YCTDLRDYWGQGTLVTVSS

20 SEQ ID NO: 45: Heavy chain variable region

QVQLVESGGGLVEPGGSLRLSCAASGFTFSNAWMSWVRQAPGKGLEW
VGRIKTTYSGGATEFAAPVKGRFTISRDDSKNTLYLQMNSLKTEDTAVY
YCTDLRKYWGQGTLVTVSS

25 SEQ ID NO: 46: Heavy chain variable region

QVQLVESGGGLVEPGGSLRLSCAASGFTFSNYWMSWVRQAPGKGLEW
VGRIKTTISGGATDFAAPVKGRFTISRDDSKNTLYLQMNSLKTEDTAVY
YCTDLRAYWGQGTLVTVSS

30 SEQ ID NO: 47: Heavy chain variable region

QVQLVESGGGLVEPGGSLRLSCAASGFTFSRAWMSWVRQAPGKGLEW
VGRIKTTISGAATDFAAPVKGRFTISRDDSKNTLYLQMNSLKTEDTAVY
YCTDLRDYWGQGTLVTVSS

5 **SEQ ID NO: 48: Heavy chain variable region**

QVQLVESGGGLVEPGGSLRLSCAASGFTFANAWMSWVRQAPGKGLEW
VGRIKTTYSGGATDFAAPVKGRFTISRDDSKNTLYLQMNSLKTEDTAVY
YCTDLRKYWGQGTLVTVSS

10 **SEQ ID NO: 49: Heavy chain variable region**

QVQLVESGGGLVEPGGSLRLSCAASGFQFSNAWMSWVRQAPGKGLEW
VGRIKTTYSGGATDFAAPVKGRFTISRDDSKNTLYLQMNSLKTEDTAVY
YCTDLRDYWGQGTLVTVSS

15 **SEQ ID NO: 50: Heavy chain variable region**

QVQLVESGGGLVEPGGSLRLSCAASGFTFSNAHMSWVRQAPGKGLEW
VGRIKTTYSGGATDFAAPVKGRFTISRDDSKNTLYLQMNSLKTEDTAVY
YCTDLRQYWGQGTLVTVSS

20 **SEQ ID NO: 51: Heavy chain variable region**

QVQLVESGGGLVEPGGSLRLSCAASGFTFANAWMSWVRQAPGKGLEW
VGRIKTTYSGGATEFAAPVKGRFTISRDDSKNTLYLQMNSLKTEDTAVY
YCTDLRQYWGQGTLVTVSS

25 **SEQ ID NO: 52: Heavy chain variable region**

QVQLVESGGGLVEPGGSLRLSCAASGFTFSNAWMSWVRQAPGKGLEW
VGRIKTTYSGGATDFAAPVKGRFTISRDDSKNTLYLQMNSLKTEDTAVY
YCTDLRSYWGQGTLVTVSS

30 **SEQ ID NO: 53: Heavy chain variable region**

QVQLVESGGGLVEPGGSLRLSCAASGFQFSNAWMSWVRQAPGKGLEW
VGRIKTTISGGATEFAAPVKGRFTISRDDSKNTLYLQMNSLKTEDTAVY
YCTDLRDYWGQGTLVTVSS

5 **SEQ ID NO: 54: Heavy chain variable region**

QVQLVESGGGLVEPGGSLRLSCAASGFVFSNAWMSWVRQAPGKGLEW
VGRIKTTISGGATDFAAPVKGRFTISRDDSKNTLYLQMNSLKTEDTAVY
YCTDLRK YWGQGTLVTVSS

10 **SEQ ID NO: 55: Heavy chain variable region**

QVQLVESGGGLVEPGGSLRLSCAASGFHFSNAWMSWVRQAPGKGLEW
VGRIKTTISGGATDFAAPVKGRFTISRDDSKNTLYLQMNSLKTEDTAVY
YCTDLRAYWGQGTLVTVSS

15 **SEQ ID NO: 56: Heavy chain variable region**

QVQLVESGGGLVEPGGSLRLSCAASGFKFSNAWMSWVRQAPGKGLEW
VGRIKTTISGGKTEFAAPVKGRFTISRDDSKNTLYLQMNSLKTEDTAVY
YCTDLRRYWGQGTLVTVSS

20 **SEQ ID NO: 57: Heavy chain variable region**

QVQLVESGGGLVQPGGSLRLSCAVSGFAFRRYAMSWVRQAPGKGLEW
VSAISASGDRTHNTDSVKGRFSISRDN SKNTLYLQMNSLRAEDTAVYFC
AKGIAKRGKNYFDPWGQGTLVTVSS

25 **SEQ ID NO: 58: Heavy chain variable region**

QVQLVESGGGLVQPGGSLRLSCAVSGFQFRRYAMSWVRQAPGKGLEW
VSAISASGDRTHNTDSVKGRFSISRDN SKNTLYLQMNSLRAEDTAVYFC
AKGIAKSGKNYFDPWGQGTLVTVSS

30 **SEQ ID NO: 59: Heavy chain variable region**

QVQLVESGGGLVQPGGSLRLSCAVSGFTFRRYAMSWVRQAPGKGLEW
VSAISASGDRTHNTDSVKGRFSISRDN SKNTLYLQMNSLRAEDTAVYFC
AKGIAKSGKNYFDPWGQGTLVTVSS

5 **SEQ ID NO: 60: Heavy chain variable region**

QVQLVESGGGLVQPGGSLRLSCAVSGFKFRRYAMSWVRQAPGKGLEW
VSSISASGDRTHNTDSVKGRFSISRDN SKNTLYLQMNSLRAEDTAVYFC
AKGLAASGKNYFDPWGQGTLVTVSS

10 **SEQ ID NO: 61: Heavy chain variable region**

QVQLVESGGGLVQPGGSLRLSCAVSGFRFRRYAMSWVRQAPGKGLEW
VSAISASGDRTHNTDSVKGRFSISRDN SKNTLYLQMNSLRAEDTAVYFC
AKGIYASGKHYFDPWGQGTLVTVSS

15 **SEQ ID NO: 62: Heavy chain variable region**

QVQLVESGGGLVQPGGSLRLSCAVSGFTFRRYAMSWVRQAPGKGLEW
VSAISASGDRTKNTDSVKGRFSISRDN SKNTLYLQMNSLRAEDTAVYFC
AKGTAASGRNYFDPWGQGTLVTVSS

20 **SEQ ID NO: 63: Heavy chain variable region**

QVQLVESGGGLVQPGGSLRLSCAVSGFAFRRYAMSWVRQAPGKGLEW
VSSISASGDRTKNTDSVKGRFSISRDN SKNTLYLQMNSLRAEDTAVYFC
AKGIAASGKNYFDPWGQGTLVTVSS

25 **SEQ ID NO: 64: Heavy chain variable region**

QVQLVESGGGLVQPGGSLRLSCAVSGFTFRRYAMSWVRQAPGKGLEW
VSAISASGDRTHNTDSVKGRFSISRDN SKNTLYLQMNSLRAEDTAVYFC
AKGIFASGKH YFDPWGQGTLVTVSS

30 **SEQ ID NO: 65: Heavy chain variable region**

QVQLVESGGGLVQPGGSLRLSCAVSGFTFG**RYAMS**WVRQAPGKGLEW
VS**SAISASGDRHHNTDSVKGR**FSISRDN SKNTLYLQMNSLRAEDTAVYF
CAK**GIAKSGKNYFDP**WGQGLTVTVSS

5 **SEQ ID NO: 66: Heavy chain variable region**

QVQLVESGGGLVQPGGSLRLSCAVSGFQFRRYAMSWVRQAPGKGLEW
VSDISASGDRTHNTDSVKGRFSISRDN SKNTLYLQMNSLRAEDTAVYFC
AKGIARSGKNYFDPWGQGLTVTVSS

10 **SEQ ID NO: 67: Heavy chain variable region**

QVQLVESGGGLVQPGGSLRLSCAVSGFTFRRYAMSWVRQAPGKGLEW
VSAISASGDRTLNTDSVKGRFSISRDN SKNTLYLQMNSLRAEDTAVYFC
AKGTAARGKNYFDPWGQGLTVTVSS

15 **SEQ ID NO: 68: Heavy chain variable region**

QVQLVESGGGLVQPGGSLRLSCAVSGFAFRRYAMSWVRQAPGKGLEW
VSAISAFGDRTHNTDSVKGRFSISRDN SKNTLYLQMNSLRAEDTAVYFC
AKGTAASGKNFFDPWGQGLTVTVSS

20 **SEQ ID NO: 69: Heavy chain variable region**

QVQLVESGGGLVQPGGSLRLSCAVSGFTFRRYAMSWVRQAPGKGLEW
VSAISASGDRTKNTDSVKGRFSISRDN SKNTLYLQMNSLRAEDTAVYFC
AKGTAASGKNFFDPWGQGLTVTVSS

25 **SEQ ID NO: 70: Heavy chain variable region**

QVQLVESGGGLVQPGGSLRLSCAVSGFTFS**RYAMS**WVRQAPGKGLEW
VS**SAISASGDRTKNTDSVKGR**FSISRDN SKNTLYLQMNSLRAEDTAVYFC
AK**GTAAGKNYFDP**WGQGLTVTVSS

30 **SEQ ID NO: 71: Heavy chain variable region**

QVQLVESGGGLVQPGGSLRLSCAVSGFQFRRYAMSWVRQAPGKGLEW
VSAISAHGDRTHNTDSVKGRFSISRDN SKNTLYLQMNSLRAEDTAVYFC
AKGTAASGKNYFDPWGQGTLVTVSS

5 **SEQ ID NO: 72: Heavy chain variable region**

QVQLVESGGGLVQPGGSLRLSCAVSGFTIRRYAMSWVRQAPGKGLEWV
SYISASGDRTHNTDSVKGRFSISRDN SKNTLYLQMNSLRAEDTAVYFCA
KGTANS GKNYFDPWGQGTLVTVSS

10 **SEQ ID NO: 73: Heavy chain variable region**

QVQLVESGGGLVQPGGSLRLSCAVSGFTFRRYAMSWVRQAPGKGLEW
VSAISASGDRTHNTDSVKGRFSISRDN SKNTLYLQMNSLRAEDTAVYFC
AKGTAARGKNYFDPWGQGTLVTVSS

15 **SEQ ID NO: 74: Heavy chain variable region**

QVQLVESGGGLVQPGGSLRLSCAVSGFTFERYAMSWVRQAPGKGLEW
VSAISASGDRTQNTDSVKGRFSISRDN SKNTLYLQMNSLRAEDTAVYFC
AKGTAASGRNYFDPWGQGTLVTVSS

20 **SEQ ID NO: 75: Heavy chain variable region**

QVQLVESGGGLVQPGGSLRLSCAVSGFEFRRYAMSWVRQAPGKGLEW
VSAISAGGDRTANTDSVKGRFSISRDN SKNTLYLQMNSLRAEDTAVYFC
AKGTAARGKNYFDPWGQGTLVTVSS

25 **SEQ ID NO: 76: Heavy chain variable region**

QVQLVESGGGLVQPGGSLRLSCAVSGFAFRRYAMSWVRQAPGKGLEW
VSAISAGDRTQNTDSVKGRFSISRDN SKNTLYLQMNSLRAEDTAVYFC
AKGTAASGKNYFDPWGQGTLVTVSS

30 **SEQ ID NO: 77: Heavy chain variable region**

QVQLVESGGGLVQPGGSLRLSCAVSGFSFRRYAMSWVRQAPGKGLEW
VSAISASGDRTLNTDSVKGRFSISRDN SKNTLYLQMNSLRAEDTAVYFC
AKGTAARGKNYFDPWGQGTLVTVSS

5 **SEQ ID NO: 78: Heavy chain variable region**

QVQLVESGGGLVQPGGSLRLSCAVSGFEFRRYAMSWVRQAPGKGLEW
VSAISASGDRTDNTDSVKGRFSISRDN SKNTLYLQMNSLRAEDTAVYFC
AKGIARSGKNFFDPWGQGTLVTVSS

10 **SEQ ID NO: 79: Heavy chain variable region**

QVQLVESGGGLVQPGGSLRLSCAVSGFNFRYYAMSWVRQAPGKGLEW
VSAISASGDRTHNTDSVTGRFSISRDN SKNTLYLQMNSLRAEDTAVYFC
AKGLAASGKNYFDPWGQGTLVTVSS

15 **SEQ ID NO: 80: Heavy chain variable region**

QVQLVESGGGLVQPGGSLRLSCAVSGFAFRRYAMSWVRQAPGKGLEW
VSAISAHGDRTHNTDSVKGRFSISRDN SKNTLYLQMNSLRAEDTAVYFC
AKGTAASGKNYFDPWGQGTLVTVSS

20 **SEQ ID NO: 81: Heavy chain variable region**

QVQLVESGGGLVQPGGSLRLSCAVSGFTFRRYAMSWVRQAPGKGLEW
VSAISASGDRTHNTDSVKGRFSISRDN SKNTLYLQMNSLRAEDTAVYFC
AKGLAASGKNFFDPWGQGTLVTVSS

25 **SEQ ID NO: 82 Heavy chain variable region**

QVQLVESGGGLVQPGGSLRLSCAVSGFAFRRYAMSWVRQAPGKGLEW
VSSISASGDRTHNTDSVKGRFSISRDN SKNTLYLQMNSLRAEDTAVYFC
AKGLASSGKNYFDPWGQGTLVTVSS

30 **SEQ ID NO: 83: Heavy chain variable region**

QVQLVESGGGLVQPGGSLRLSCAVSGFQFRRYAMSWVRQAPGKGLEW
VSSISASGDRTHNTDSVKGRFSISRDN SKNTLYLQMNSLRAEDTAVYFC
AKGLAASGKNYFDPWGQGTLVTVSS

5 **SEQ ID NO: 84: Heavy chain variable region**

QVQLVESGGGLVQPGGSLRLSCAVSGFQFRRYAMSWVRQAPGKGLEW
VSAISASGDRYHNTDSVKGRFSISRDN SKNTLYLQMNSLRAEDTAVYFC
AKGTAASGKNYFDPWGQGTLVTVSS

10 **SEQ ID NO: 85: Heavy chain variable region**

QVQLVESGGGLVQPGGSLRLSCAVSGFKFRRYAMSWVRQAPGKGLEW
VSSAISASGDYTHNTDSVKGRFSISRDN SKNTLYLQMNSLRAEDTAVYFC
AKGIAKSGKNYFDPWGQGTLVTVSS

15 **SEQ ID NO: 86: Heavy chain variable region**

QVQLVESGGGLVQPGGSLRLSCAVSGFAFRRYAMSWVRQAPGKGLEW
VSSAISASGDRTRNTDSVKGRFSISRDN SKNTLYLQMNSLRAEDTAVYFC
AKGIATSGKNYFDPWGQGTLVTVSS

20 **SEQ ID NO: 87: Heavy chain variable region**

QVQLVESGGGLVQPGGSLRLSCAVSGFTFKRYAMSWVRQAPGKGLEW
VSAISASGDRSHNTDSVKGRFSISRDN SKNTLYLQMNSLRAEDTAVYFC
AKGLAARGKNYFDPWGQGTLVTVSS

25 **SEQ ID NO: 88: Heavy chain variable region**

QVQLVESGGGLVQPGGSLRLSCAVSGFNFRRYAMSWVRQAPGKGLEW
VSAISASGDRTHNTDSVKGRFSISRDN SKNTLYLQMNSLRAEDTAVYFC
AKGTAARGKNYFDPWGQGTLVTVSS

30 **SEQ ID NO: 89: Heavy chain variable region**

QVQLVESGGGLVQPGGSLRLSCAVSGFTFRRYAMSWVRQAPGKGLEW
VSAISASGDRTHNTDSVKGRFSISRDN SKNTLYLQMNSLRAEDTAVYFC
AKGIAASGKNFFDPWGQGTLVTVSS

5 **SEQ ID NO: 90: Heavy chain variable region**

QVQLVESGGGLVQPGGSLRLSCAVSGFTFRRYAMSWVRQAPGKGLEW
VSAISASGDRTHNTDSVKGRFSISRDN SKNTLYLQMNSLRAEDTAVYFC
AKGIAARGKNYFDPWGQGTLVTVSS

10 **SEQ ID NO: 91: Heavy chain variable region**

QVQLVESGGGLVQPGGSLRLSCAVSGFRFRRYAMSWVRQAPGKGLEW
VSAISASGDRTHNTDSVKGRFSISRDN SKNTLYLQMNSLRAEDTAVYFC
AKGIAARGKNFFDPWGQGTLVTVSS

15 **SEQ ID NO: 92: Heavy chain variable region**

EVQLVETGAEVKKPGASVKVSKASDYIFTKYDINWVRQAPGQGLEW
MGWMSANTGNTGYAQKFQGRVTMTRDTSINTAYMELSSLTSGDTAVY
FCARSSLFKTETAPYYHFALDVWGQGTTVTVSS

20 **SEQ ID NO: 93: Heavy chain variable region**

EVQLVESGGDLVKPGGSLRLSCAASGFTFSNAWMSWVRQAPGKGLEW
VGRVKTTVSGGTTDYAAAVKGRFTISRDDSKNTLYLQMNSLKTEDTAI
YYCTIDLRDYWGQGTLVTVSS

25 **SEQ ID NO: 94: Heavy chain variable region**

EVQLVESGGGLVQPGGSLRLSCAASGFTFSRYAMSWVRQAPGKGLEWV
SSINTSGGNTFYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAIYYCAK
GIAATGKNYFDPWGQGTLVTVSS

30 **SEQ ID NO: 95: Heavy chain variable region**

QVQLVESGGGLVQPGGSLRLSCAASGFTFSRYAMSWVRQAPGKGLEW
VSSINTSGGNTFYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYC
VKGIAAAGKNWFGPWGQGT LVTVSS

5 **SEQ ID NO: 96: Heavy chain variable region**

QVQLVESGGGLVQPGGSLRLSCAASGFTFSRYAMSWVRQAPGKGLEWV
SSINTSGGNTYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCA
KGIAASGKNYFDPWGQGT LVTVSS

10 **SEQ ID NO: 97: Heavy chain variable region**

EVQLVESGGGVVQPGRSLRLSCAASGFTFSNYGMHWVRQAPGKGLEW
VAVISYDGSTKYSADSLKGRFTISRDN SKNTLYLQMNSLRADDTAVYYC
AKEGWSFDSSGYRSWFDSWGQGT LVTVSS

15 **SEQ ID NO: 98: Heavy chain variable region**

QLQLQESGPGLVKPSETLSLTCTVSGGSISSSSYYWGWRQPPGKGLEWI
GSIYYSGSTYYNPSLKSRTISVDTSKNQFSLKLSSVTAADTAVYYCARS
FRGGYTAFDVWGQGT LVTVSS

20 **SEQ ID NO: 99: IGKV1-39**

DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYA
ASSLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQSYSTP

SEQ ID NO: 100: IGKV1-39/jk5

25 DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYA
ASSLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQSYSTPPITFGQG
TRLEIK

SEQ ID NO: 101: Isoform A human TGF- β RII

30 MGRGLLRGLWPLHIVLWTRIASTIPPHVQKSVNNDMIVTDNNGAVKFP
QLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITL
ETVCHDPKLPYHDFILEDAAAPK CIMKEKKKPGETFFMCSCSSDECNDNI

IFSEEYNTSNPDLLLVIQVVTGISLLPPLGVAISVIIIIFYCYRVNRQQKLSST
WETGKTRKLMFSEHCAIILEDDRSDISSTCANNINHNTPELLDTLVG
KGRFAEVYKAKLKQNTSEQFETVAVKIFPYEEYASWKTEKDIFSDINLK
HENILQFLTAEERKTELGKQYWLITAFHAKGNLQEYLTRHVISWEDLRK
5 LGSSLARGIAHLHSDHTPCGRPKMPIVHRDLKSSNILVKNDLTCCCLCDFG
LSRLDPTLSVDDLANSQGQVGTARYMAPEVLESRMNLENVESFKQTDV
YSMALVLWEMTSRCNAVGEVKDYEPFGSKVREHPCVESMKDNVLRD
RGRPEIPSWLHNHQQIQMVCELTTECWHDHPEARLTAQCVAERFSELEH
LDRLSGRSCSEEKIPEDGSLNTTK

10

SEQ ID NO: 102: Extracellular domain of isoform A of human TGF- β RII
TIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSN
CSITSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAAAPK
CIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPDLLLVIQV

15

SEQ ID NO: 103: Isoform B human TGF β RII

MGRGLLRGLWPLHIVLWTRIASTIPPHVQKSDVEMEAQKDEIICPSCNRT
AHPLRHINNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSIT
SICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAAAPKCIMK
20 EKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPDLLLVIQVVTGISLLPPL
GVAISVIIIIFYCYRVNRQQKLSSTWETGKTRKLMFSEHCAIILEDDRSDI
SSTCANNINHNTPELLDTLVGKGRFAEVYKAKLKQNTSEQFETVAV
KIFPYEEYASWKTEKDIFSDINLKHENILQFLTAEERKTELGKQYWLITAF
HAKGNLQEYLTRHVISWEDLRKLGSSLARGIAHLHSDHTPCGRPKMPIV
25 HRDLKSSNILVKNDLTCCCLCDFGLSLRLDPTLSVDDLANSQGQVGTARYM
APEVLESRMNLENVESFKQTDVYSMALVLWEMTSRCNAVGEVKDYEP
PFGSKVREHPCVESMKDNVLRDRGRPEIPSWLHNHQQIQMVCELTTECW
DHDPEARLTAQCVAERFSELEHLDRLSGRSCSEEKIPEDGSLNTTK

30

SEQ ID NO: 104: Extracellular domain of isoform B of human TGF- β RII
TIPPHVQKSDVEMEAQKDEIICPSCNRTAHPLRHINNDMIVTDNNGAVKFP
QLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENIT

LETVCHDPKLPYHDFILEDAAAPKCIMKEKKKPGETFFMCSCSSDECND
NIIFSEEYNTSNPDLLL VIFQ

5 **SEQ ID NO: 105: HCDR1 according to Kabat**
NAWMS

SEQ ID NO: 106: HCDR2 according to Kabat
RIKTTISGGATQFAAPVKG

10 **SEQ ID NO: 107: HCDR3 according to Kabat**
DLRDY

SEQ ID NO: 108: HCDR1 according to Kabat
RYAMS
15

SEQ ID NO: 109: HCDR2 according to Kabat
AISAGGDRTANTDSVKG

SEQ ID NO: 110: HCDR3 according to Kabat
GTAARGKNYFDP
20

SEQ ID NO: 111: HCDR1 according to Kabat
RYAMS

25 **SEQ ID NO: 112: HCDR2 according to Kabat**
AISASGDRTKNTDSVKG

SEQ ID NO: 113: HCDR3 according to Kabat
GTAAAGKNYFDP
30

SEQ ID NO: 114: HCDR1 according to Kabat
RYAMS

- SEQ ID NO: 115: HCDR2 according to Kabat
AISASGDRYHNTDSVKG
- 5 SEQ ID NO: 116: HCDR3 according to Kabat
GTAASGKNYFDP
- SEQ ID NO: 117: HCDR1 according to Kabat
RYAMS
- 10 SEQ ID NO: 118: HCDR2 according to Kabat
AISASGDRTHNTDSVKG
- SEQ ID NO: 119: HCDR3 according to Kabat
- 15 GTAARGKNYFDP
- SEQ ID NO: 120: HCDR1 according to Kabat
NYWMS
- 20 SEQ ID NO: 121: HCDR2 according to Kabat
RIKTTYSGGATDFAAPVKG
- SEQ ID NO: 122: HCDR3 according to Kabat
DLRDY
- 25 SEQ ID NO: 123: HCDR1 according to Kabat
RYAMS
- SEQ ID NO: 124: HCDR2 according to Kabat
- 30 SISASGDRTHNTDSVKG
- SEQ ID NO: 125: HCDR3 according to Kabat

GLAASGKNYFDP

**SEQ ID NO: 126: HCDR1 according to Kabat
RYAMS**

5

**SEQ ID NO: 127: HCDR2 according to Kabat
AISASGDRTDNTDSVKG**

10

**SEQ ID NO: 128: HCDR3 according to Kabat
GIARSGKNFFDP**

**SEQ ID NO: 129: HCDR1 according to Kabat
RAWMS**

15

**SEQ ID NO: 130: HCDR2 according to Kabat
RIKTTISGAATDFAAPVKG**

**SEQ ID NO: 131: HCDR3 according to Kabat
DLRDY**

20

**SEQ ID NO: 132: HCDR1 according to Kabat
RYAMS**

**SEQ ID NO: 133: HCDR2 according to Kabat
AISASGDRTLNTDSVKG**

25

**SEQ ID NO: 134: HCDR3 according to Kabat
GTAARGKNYFDP**

30

SEQ ID NO: 135: Light chain variable region

EIVLTQSPATLSLSPGERATLSCRASQSVRSYLA WYQQKPGQAPRLLIYD
ASN RATGIPARFSGSGGTDFLTITSSLEPEDFAVYYCQQR SNWPPTFGQ
GTKVEIK

5

SEQ ID NO:136: Heavy chain variable region

EVQLVESGGGVVQPGRSLRLSCAASGFTFSNYGMHWVRQAPGKGLEW
VAVISYDGSTKYSADSLKGRFTISRDN SKNTLYLQMNSLRADDTAVYYC
AKEGWSFDSSGYRSWFDSWGQGT LVTVSS