ANTI-TGF-BETA RECEPTOR II ANTIBODIES

This application claims priority to U.S. application nos. 61/198,697 filed 7 November 2008 and 61/170,369 filed 17 April 2009.

The present invention is in the field of medicine, particularly in the field of antibodies that bind human transforming growth factor beta receptor II (TGFβRII), pharmaceutical compositions comprising antibodies and methods of using the antibodies, for example, for treating cancer, fibrosis, and fibrotic diseases.

TGFβs are pleiotropic cytokines that regulate cell growth and differentiation, motility, extracellular matrix production, and immune functions. TGFβs have three mammalian isoforms, TGFβ-1, TGFβ-2 and TGFβ-3, each with distinct functions *in vivo*. All three TGFβs use the same receptor signaling system. The binding of TGFβs to TGFβRII is a crucial step in initiating activation of the TGFβ signaling pathway, leading to phosphorylation of Smad2, and translocation of the activated Smad2/Smad4 complex to the nucleus to modulate gene expression.

Human monoclonal antibodies (mAbs) that bind human TGFβRII with high affinity (K_D of 8.06×10^{-10} and 1.91×10^{-9} M) to treat kidney disease and tissue fibrosis are disclosed in JP 2004/121001A. The application also discloses that the mAbs suppress the TGFβ-induced growth of keratinocytes (average value of IC_{50} of 2.17-3.89, 3.17-4.95, and 3.21-5.07 μg/ml). Use of a fully human monoclonal antibody to TGFβRII was reported to be effective to reduce the deposit of extracellular matrix in rat anti-Thy-1 nephritis. (Kasuga, H., et al., Kidney Int'l, Vol. 60 (2001) 1745-1755.)

To date, there has been no disclosure of highly specific, high affinity anti-TGFβRII antibodies that specifically bind the extracellular domain of human TGFβRII with very high affinity, block the binding of human TGFβ1, TGFβ2, and TGFβ3 to human TGFβRII, inhibit angiogenesis, suppress tumor cell growth, inhibit migration and invasion of cancer cells, reduce collagen deposition and liver function, inhibit ligand induced regulation of T cells, or inhibit tumor growth in combination with cytotoxic agents, and are therefore needed.

The present invention seeks to provide novel isolated anti-TGFβRII mAbs that address these needs. The TGF beta RII is mammalian, and is preferably human. The antibodies of the present invention are capable of one or more of the following activities: 1) displaying high affinity binding toward the extracellular domain of human TGFβRII;

2) blocking the binding of TGFβRII ligands (TGFβ1, TGFβ2, and TGFβ3) to TGFβRII, thereby inhibiting TGFβ-induced Smad2 phosphorylation; 3) internalizing TGFβRII, which can act as a signaling down-regulation mechanism independent of ligand-receptor interaction; 4) inhibiting ligand-induced TGFβRII signaling pathways; 5) inhibiting TGFβRII-mediated cellular activities; 6) inhibiting tumor growth *in vitro* and *in vivo*; and also more preferably are additionally capable of one or more of the following: 7) inhibiting angiogenesis by reducing TGFβ-induced vascular endothelial growth factor A (VEGF-A) secretion; 8) inhibiting migration and invasion of cancer cells, 9) reducing collagen deposition and liver function; 10) inhibiting ligand induced regulation of T cells to form Treg cells that have immunosuppressive effects; or 11) inhibiting tumor growth in combination with cytotoxic agents.

A high affinity monoclonal antibody that specifically binds to TGFβRII and neutralizes TGFβRII-mediated activity would be particularly useful as a therapeutic bioagent for the treatment of TGFβ signaling mediated diseases.

According to a first aspect of the present invention, there is provided isolated antibodies that specifically bind the extracellular domain of human TGFβRII with a K_D of less than 100 pM at room temperature (20-25°C).

In one aspect, the antibodies of the present invention block binding of human TGFβ1, TGFβ2, or TGFβ3 to human TGFβRII with an IC_{50} of less than 1.0 nM as determined by ELISA.

In another aspect, the antibodies of the present invention inhibit TGFβ-induced Smad2 phosphorylation with an IC_{50} of less than 30 nM.

In yet another aspect, the antibodies of the present invention comprise an antibody that specifically binds to TGFβRII comprising:

i) a CDRH1 having the sequence GGSISNSYF (SEQ ID NO: 1), a CDRH2 having the sequence SFYYGEKTYYNPSLKS (SEQ ID NO: 2), a CDRH3 having the sequence GPTMIRGVIDS (SEQ ID NO: 3), a CDRL1 having the sequence RASQSVRSYLA (SEQ ID NO: 10), a CDRL2 having the sequence DASNRAT (SEQ ID NO: 11), and a CDRL3 having the sequence QQRSNWPPT (SEQ ID NO: 12); or

ii) a CDRH1 having the sequence GGSISSSSY (SEQ ID NO: 7), a CDRH2 having the sequence SFYYSGITYYSPSLKS (SEQ ID NO: 8), a CDRH3 having the sequence GFTMIRGALDY (SEQ ID NO: 9), a CDRL1 having the sequence RASQSVRSFLA (SEQ ID NO: 16), a CDRL2 having the sequence DASNRAT (SEQ ID NO: 11), and a CDRL3 having the sequence QQRSNWPPT (SEQ ID NO: 12).

In another aspect, the antibodies of the present invention comprise:

i) a HCVR amino acid sequence:
QLQVQESGPGLVKPSETLSLTCTVSGGSISNSYFSWGWIRQPPGKG
LEWIGSFYYGEKTYYNPSLKSRATISIDTSKSQFSLKLSSVTAADTA
VYYCPRGPTMIRGVIDSWGQGTLVTVSS (SEQ ID NO: 25) and
a LCVR amino acid sequence:
EIVLTQSPATLSLSPGERATLSCRASQSVRSYLAWYQQKPGQAPRL
LIYDASNRATGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQRSN
WPPTFGQGTKVEIK (SEQ ID NO: 27); or

ii) a HCVR amino acid sequence:

QLQLQESGPGLVKPSETLSLTCTVSGGSISSSSYSWGWIRQPPGKGL
EWIGSFYYSGITYYSPSLKSRIIISEDTSKNQFSLKLSSVTAADTAVY
YCASGFTMIRGALDYWGQGTLVTVSS (SEQ ID NO: 33), and

a LCVR amino acid sequence:

EIVLTQSPATLSLSPGERATLSCRASQSVRSFLAWYQQKPGQAPRL
LIYDASNRATGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQRSN
WPPTFGQGTKVEIK(SEQ ID NO: 35).

In another aspect, the antibodies of the present invention comprise a HCVR amino acid sequence:

QLQVQESGPGLVKPSETLSLTCTVSGGSISNSYFSWGWIRQPPGKGLEWIG
SFYYGEKTYYNPSLKSRATISIDTSKSQFSLKLSSVTAADTAVYYCPRGPT
MIRGVIDSWGQGTLVTVSS (SEQ ID NO: 25) and

a LCVR amino acid sequence:

EIVLTQSPATLSLSPGERATLSCRASQSVRSYLAWYQQKPGQAPRLLIYDA
SNRATGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQRSNWPPTFGQGT
KVEIK (SEQ ID NO: 27).

In another aspect, the antibodies of the present invention comprise:

i) a heavy chain of SEQ ID NO: 37 and a light chain of SEQ ID NO: 4;

or

ii) a heavy chain of SEQ ID NO: 6 and a light chain of SEQ ID NO: 14.

In another aspect, the antibodies of the present invention comprise two heavy chains of SEQ ID NO: 37 and two light chains of SEQ ID NO: 4.

In another aspect, the present invention comprises a human TGFβRII-binding fragment.

It is contemplated that any of the antibodies of the present invention may be administered to a subject in need thereof. Accordingly, one aspect of the invention provides a pharmaceutical composition comprising an antibody or fragment of the present invention and a pharmaceutically acceptable carrier, diluent or excipient.

In a preferred aspect of the invention, the antibody or a functional fragment thereof competes for binding to the extracellular domain of TGFβRII in a competition ELISA assay with a competing antibody, wherein said competing antibody binds TGFβRII with a K_D of less than 100 pM at room temperature (20-25°C).

In another preferred aspect of the invention, the antibody of the invention blocks binding of human TGFβ1, TGFβ2, or TGFβ3 to human TGFβRII with an IC_{50} of less than 1.0 nM as determined by ELISA.

It is also contemplated that the mAbs of the present invention may be used for treating fibrosis or fibrotic diseases of the lungs, liver, and kidneys. In one aspect, a method is provided for treating fibrosis or fibrotic diseases of the lungs, liver, and kidneys comprising administering to a subject in need of such treatment an effective amount of a mAb of the present invention.

One aspect of the present invention provides the antibodies of the present invention for use as a medicament. One aspect of the present invention provides the antibodies of the present invention for use in the treatment of cancer. A further aspect of the invention provides antibodies for use in the treatment of breast, lung or pancreatic

cancer. The antibodies of the invention may be used in the treatment of cancer together with an anti-cancer agent. Another aspect of the present invention provides a product containing the antibody or fragment and an additional anti-cancer agent for treatment in combination for simultaneous, separate or sequential use in therapy.

A preferred aspect of the invention provides an isolated antibody that specifically binds the extracellular domain of hTGFβRII, comprising a CDRH1 having the sequence GGSISX$_1$SX$_2$X$_3$ (SEQ ID NO: 17), wherein X$_1$ is N or S, X$_2$ is Y or S, and X$_3$ is F or Y; a CDRH2 having the sequence SFYYX$_1$X$_2$X$_3$TYYX$_4$PSLKS (SEQ ID NO: 18), wherein X$_1$ is G or S, X$_2$ is E or G, X$_3$ is K or I, X$_4$ is N or S; a CDRH3 having the sequence GX$_1$TMIRGX$_2$X$_3$DX$_4$ (SEQ ID NO: 42), wherein X$_1$ is P or F, X$_2$ is V or A, X$_3$ is I or L, X$_4$ is S or Y; a CDRL1 having the sequence RASQSVRSX$_1$LA (SEQ ID NO: 20), wherein X$_1$ is Y, or F; a CDRL2 having the sequence DASNRAT (SEQ ID NO: 11); and a CDRL3 having the sequence QQRSNWPPT(SEQ ID NO:12).

Another aspect of the present invention provides a method of treating cancer in a patient comprising administering to the patient an effective amount of the antibodies of the invention. The cancer may be breast, lung or pancreatic cancer. The antibodies may be administered to the patient, with an effective amount or another anti-cancer agent, simultaneously, separately or sequentially. The anti-cancer agent may be cyclophosphamide.

Another aspect of the invention provides an isolated antibody that specifically binds to the extracellular domain of human TGFβ receptor II (TGFβRII) comprising: a CDRH1 having the sequence GGSISNSYF (SEQ ID NO: 1), a CDRH2 having the sequence SFYYGEKTYYNPSLKS (SEQ ID NO: 2), a CDRH3 having the sequence GPTMIRGVIDS (SEQ ID NO: 3), a CDRL1 having the sequence RASQSVRSYLA (SEQ ID NO: 10), a CDRL2 having the sequence DASNRAT (SEQ ID NO: 11), and a CDRL3 having the sequence QQRSNWPPT (SEQ ID NO: 12); or a CDRH1 having the sequence GGSISSSSY (SEQ ID NO: 7), a CDRH2 having the sequence SFYYSGITYYSPSLKS (SEQ ID NO: 8), a CDRH3 having the sequence GFTMIRGALDY (SEQ ID NO: 9), a CDRL1 having the sequence RASQSVRSFLA (SEQ ID NO: 16), a CDRL2 having the sequence DASNRAT (SEQ ID NO: 11), and a

CDRL3 having the sequence QQRSNWPPT (SEQ ID NO: 12), or a TGFβRII-binding fragment of the antibody.

Another aspect of the invention comprises an antibody of the invention, comprises a HCVR amino acid sequence:

QLQVQESGPGLVKPSETLSLTCTVSGGSISNSYFSWGWIRQPPGKGLEWIGSFYYG EKTYYNPSLKSRATISIDTSKSQFSLKLSSVTAADTAVYYCPRGPTMIRGVIDSWG QGTLVTVSS (SEQ ID NO: 25) and a LCVR amino acid sequence: EIVLTQSPATLSLSPGERATLSCRASQSVRSYLAWYQQKPGQAPRLLIYDASNRAT GIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQRSNWPPTFGQGTKVEIK (SEQ ID NO: 27); or a HCVR amino acid sequence: QLQLQESGPGLVKPSETLSLTCTVSGGSISSSSYSWGWIRQPPGKGLEWIGSFYYS GITYYSPSLKSRIIISEDTSKNQFSLKLSSVTAADTAVYYCASGFTMIRGALDYWG QGTLVTVSS (SEQ ID NO: 33), and a LCVR amino acid sequence: EIVLTQSPATLSLSPGERATLSCRASQSVRSFLAWYQQKPGQAPRLLIYDASNRAT GIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQRSNWPPTFGQGTKVEIK (SEQ ID NO: 35), or a TGFβRII-binding fragment of the antibody.

Another aspect of the invention comprises an antibody of the invention comprising a heavy chain of SEQ ID NO: 37 and a light chain of SEQ ID NO: 4; or a heavy chain of SEQ ID NO: 6 and a light chain of SEQ ID NO: 14.

An "isolated antibody" is an antibody that (1) has been partially, substantially, or fully purified from a mixture of components; (2) has been identified and separated and/or recovered from a component of its natural environment; (3) is monoclonal; (4) is free of other proteins from the same species; (5) is expressed by a cell from a different species; or (6) does not occur in nature. Contaminant components of its natural environment are materials which would interfere with diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. Examples of isolated antibodies include an antibody that has been affinity purified, an antibody that has been made by a hybridoma or other cell line *in vitro*, and a human antibody derived from a transgenic mouse.

As used herein, the term "antibody" refers to immunoglobulin molecules comprising 4 polypeptide chains, two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds. Each heavy chain is comprised of a heavy chain variable

region (abbreviated herein as HCVR or VH) and a heavy chain constant region. The heavy chain constant region contains three domains, CH1, CH2 and CH3. Each light chain is comprised of a light chain variable region (abbreviated herein as LCVR or VL) and a light chain constant region. The light chains of antibodies (immunoglobulins) from any vertebrate species can be assigned to one of two clearly distinct types, called kappa (K) and lambda (λ), based on the amino acid sequences of their constant domains. The variable regions of kappa light chains are referred to herein as VK. The expression VL, as used herein, is intended to include both the variable regions from kappa-type light chains (VK) and from lambda-type light chains. The light chain constant region is comprised of one domain, CL. The VH and VL regions include regions of hypervariability, termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FR). Each VH and VL is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4.

"CDRH1" refers to the first CDR region in an antibody heavy chain, "CDRH2" refers to the second CDR region in an antibody heavy chain, and "CDRH3" refers to the third CDR region in an antibody heavy chain. "CDRL1" refers to the first CDR region in an antibody light chain, "CDRL2" refers to the second CDR region in an antibody light chain, and "CDRL3" refers to the third CDR region in an antibody light chain.

The term "antigen-binding fragment" refers to a portion or fragment of an intact antibody, comprising the antigen-binding or variable region thereof. Examples of antibody fragments include less than full length antibodies, *e.g.,* a Fab fragment, $F(ab')_2$, or a single-chain variable fragment (scFv). Likewise encompassed by the invention are diabodies, linear antibodies, single-chain antibodies, fusion proteins, recombinant proteins, and multivalent or multispecific antibodies formed or partly formed from an antigen-binding fragment of the present invention.

The term "TGF-beta receptor II" or "TGFβRII" as used herein refers to a cell surface receptor that binds a ligand, including, but not limited to, TGFβ1, TGFβ2, and TGFβ3, and as a result initiates a signal transduction pathway within the cell. Human TGFβRII is a transmembrane protein having amino acid residues 23-166 (143 aa).

The antibodies of the present invention bind human TGFβRII, more specifically the extracellular domain of human TGFβRII, and block binding of human TGFβ1, TGFβ2, and TGFβ3 to human TGFβRII.

The antibodies of the present invention also include those for which binding characteristics have been improved by direct mutation, methods of affinity maturation, phage display, or chain shuffling by methods known in the art. The antibodies of the invention include any combination of heavy and light chains (either full length or portions thereof) from the antibodies of the invention, referred to as TGF1 and TGF3.

The antibodies of the present invention can be used as a template or parent antibody to make additional antibodies of the invention using a variety of techniques including CDR-grafting, veneering or resurfacing, and chain shuffling (e.g., as disclosed in U.S. Patent 5,565,332). The human antibodies of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (e.g., mutations introduced by random or site-specific mutagenesis in vitro or by somatic mutation in vivo), for example in the CDRs. The human antibody can have at least one position replaced with an amino acid residue, e.g., an activity enhancing amino acid residue which is not encoded by the human germline immunoglobulin sequence, and in so doing generate further variable region amino acid sequences derived from the sequences herein provided.

In one approach the parent antibody CDRs are grafted into a human framework that has high sequence identity with the parent antibody framework. The sequence identity of the new framework will generally be at least 80%, at least 85%, or at least 90% with the corresponding framework in the parent antibody. This grafting may result in reduction in binding affinity compared to the parent antibody. If so, the framework can be back-mutated to the parent framework at certain positions based on specific criteria published by Queen (Queen, et al., Proc. Natl. Acad. Sci. USA 88, 2869 (1991)). Further methods that may be used include, for example, Jones et al., Nature, 321:522 (1986); Riechmann et al., Nature , 332:323-327 (1988); and Verhoeyen et al., Science, 239:1534 (1988).

Up to all 20 alternative naturally occurring amino acids may be introduced at a specific substitution site. The in vitro selection process defined here may then be suitably used to screen these additional variable region amino acid sequences for Fab fragments having the claimed cross reactivity and in vitro. In this way further Fab fragments are identified that are suitable for preparing a humanized antibody in accordance with the present invention. Preferably the amino acid substitution within the frameworks is restricted to one, two or three positions within one or each of the framework sequences disclosed herein. Preferably amino acid substitution within the CDRs is restricted to one to three positions within one or each CDR, more preferably substitution at one or two amino acid positions within one or each CDR is performed. Further preferred, amino acid substitution is performed at one or two amino acid positions in the CDRs of the heavy chain variable region. A suitable methodology for combining CDR and framework substitutions to prepare alternative antibodies according to the present invention, using an antibody described herein as a parent antibody, is provided in Wu et al., J. Mol. Biol., 294:151-162.

The term "K_D" refers to the dissociation constant of a particular antibody-antigen interaction. It is calculated by the formula: $k_{off}/k_{on} = K_D$. The term "k_{on}" refers to the association or on rate constant, or specific reaction rate, of the forward, or complex-forming, reaction, measured in units: $M^{-1}sec^{-1}$. The term "k_{off}" refers to the dissociation or off rate constant, or specific reaction rate, for dissociation of an antibody from the antibody/antigen complex, measured in units: 1/second. The binding affinity of an antibody of the present invention is often correlated with a lower k_{off} more so than a higher k_{on}, however, not being bound by theory, both improved k_{off} and k_{on}, embodiments are encompassed. In a more preferred aspect, antibodies of the present invention are high potency antibodies, or fragments thereof, generally exhibiting low k_{off} values.

In certain aspects, the antibodies of the present invention have a K_D of about 1 pM to about 200 pM, about 5 pM to about 100pM or about 10pM to about 80 pM.

As used herein, the terms "blocks binding" and "inhibits binding," used interchangeably, refer to blocking/inhibition of binding of a cytokine to its receptor, resulting in complete or partial inhibition or reduction of a biological function of the

cytokine/receptor signal pathway. Blocking/inhibition of binding of TGFβ to TGFβRII is assessed by measuring the complete or partial inhibition or reduction of one or more *in vitro* or *in vivo* indicators of TGFβ activity such as, receptor binding, an inhibitory effect on cell growth, chemotaxis, apoptosis, intracellular protein phosphorylation, or signal transduction. The ability to block the binding TGFβ to TGFβRII may be measured by ELISA as described herein. The ability to inhibit TGFβ activity may be assessed by measuring the inhibition of Smad2 phosphorylation in a cell, for example, in human MDA-MB-231 cells as described herein.

The antibodies of the present invention block binding of human TGFβ1, TGFβ2, or TGFβ3 to human TGFβRII with an IC_{50} of about 0.05 nM to about 1.0 nM, about 0.08 nM to about 0.75 nM, or about 0.10 nM to about 0.60 nM.

The antibodies of the present invention inhibit TGFβ-induced Smad2 phosphorylation with an IC_{50} of less than or equal to about 2.0 nM to about 30 nM, about 3.0 nM to about 15.0 nM or about 4.0 nM to about 7.5 nM in an *in vitro* blocking assay, for example, in an *in vitro* MDA-MB-231 cell blocking assay as described herein.

Antibodies may have a glycosylation pattern that is different or altered from that found in the native species. As is known in the art, glycosylation patterns may depend on the sequence of an antibody (e.g., the presence or absence of particular glycosylation amino acid residues), or the host cell, or the organism in which the protein is produced. It is contemplated that the antibodies of the present invention include the antibodies disclosed herein as well as glycosylation variants thereof.

The present invention also includes expression vectors comprising any of the polynucleotides described herein. Exemplary vectors include plasmids, phagemids, cosmids, viruses and phage nucleic acids or other nucleic acid molecules that are capable of replication in a prokaryotic or eukaryotic host such as a cell, *e.g.*, a mammalian cell. The vector may be an expression vector, wherein the polynucleotide encoding the antibody is operably linked to expression control elements. Typical expression vectors contain transcription and translation terminators, initiation sequences, and promoters useful for regulation of the expression of the nucleic acid molecules of the invention. The vectors may also contain genetic expression cassettes containing an independent terminator sequence, sequences permitting replication of the vector in both eukaryotes and prokaryotes, *i.e.*, shuttle vectors and selection markers for both prokaryotic and

eukaryotic systems. The vectors typically contain a marker to provide a phenotypic trait for selection of transformed hosts such as conferring resistance to antibiotics such as ampicillin or neomycin.

Suitable promoters include constitutive promoters and inducible promoters. Representative promoters include promoters derived from the human cytomegalovirus, metallothionein promoter, SV-40 early promoter, SV-40 later promoter, murine mammary tumor virus promoter, Rous sarcoma virus promoter and polyhedrin promoter.

The invention also includes recombinant cells containing a nucleic acid molecule or an expression vector of the invention. "Recombinant cell" means a non-human multicellular organism or a "host cell," which refers to a cell or population of cells into which a nucleic acid molecule or vector of the invention is introduced. A host cell of the present invention may be a eukaryotic cell or cell line, such as a plant, animal, vertebrate, mammalian, rodent, mouse, primate, or human cell, or cell line.

In one aspect, a host of the present invention may be prokaryotic or eukaryotic. Suitable prokaryotic hosts include, for example, *Escherichia coli*, such as *E. coli* SG-936, *E. coli* HB 101, *E. coli* W3110, *E. coli* X1776, *E. coli* X2282, *E. coli* DH1, and *E. coli* MRC1, *Pseudomonas*, *Bacillus*, such as *Bacillus subtilis*, and *Streptomyces*. Suitable eukaryotic cells include yeast and other fungi, insect cells, plant cells, human cells, and animal cells, including mammalian cells, such as hybridoma lines, COS cells, NS0 cells and CHO cells.

The invention includes methods of producing an antibody by culturing a recombinant cell expressing one or more nucleic acid sequences encoding an antibody of the present invention, and recovering the antibody from the culture medium. An antibody so expressed is typically purified or isolated after expression. Antibodies may be isolated or purified in a variety of ways known to those skilled in the art. Standard purification methods include chromatographic techniques, electrophoretic, immunological, precipitation, dialysis, filtration, concentration, and chromatofocusing techniques. As is well known in the art, a variety of natural proteins bind antibodies, for example bacterial proteins A, G, and L, and these proteins may find use in the present invention for purification. Purification can often be enabled by a particular fusion partner. For example, proteins may be purified using glutathione resin if a GST fusion is employed, Ni^{+2} affinity chromatography if a His-Tag is employed or immobilized anti-Flag

antibody if a His-Tag is used. The antibody can be purified by separating it from the culture medium. Antibodies comprising more than one chain can be produced by expressing each chain together in the same host; or as separate chains, which are assembled before or after recovery from the culture medium.

Antibodies may be screened using a variety of methods, including, but not limited to, *in vitro* assays, *in vitro* cell-based assays, *in vivo* assays, and selection technologies. Properties of antibodies that may be screened include, but are not limited to, biological activity, stability, solubility, and binding affinity for the target. Multiple properties may be screened simultaneously or individually. Proteins may be purified or unpurified, depending on the requirements of the assay. In one aspect, the screen is a qualitative or quantitative binding assay for binding of antibodies to a protein or nonprotein molecule that is known or thought to bind the antibody. In one aspect, the screen is a binding assay for measuring binding to the target antigen. Automation and high-throughpot screening technologies may be utilized in the screening procedures. Screening may employ the use of a fusion protein or labeled-protein. Binding assays can be carried out using a variety of methods known in the art, including, but not limited to, ELISA. As used herein, "competes for binding" refers to the situation in which an antibody reduces binding or signaling by at least about 20%, 30%, 50%, 70% or 90% as measured by a technique available in the art, e.g., competition ELISA or Kd measurement with BIAcore, but is not intended to completely eliminate binding.

One apparatus well known in the art for measuring binding interactions is a BIAcore™ 2000 instrument which is commercially available through Pharmacia Biosensor (Uppsala, Sweden).

This invention includes a pharmaceutical composition comprising an antibody of the invention described herein and a pharmaceutically acceptable carrier, diluent, or excipient. The pharmaceutical composition can optionally contain other therapeutic ingredients. As used herein, "pharmaceutically acceptable carrier" includes solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like that are physiologically compatible.

Examples of pharmaceutically acceptable carriers include water, saline, phosphate buffered saline, dextrose, glycerol, ethanol and the like, as well as combinations thereof. Pharmaceutically acceptable carriers may further include minor amounts of auxiliary

substances such as wetting or emulsifying agents, preservatives or buffers, which enhance the shelf life or effectiveness of the antibody, as well as isotonic agents such as sugars, polyalcohols such as mannitol and sorbitol, and sodium chloride.

The pharmaceutical compositions of the present invention may be formulated in a variety of ways, including, for example, liquid, semi-solid and solid dosage forms, such as liquid solutions (*e.g.*, injectable and infusible solutions), dispersions or suspensions, powders, liposomes and suppositories. The compositions are preferably in the form of injectable or infusible solutions.

The preferred mode of administration is parenteral (*e.g.*, intravenous, subcutaneous, intraperitoneal, intramuscular). Particularly preferred modes are intravenous infusion or injection, intramuscular injection and subcutaneous injection. Said compositions are designed in accordance with conventional techniques as in *e.g.*, Remington, *The Science and Practice of Pharmacy*, 19th Edition, Gennaro, Ed., Mack Publishing Co., Easton, PA 1995 which provides a compendium of formulation techniques as are generally known to practitioners.

Effective doses of the compositions of the present invention for treatment of a disease or disorder as described herein vary depending upon many different factors, including means of administration, target site, physiological state of the subject, whether the subject is human or an animal, other medications administered, and whether treatment is prophylactic or therapeutic. Treatment dosages may be titrated using routine methods known to those of skill in the art to optimize safety and efficacy.

The terms "treat," "treating," and "treatment" refer to therapeutic treatment, wherein the object is to slow down (lessen) an undesired physiological change associated with a disease or disorder. Beneficial or desired clinical results include, but are not limited to, alleviation of symptoms, diminishment of the extent of a disease or disorder, stabilization of a disease or disorder (*i.e.*, where the disease or disorder does not worsen), delay or slowing of the progression of a disease or disorder, and remission (whether partial or total) of the disease or disorder, whether detectable or undetectable.

"Treatment" can also mean prolonging survival as compared to expected survival if not receiving treatment. Those in need of treatment include those already with the disease or disorder as well as those prone to having the disease or disorder.

The pharmaceutical compositions of the present invention may include a "therapeutically effective amount" of an anti-TGFβRII antibody of the present invention. A "therapeutically effective amount" means an amount effective at dosages and for periods of time necessary to achieve the desired therapeutic result. A therapeutically effective amount of the antibody may vary according to factors such as the disease state, age, sex, weight of the individual, and the ability of the antibody or antibody portion to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the antibody or antibody portion are outweighed by the therapeutically beneficial effects.

Dosage regimens may be adjusted to provide the optimum desired response. For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form means a dose containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic or prophylactic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

An exemplary, non-limiting range for a therapeutically effective amount of an antibody of the invention is 0.1-50 mg/kg. In another aspect the effective amount of an antibody is 3-35 mg/kg. In another aspect, the effective amount is 10-25 mg/kg. In another aspect, the effective amount is 5-20 mg/kg. In another aspect, the effective amount is 3-15 mg/kg. In another aspect, the effective amount is 2-10 mg/kg. In another aspect, the effective amount is 5-10 mg/kg. In another aspect the effective amount of an antibody is 1-10 mg/kg. It is to be noted that dosage values may vary with the type and severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that dosage ranges set forth herein are

exemplary only and are not intended to limit the scope or practice of the claimed composition.

The antibodies of the present invention can be used for treating cancer. Cancer is considered to be a large group of diseases classified by the tissue of origin and the degree of tumor progression. Cancer can also be classified as primary tumors and metastatic tumors, as well as refractory or recurrent tumors. Refractory tumors are tumors that fail to respond or are resistant to treatment with chemotherapeutic agents alone, antibodies alone, radiation alone or combinations thereof. Recurrent tumors are tumors that appear to be inhibited by treatment with such agents, but recur up to five years, sometimes up to ten years or longer after treatment is discontinued.

Cancer that may be treated also includes tumors that are not vascularized, or not yet substantially vascularized, as well as vascularized tumors. Cancer may be comprised of non-solid tumors or solid tumors.

Anti-TGFβRII antibodies of the invention can also be used to treat TGFβRII-related disorders, diseases, or conditions that include chronic and acute disorders or diseases, including those pathological conditions that predispose the mammal to the disorder. Disorders to be treated herein include fibrosis caused by an arterial injury, an infection, rheumatoid arthritis, diabetes or a diabetic condition, or a malignancy, diseases characterized by accumulation of extracellular matrix, diseases caused by TGFβRII signaling, conditions caused by suppression of the immune system due to TGFβRII mediated activity, acute immune deficiencies resulting from severe injuries, burns, and illnesses such as viral or bacterial infections, and multi-organ systemic illnesses due to TGFβRII-mediated activity.

TGFβs play a significant role in self-renewal, proliferation and differentiation of hematopoietic stem cells. The antibodies of the present invention may be used for the enrichment and regeneration of stem cells, and facilitating of stem cell-based therapeutics in post-myocardial infarction, neuronal disorders and various types of tissue regeneration.

The antibodies of the present invention may be administered alone, or in combination with an anti-neoplastic agent other than anti-human TGFβRII antibodies, including chemotherapeutic agents, radiation, other TGFβRII antagonists, TGFβ antagonists, anti-angiogenesis agents, antibodies to other targets, and small molecules. Anti-TGFβRII antibodies are especially useful in treating anti-VEGF-A resistant tumors.

The administration of the antibodies with other antibodies and/or treatments may occur simultaneously, or separately, via the same or different route, at the same or different times.

The methods of treatment described herein can be used to treat any suitable mammal, including primates, such as monkeys and humans, horses, cows, cats, dogs, rabbits, and rodents such as rats and mice.

The following examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way.

EXAMPLES

Materials and Cell Lines

Human TGFβ1, TGFβ2, and TGFβ3 may be produced recombinantly and purified or may be purchased, for example from R&D Systems. Recombinant TGFβRII Fc fusion proteins (TGFβRII-Fc) and soluble recombinant TGFβRII alkaline phosphatase (TGFβRII-AP) proteins may be expressed in stably-transfected cells and purified from cell culture supernatants following the procedures known to one skilled in the art (Tessler, *J. Biol. Chem.*, 269:12456-12461 (1994)).

The human cancer cell lines BXPC-3, PANC-1, MDA-MB-231 and mouse tumor cell lines EMT6, 4T1, CT26, B16-F10 and myeloma cell lines P3-X63-Ag8.653 may be obtained from the American Type Tissue Culture Collection (Manassas, VA). MDA-MB-231 luciferase transfectant cell line may be obtained from Sunnybrook Health Sciences Centre. Cells may be maintained in RPMI1640 or IMDM medium (Invitrogen/Life Technologies, Inc., Rockville, MD) containing 10% fetal calf serum (FCS, Hyclone, Logan, UT). All cells may be maintained at 37 °C in a humidified, 5% CO_2 atmosphere.

Generation of anti-TGFβRII mAbs

Anti-TGFβRII mAbs may be generated essentially by standard hybridoma technology (Harlow & Lane, ed., *Antibodies: A Laboratory Manual*, Cold Spring Harbor, pages 211-213 (1998)) using human immunoglobulin transgenic mice (Medarex, San Jose, CA), which produce human immunoglobulin gamma heavy and kappa light chains, or Lewis rats (Charles River Laboratories, Wilmington, MA). Briefly, mice or rats are

immunized subcutaneously (s.c.) with recombinant human or mouse TGFβRII-Fc protein emulsified with complete Freund's adjuvant. Animals are intraperitoneally (i.p.) boosted three times with the same TGFβRII-Fc protein in incomplete Freund's adjuvant. The animals are rested for a month before they receive a final i.p. boost of 50 micrograms (μg) of TGFβRII-Fc protein in phosphate buffer solution (PBS). Splenocytes are harvested from the immunized mice and fused with P3-X63-Ag8.653 plasmacytoma cells using polyethylene glycol (PEG, MW: 1450 KD). After fusion, the cells are resuspended in HAT (hyposanthine, aminopterin, thymidine) medium supplemented with 10% fetal bovine serum (FBS) and distributed to 96 well plates at a density of 200 microliters per well for establishment of hybridoma cells.

At day 10 to 12 post-fusion, the hybridomas are screened for antibody production and specific binding activity of culture supernatants with TGFβRII protein in ELISA-based binding and blocking assays. Specifically, hybridomas producing anti-TGFβRII mAbs are first identified by detection of TGFβRII-bound antibody with a goat anti-human kappa light chain or anti-mouse IgG horse radish peroxidase (HRP) conjugated antibody according to the following procedure. Human TGFβRII-Fc or mouse TGFβRII-Fc is coated at 100 ng/well on 96 microtiter plates at 4°C overnight. The coated plates are blocked with the blocking buffer (PBS 0.05% TWEEN® 20 containing 5% dry milk) at room temperature for 2 hours. Hybridoma supernatants or purified antibodies are diluted in PBS with 2% bovine serum albumin (BSA) and 0.05% TWEEN® 20 (ELISA buffer) and incubated in TGFβRII--coated, 96-well, microtiter plates for 30 minutes. Plates are washed with the ELISA buffer and incubated with goat anti-human kappa light chain or anti-mouse IgG-HRP conjugate for 30 minutes. TMB (3,3', 5,5'-tetra-methylbenzidine) substrate is used for color development following the manufacturer's instructions. The absorbance at 450 nanometers (nm) is read for quantification of binding activity of antibodies. For identification of hybridomas producing neutralizing anti-TGFβRII mAbs, an ELISA based blocking assay is performed according to the following procedure. TGFβ1, TGFβ2, or TGFβ3 is coated at 200 ng per well on 96-well plates, and wells are then blocked with the blocking buffer. Hybridoma supernatants are incubated with ELISA buffer containing TGFβRII-AP in TGFβ-coated, 96-well microtiter plates for 1 hour. After washing, p-nitrophenyl phosphate (PNPP) substrate for AP is added to the wells for color development following the manufacturer's instructions. The absorbance at

405 nm is read for quantification of TGFβRII-binding to TGFβ1, TGFβ2, and TGFβ3. Optical density (OD) values are read on a microtiter plate reader (Molecular Devices Corp., Sunnyvale, CA).

The positive hybridomas are subcloned three times by a limiting dilution culture for establishment of monoclonal hybridoma cell lines.

Table 1 shows the amino acid sequences of the light chain and heavy chain CDRs of mAbs TGF1 and TGF3.

Table 1 Amino acid sequences of the light chain and heavy chain CDRs of anti-human TGFβRII mAbs

	mAb TGF1	**mAb TGF3**
CDRH1	GGSISNSYF (SEQ ID NO: 1)	GGSISSSSY (SEQ ID NO: 7)
CDRH2	SFYYGEKTYYNPS LKS (SEQ ID NO: 2)	SFYYSGITYYSPSL KS (SEQ ID NO: 8)
CDRH3	GPTMIRGVIDS (SEQ ID NO: 3)	GFTMIRGALDY (SEQ ID NO: 9)
CDRL1	RASQSVRSYLA (SEQ ID NO: 10)	RASQSVRSFLA (SEQ ID NO: 16)
CDRL2	DASNRAT (SEQ ID NO: 11)	DASNRAT (SEQ ID NO: 11)
CDRL3	QQRSNWPPT (SEQ ID NO: 12)	QQRSNWPPT (SEQ ID NO: 12)

The SEQ ID NOs of the amino acid sequences and the DNA sequences encoding the amino acid sequences of HCVRs, LCVRs, the heavy chains (HCs), and the light chains (LCs) for mAbs TGF1 and TGF3 are provided in Table 2 below.

Table 2 SEQ ID NOs of the amino acid sequences and the encoding DNA sequences of anti-human TGFβRII mAbs

Amino acid Sequences	**mAb TGF1**	**mAb TGF3**
HCVR	(SEQ ID NO: 25)	(SEQ ID NO: 33)
LCVR	(SEQ ID NO: 27)	(SEQ ID NO: 35)
HC	(SEQ ID NO: 37) (SEQ ID NO: 30)*	(SEQ ID NO: 6) (SEQ ID NO: 31)*
LC	(SEQ ID NO: 4) (SEQ ID NO: 32)*	(SEQ ID NO: 14) (SEQ ID NO: 29)*

DNA Sequences	mAb TGF1	mAb TGF3
HCVR	(SEQ ID NO: 26)	(SEQ ID NO: 34)
LCVR	(SEQ ID NO: 28)	(SEQ ID NO: 36)
HC**	(SEQ ID NO: 38)	(SEQ ID NO: 13)
LC**	(SEQ ID NO: 5)	(SEQ ID NO: 15)

* Amino acid sequences with a secretory signal sequence.
**cDNA Sequences include a secretory signal sequence.

Engineering and expression of human IgG1 anti-human TGFβ Receptor II antibodies.

The DNA sequences encoding the heavy chain and light chain variable regions of the anti-TGFβRII mAbs may be amplified by PCR for cloning into expression vectors. The heavy chain variable regions may be fused in frame to the human immunoglobulin heavy chain gamma1 constant region in vector pEE6.1 (Lonza Biologics plc, Slough, Berkshire, UK). The entire human light chain cDNA may be cloned directly into vector pEE12.1 (Lonza Biologics PLC, Slough, Berkshire, UK). Engineered immunoglobulin expression vectors may be stably transfected in NS0 myeloma cells by electroporation and selected in glutamine synthetase selection medium. Stable clones may be screened for antibody expression by anti-human TGFβRII specific binding ELISA. Positive clones may be cultured into serum-free medium culture for antibody production in spinner flasks or bioreactors. Full length IgG1 antibody may be purified by protein an affinity chromatography (Poros A, PerSeptive Biosystems Inc., Foster City, CA) and eluted into a neutral buffered saline solution.

The cDNA encoding the heavy and light chain variable regions of the anti-human TGFβRII mAbs TGF1 and TGF3 may be cloned and fused in frame to the human immunoglobulin heavy chain gamma1 constant region in GS (glutamine synthetase) expression vector. Engineered immunoglobulin expression vectors may be stably transfected in CHO cells. Stable clones may be verified for expression of antibody specifically binding to human TGFβRII. Positive clones may be expanded into serum-free medium culture for antibody production in bioreactors. Full length IgG1 antibody may be purified by protein A affinity chromatography and eluted into a neutral buffered saline solution.

Anti-TGFβRII mAbs bind to TGFβRII and block TGFβRII binding to its ligands.

The binding and blocking activity of purified anti-TGFβRII mAbs is determined in ELISA as described in "*Generation of anti-TGFβRII mAbs*" above. ED_{50} and IC_{50} of the antibodies are analyzed using GraphPad Prism® software 3.03 (GraphPad Software Inc., San Diego, CA). Anti-human TGFβRII mAbs TGF1 and TGF3 each separately exhibit binding activity to human TGFβRII with ED_{50}s of 0.031-0.059 nM in an ELISA-based binding assay whereas normal human IgG has no binding activity to the receptor. Purified mAbs TGF1 and TGF3 each separately effectively block the binding of human TGFβ1, TGFβ2, or TGFβ3 to human TGFβRII with IC_{50}s of 0.10-0.54 nM.

The binding and blocking characteristics of the anti-human TGFβRII antibodies are summarized in Table 3.

Table 3 Binding and Blocking Characteristics of anti-human TGFβRII antibodies

Clone	Binding Activity to Human TGFβRII (ED_{50}) by ELISA	Binding Affinity to Human TGFβRII (KD value) by Biacore Analysis	Blocking Activity to Human TGFβRII binding to human TGFβ1, 2, or 3 (IC_{50}) by ELISA
TGF1	0.059 nM	0.011 nM	0.12 nM: TGFβ1 0.54 nM: TGFβ2 0.19 nM: TGFβ3
TGF3	0.031 nM	0.019 nM	0.10 nM: TGFβ1 0.41 nM: TGFβ2 0.13 nM: TGFβ3

The binding activity of anti-mouse TGFβRII mAb MT1 to mouse TGFβRII has an ED_{50} of 0.054 nM and the blocking activity of mAb MT1 to mouse TGFβRII binding to mouse TGFβ1, TGFβ2, or TGFβ3 has an IC_{50}s value of 0.12-0.54 nM.

The binding and blocking characteristics of mAb MT1 are summarized in Table 4.

Table 4 Binding and Blocking Characteristics of anti-mouse TGFβRII mAb MT1

Binding Activity to Murine TGFβRII (ED_{50}) in ELISA	Binding Affinity to Murine TGFβRII (K_D value) Biacore Analysis	Blocking Activity to Murine TGFβRII binding to Murine TGFβ1, 2, or 3 (IC_{50}) in ELISA
0.054 nM	0.033 nM	0.12 nM: TGFβ1 0.54 nM: TGFβ2 0.19 nM: TGFβ3

Binding Affinity of Anti-TGFβRII mAbs.

The binding affinities of anti-TGFβRII mAbs are determined by surface plasmon resonance technology using BIAcore™ 2000 at room temperature (20 – 25 °C) (Pharmacia, Piscataway, NJ). Kinetic analyses of the mAbs are performed by immobilization of a fusion protein of recombinant extracellular domain of either mouse TGFβRII (SEQ ID NO: 41), or the extracellular domain of human TGFβRII linked, respectively, with either mouse or human Fc or heavy chain constant region, onto a sensor surface at a concentration of from 5 to 100 nM. Anti-human TGFβRII mAbs TGF1 and TGF3 exhibit a high affinity, with K_D values of 11, 78, 19 pM, respectively. Anti-murine TGFβRII mAb MT1 exhibits a high affinity, with a K_D value of 33 pM.

The kinetics of the mAbs are summarized in Table 5.

Table 5 Kinetics of Anti-human TGFβRII mAbs

mAb	K_{on} (1/Ms)	K_{off} (1/s)	K_D (M)
TGF1	1.5×10^{6}	1.7×10^{-5}	1.1×10^{-11}
TGF3	1.4×10^{6}	2.7×10^{-5}	1.9×10^{-11}

Species specificity of anti-human TGFβRII mAbs.

The specificity of anti-human TGFβRII mAbs is determined by measuring the reactivity of the antibodies to human TGFβRII or mouse TGFβRII by ELISA. Anti-

human TGFβRII mAb TGF1 exhibits no cross-reactivity with mouse TGFβRII, whereas mAb TGF3 exhibits intermediate or minimal cross reactivity with mouse TGFβRII. However, mAbTGF3 does not block human TGFβ1 binding to mouse TGFβRII.

Binding of anti-TGFβRII mAbs to native TGFβRII on TGFβRII expressing cells.

Binding activity of anti-human TGFβRII mAb TGF1 and fluorescein isothiocyanate (FITC)-labeled goat anti-human IgG antibody may be determined by a staining assay with 293-human TGFβRII transfectant cells and human carcinoma cells. Specifically, aliquots of transfectant cells, carcinoma cells, spleen cells, or lymph node cells are harvested from subconfluent cultures and incubated with fluorescein-labeled or unlabeled primary antibodies to desired molecules in PBS with 1% BSA (staining buffer) for 1 hour on ice. A matched IgG isotype is used as a negative control. Cells are washed twice with the staining buffer and then incubated with FITC, Phycoerythrin (PE) or Alxas Red labeled species-specific secondary antibody to primary antibody (BioSource International, Camarillo, CA) in the buffer for 30 min on ice. Cells are washed as above and analyzed on a flow cytometer. Dead cells and debris are eliminated from the analysis on the basis of forward and sideways light scatter. The mean fluorescent intensity units (MFIU) are calculated as the mean log fluorescence multiplied by the percentage of positive population. The mean fluorescent intensity ratio (MFIR) is calculated to quantitate relative expression levels of TGFβRII in the cell lines. The MFIR is the mean fluorescence intensity (MFI) of cells stained with TGFβRII specific mAb divided by the MFI of cells stained with an isotype control antibody.

Anti-human TGFβRII mAb TGF1 demonstrates binding reactivity with the 293-human TGFβRII transfectant cells and MDA-MB-231 human breast carcinoma cells with MFIRs of 46 and 209, respectively whereas normal human IgG has no reactive with the cells. The results indicate that mAb TGF1 has specific reactivity with native human TGFβRII expressed on the cell surface.

Inhibitory activity of anti-TGFβRII mAbs on activation of TGFβRII downstream kinase Smad2 in response to TGFβ1.

Phosphorylation of Smad2 (p-Smad2) induced by TGFβ is a typical downstream signaling pathway of TGFβ signaling through TGFβRII that mediates cellular biological responses such as proliferation, motility, survival, and differentiation in variety of cell types. The ability of anti-human TGFβRII and anti-human mouse TGFβRII mAbs to inhibit p-Smad2 activation may be determined by using 4T1 murine breast cancer cells and MDA-MB-231 human breast carcinoma cells according to the following procedure. Briefly, cells are grown to 80% confluence in FCS-containing medium. After replacing the culture medium with serum free medium, cells are treated with antibody or isotype control in the presence of 10 ng/mL TGFβ for 1 hour. After washing, cell lysates are prepared with lysis buffer and subjected to electrophoresis and Electro-Transfer to nitrocellulose membrane. Phosphorylated Smad2 and Smad2 are detected by Western blot using anti-phospho-Smad2 and Smad2 monoclonal antibodies (Millipore Corporate) and electrogenerated chemiluminescence system (ECL), and imaged and quantified by densitometry using a Fuji Image Analyzer.

Anti-TGFβRII mAbs TGF1 and MT1 reduce TGFβ-induced phosphorylation of Smad2 in human MDA-MB-231 and mouse 4T1 breast cancer cells in a dose-dependent manner. The IC_{50}s of mAbs TGF1 and MT1 in the p-Smad2 inhibition assays is determined to be 5 ± 0.5 nM, whereas, mAbTGF3 exhibits an IC_{50} lower than 25 ± 0.5 nM.

Inhibitory activity of anti-TGFβRII mAbs on in vitro migration and invasion of tumor cells.

The inhibitory effect of anti-TGFβRII mAbs on the invasiveness of tumor cells may be determined by *in vitro* migration and invasion assays. Briefly, carcinoma cells are loaded at a density of 5×10^3 per well into upper chambers inserted in Collagen I and IV coated lower chambers of 48-well plates in serum-free medium. The cells are treated with mAbs TGF1 or MT1 at doses of 3, 10, and 30 μg/mL in the presence of 10 ng/mL of TGFβ at 37 °C for 24-48 hours. 25 μg/mL TGFβRII-Fc or isotype IgG are used in assays as positive and negative control. The same conditions are used in the invasion assay with the exception that Matrigel-coated upper chambers are used. After incubation, migrated cells in the opposite sides of upper chambers are fixed with 10% buffered neutral

formalin, and stained with 2 μg/mL Hoechst 33342, trihydrochloride, trihydrate solution (Invitrogen) and counted at 20X magnification using a Zeiss Digital Image Camera and software Image-Pro Plus 5.1.

Anti-TGFβRII mAbs TGF1 and MT1 significantly inhibited the migration of BXPC-3 human pancreatic carcinoma cells and the invasion of 4T1 murine breast carcinoma cells by 100% ($P<0.0001$) and 93% ($P<0.0005$), respectively, when compared to IgG treated control.

These results demonstrate the inhibitory effect of the anti-TGFβRII antibodies of the present invention on invasiveness of cancer cells bearing TGFβRII on their surface.

Inhibitory activity of anti-TGFβRII mAbs on VEGF-A secretion in tumor cells.

TGFβs play a role in promoting angiogenesis during progression of pathological conditions through stimulation of VEGF-A secretion in tumor cells and modulation of endothelial cell functions. The inhibitory effect of anti-TGFβRII mAbs on the TGFβ-induced secretion of VEGF-A in tumor cells may be determined in cell culture.

Briefly, tumor cells are cultured in serum-free medium at 37°C in an incubator under 5% CO_2 in the presence or absence of 10 ng/mL TGFβ and a serial dilution of the mAbs for 48 hours. Alteration of VEGF-A secretion in conditioned culture supernatants is determined using an ELIKON kit (R&D Systems) per manufacturer's instructions.

Anti-human TGFβRII mAb TGF1 at 10 μM/mL inhibits TGFβ-induced production of VEGF-A in MDA-MB-231 human breast tumor cells by 63% ($P<0.01$). Anti-mouse TGFβRII mAb MT1 at 10 μM/mL inhibits TGFβ-induced production of VEGF-A in 4T1 mouse breast tumor cells 30% ($P<0.02$).

These results demonstrate that anti-TGFβRII mAbs of the present invention inhibit angiogenesis by reducing TGFβ-induced VEGF-A secretion.

Inhibitory activity of anti-TGFβRII mAbs on in vitro TGFβ-induced Treg conversion.

TGFβ has been shown to be capable of inducing naïve T cells to form regulatory T (Treg) cells that have immunosuppressive capacity to negatively control immune response. The inhibitory effect of anti-TGFβRII mAbs on the TGFβ-induced regulatory cell conversion may be evaluated *in vitro* as follows.

Briefly, purified naïve CD4+ cells are stimulated with 1 μg/mL anti-CD3 antibody and purified antigen presenting cells (APC) in the presence or absence of 10 ng/mL TGFβ and a serial dilution of mAb MT1 in complete RPMI medium at 37°C in an incubator under 5% CO_2 for 7 days. Cells are then harvested for staining of CD25+/Foxp3+ Treg cells and stained cells are analyzed on a flow cytometer.

Anti-mouse TGFβRII mAb MT1 at 10 μM/mL reduces the number of TGFβ-induced Treg cells *in vitro* by 75% ($P<0.005$) compared to control IgG treated cells.

Inhibitory activity of anti-TGFβRII mAbs on tumor growth and metastasis.

The antitumor efficacy of anti-TGFβRII mAbs may be tested in subcutaneous or intravenous metastasis tumor models.

Athymic nude mice (Charles River Laboratories, Wilmington, MA), Balb/c mice, or C57B6 mice (Charles River Laboratories, Wilmington, MA) may be used for inoculation with mouse or human carcinoma cells. For treatment of established tumors in subcutaneous models, tumors may be allowed to grow to approximately 200 mm^3 in size, and then mice may be randomized into groups of 12-15 animals per group. In lung metastasis models, mice may be injected intravenously with tumor cells via tail vein. Animals may receive i.p. administered anti-TGFβRII mAb at a dose of 10-40 mg/kg three times each week. Mice in control groups may receive an equal volume of saline or normal IgG solution. Treatment of animals may be continued for the duration of the experiment. Tumors may be measured twice each week with calipers. Tumor volumes may be calculated using the formula $[\pi/6 (w1 \times w2 \times w2)]$, where "w1" represents the largest tumor diameter and "w2" represents the smallest tumor diameter.

Tumor volume data may be analyzed using repeated-measures ANOVA (RM-ANOVA) to determine the significant differences in tumor sizes among treatments, time points, and treatment-time interactions. Comparisons of *in vitro* tumor cell growth between treatment and control may be conducted using the two-tailed Student's t test. A P value of less than 0.05 is considered to be statistically significant.

Mice bearing tumors are treated with mAb TGF1 at a dose of 40 mg/kg three times each week 24 hour post intravenous injection of tumor cells or after primary tumors are established. The systemic administration of mAb TGF1 suppresses subcutaneous

primary tumor growth of PANC-1 pancreatic carcinoma xenografts (T/C=69%, ANOVA p<0.03), BXPC-3 pancreatic carcinoma xenografts (T/C=30%, ANOVA p<0.0001), and MDA-MB-231 breast carcinoma xenografts (T/C=63%, ANOVA p<0.01).

Anti-mouse TGFβRII mAb MT1 is tested in mouse syngenetic tumor models for determining antitumor activity against primary and metastatic tumors in immunocompetent mice. Mice are injected intravenously (i.v.) with mouse 4T1, CT26 or B16 F10 carcinoma cells or subcutaneously (s.c.) with EMT6 mouse tumor cells. Mice receive administration of mAb MT1 at a dose of 40 mg/kg three times each week 24 hours post i.v. inoculation or after primary subcutaneous tumors are established.

The systemic administration of mAb MT1 significantly suppresses pulmonary metastasis of 4T1, CT26, and B16 F10 tumors by 84% (P<0.0001), 94% (P<0.0001), and 63% P<0.001), respectively. Anti-mouse TGFβRII mAb MT1 inhibits primary tumor growth by 28% (P<0.05) and spontaneous pulmonary metastasis by 84% (P<0.0001) in the EMT6 s.c. tumor model.

Myeloid cells with a Gr-1/CD11b+ phenotype have been reported to play a significant role in promoting metastasis and angiogenesis immunosuppression during tumor progression. CD4/CD25/Foxp3+ Treg cells have the ability to suppress the function of Natural Killer cells and cytotoxic T lymphocyte (CTL) immune effector cells against tumor cells. The inhibitory activity of mAb MT1 against immunosuppressive cells, i.e. CD4/CD25/Foxp3/TGFβRII+ Treg cells and Gr-1+/CD11b+/TGFβRII+ myeloid cells is evaluated in an EMT6 s.c. tumor model. The inhibitory effect of anti-TGFβRII antibody on Treg and Gr-1+/CD11b+ myeloid cell population in tumor-bearing mice may be determined by FACS analysis on the alteration of Gr-1+/CD11b+ population and CD4/CD25/Foxp3/TGFβRII+ and Gr-1+/CD11b+/TGFβRII+ population after treatment of mice with mAb MT1.

Anti-mouse TGFβRII mAb MT1 significantly decreases the number of Gr-1+/CD11b+/TGFβRII+ myeloid cells by 95% (P<0.0001) and CD4/CD25/Foxp3/TGFβRII+ Treg cells by 71% (P<0.0005), respectively, in treated mice bearing EMT6 tumors.

These results indicate that anti-TGFβRII antibodies may control the CD4/CD25/Foxp3/TGFβRII+ and Gr-1+/CD11b+ population by inhibition or/and depletion of TGFβRII+ Treg and myeloid cells.

Fibrosis model in mice.

TGFβ is a key regulator in the activation of hepatic stellate cells (HSC) and the differentiation of myofibroblasts, as well as the extracellular matrix accumulation that contributes to fibrosis. Liver fibrosis models in animals have been widely used as experimental models for the evaluation of activity of TGFβ signaling inhibitors to inhibit fibrosis. Collagen deposition is a known indicator of the formation of fibrosis in liver. Therapeutic activity of anti-TGFβRII antibody in protection and intervention of fibrosis may be evaluated in carbon tetrachloride (CCl_4) induced liver fibrosis models.

Briefly, C57BL6 mice may be injected i.p. with 1 mL/kg CCl_4 solution mixed with corn oil twice a week. Mice in the intervention treatment group may be administered mAb MT1 at doses of 40 mg/kg 3 times each week 14 days after mice are injected i.p. with CCl_4. Mice in the control group may be administered a control rat IgG at the same dosing. Eight weeks after CCl_4 injection, liver tissues and plasma samples may be collected from treated mice. Plasma levels of alanine aminotransferase (ALT), an indicator of liver dysfunction, may be determined by using a serum ALT kit (Pointe Scientific, Inc. MI). Liver tissues may be evaluated by immunohistochemistry (IHC) analysis with Sirius Red staining of collagen deposition.

In studies conducted essentially as described above, anti-mouse TGFβRII mAb MT1 significantly reduces collagen deposition by 95% ($P<0.0001$) in livers of mice given CCl_4 whereas the control rat IgG has no effect. Anti-mouse TGFβRII mAb MT1 protects liver from dysfunction by 85% ($P<0.001$) as measured by plasma level of ALT in mice given CCl_4 whereas mice treated with the control rat IgG have significantly higher levels of ALT.

These results suggest that anti-TGFβRII antibody MT1 is efficacious in protecting mice from injury-induced fibrosis and liver dysfunction.

***In vivo* studies on combination treatment with mAb MT1 and cyclophosphamide.**

Cyclophosphamide (CTX), a potent cytotoxic agent with the capacity to suppress hematopoietic and myeloid progenitor cells, has been reported to have inhibitory effects on myeloid cells (*See*, Honeychurch, et al., Cancer Res. 65:7493-7501 (2005)). EMT6-

tumor bearing mice may be treated with mAb MT1 alone, CTX alone, or a combination thereof. For instance, Balb/c mice or C57B6 mice (Charles River Laboratories, Wilmington, MA) may be used for inoculation with carcinoma cells. Mice with established tumors may be randomized into 12 animals per group, for example. Animals may be i.p. administered 40 mg/kg anti- TGFβRII mAb, 80 mg/kg CXT, or a combination of both 3 times each week. Mice in control groups may receive an equal volume of saline or normal IgG solution. Tumor volumes may be calculated using the formula {π/6 (w1 X w2 X w2)}, where "w1" represents the largest tumor diameter and "w2" represents the smallest tumor diameter.

Combination treatments with anti-mouse TGFβRII mAb MT1 and CTX performed essentially as described above reduces primary tumor growth by 80% ($P<0.0001$) and spontaneous pulmonary metastasis by 99.99% ($P<0.000001$) in EMT6 tumor-bearing mice compared to monotherapy with mAb MT1 28% ($P<0.05$) or CTX 62% ($P<0.0005$) in inhibition of primary tumor growth and mAb MT1 84% ($P<0.0001$) or CTX 96 % ($P<0.00001$) in inhibition of metastasis.

The results demonstrate that inhibition of a subset of TGFβRII-positive myeloid cells by anti-TGFβRII antibody in combination with myeloid cell suppressive chemotherapy is an effective strategy for intervention in tumor growth and metastasis.

I CLAIM:

1. An isolated antibody that specifically binds to the extracellular domain of human TGFβ receptor II (TGFβRII) with a K_D of less than 100 pM at room temperature (20-25°C).

2. The antibody of claim 1 that blocks binding of human TGFβ1, TGFβ2, or TGFβ3 to human TGFβRII with an IC_{50} of less than 1.0 nM as determined by ELISA.

3. The antibody of either claim 1 or claim 2 that inhibits TGFβ-induced Smad2 phosphorylation with an IC_{50} of less than 30 nM.

4. The antibody of any one of claims 1-3, that specifically binds to human TGFβRII comprising:

   i) a CDRH1 having the sequence GGSISNSYF (SEQ ID NO: 1), a CDRH2 having the sequence SFYYGEKTYYNPSLKS (SEQ ID NO: 2), a CDRH3 having the sequence GPTMIRGVIDS (SEQ ID NO: 3), a CDRL1 having the sequence RASQSVRSYLA (SEQ ID NO: 10), a CDRL2 having the sequence DASNRAT (SEQ ID NO: 11), and a CDRL3 having the sequence QQRSNWPPT (SEQ ID NO: 12);

   ii) a CDRH1 having the sequence GSGYRFTSY (SEQ ID NO: 4), a CDRH2 having the sequence IIYPGDSDTRYSPSFQG (SEQ ID NO: 5), a CDRH3 having the sequence HGRGYNGYEG (SEQ ID NO: 6), a CDRL1 having the sequence RASQGISSWLA (SEQ ID NO: 13), a CDRL2 having the sequence AASSLQS (SEQ ID NO: 14), and a CDRL3 having the sequence QQYNSYPWT (SEQ ID NO: 15); or,

   iii) a CDRH1 having the sequence GGSISSSSY (SEQ ID NO: 7), a CDRH2 having the sequence SFYYSGITYYSPSLKS (SEQ ID NO: 8), a CDRH3 having the sequence GFTMIRGALDY (SEQ ID NO: 9), a CDRL1 having the sequence RASQSVRSFLA (SEQ ID

NO: 16), a CDRL2 having the sequence DASNRAT (SEQ ID NO: 11), and a CDRL3 having the sequence QQRSNWPPT (SEQ ID NO: 12).

5. The antibody of any one of claims 1-4, comprising:

i) a HCVR amino acid sequence:
QLQVQESGPGLVKPSETLSLTCTVSGGSISNSYFSWGWIRQP
PGKGLEWIGSFYYGEKTYYNPSLKSRATISIDTSKSQFSLKLS
SVTAADTAVYYCPRGPTMIRGVIDSWGQGTLVTVSS (SEQ
ID NO: 25) and
a LCVR amino acid sequence:
EIVLTQSPATLSLSPGERATLSCRASQSVRSYLAWYQQKPG
QAPRLLIYDASNRATGIPARFSGSGSGTDFTLTISSLEPEDFA
VYYCQQRSNWPPTFGQGTKVEIK (SEQ ID NO: 27);

ii) a HCVR amino acid sequence:
QVQLVQSGAAVKKPGESLKISCKGSGYRFTSYWIGWVRQM
PGKGLEWMGIIYPGDSDTRYSPSFQGQVTISAGKSISTAYLQ
WSSLKASDTAMYYCARHGRGYNGYEGAFDIWGQGTMVT
VSS (SEQ ID NO: 29) and
a LCVR amino acid sequence:
DIQMTQSPSSLSASVGDRVTITCRASQGISSWLAWYQQKPE
KAPKSLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFA
TYYCQQYNSYPWTFGQGTKVEIK (SEQ ID NO: 31); or

iii) a HCVR amino acid sequence:
QLQLQESGPGLVKPSETLSLTCTVSGGSISSSSYSWGWIRQP
PGKGLEWIGSFYYSGITYYSPSLKSRIIISEDTSKNQFSLKLSS
VTAADTAVYYCASGFTMIRGALDYWGQGTLVTVSS (SEQ
ID NO: 33), and
a LCVR amino acid sequence:
EIVLTQSPATLSLSPGERATLSCRASQSVRSFLAWYQQKPGQ
APRLLIYDASNRATGIPARFSGSGSGTDFTLTISSLEPEDFAV
YYCQQRSNWPPTFGQGTKVEIK(SEQ ID NO: 35).

6. The antibody of claim 5, comprising:

i) a heavy chain of SEQ ID NO: 37 and a light chain of SEQ ID NO: 39;

ii) a heavy chain of SEQ ID NO: 41 and a light chain of SEQ ID NO: 43; or

iii) a heavy chain of SEQ ID NO: 45 and a light chain of SEQ ID NO: 47.

7. The antibody of claim 5, comprising:

i) a HCVR amino acid sequence: QLQVQESGPGLVKPSETLSLTCTVSGGSISNSYFSWGWIRQP PGKGLEWIGSFYYGEKTYYNPSLKSRATISIDTSKSQFSLKLS SVTAADTAVYYCPRGPTMIRGVIDSWGQGTLVTVSS (SEQ ID NO: 25) and

ii) a LCVR amino acid sequence: EIVLTQSPATLSLSPGERATLSCRASQSVRSYLAWYQQKPG QAPRLLIYDASNRATGIPARFSGSGSGTDFTLTISSLEPEDFA VYYCQQRSNWPPTFGQGTKVEIK (SEQ ID NO: 27).

8. The antibody of claim 7 comprising two heavy chains of SEQ ID NO: 37 and two light chains of SEQ ID NO: 39.

9. A human TGFβRII-binding fragment of the antibody of any of claims 1-8.

10. An isolated antibody or a functional fragment thereof, wherein said antibody competes for binding to the extracellular domain of TGFβRII in a competition ELISA assay with a competing antibody according to any one of claims 4 to 9, wherein said competing antibody binds TGFβRII with a K_D of less than 100 pM at room temperature (20-25°C).

11. The isolated antibody of claim 10, wherein said antibody blocks binding of human TGFβ1, TGFβ2, or TGFβ3 to human TGFβRII with an IC_{50} of less than 1.0 nM as determined by ELISA.

12. A pharmaceutical composition comprising an antibody or fragment of any one of claims 1-11 and a pharmaceutically acceptable carrier, diluent, or excipient.

13. A product containing an antibody or fragment, as claimed in any one of claims 1-11, and an additional anti-cancer agent for treatment in combination for simultaneous, separate or sequential use in therapy.

14. An antibody or fragment of any one of claims 1-11 for use as a medicament.

15. An antibody or fragment of any one of claims 1-11 for use in the treatment of cancer.

16. An antibody or fragment as claimed in claim 15 wherein the cancer is breast, lung or pancreatic.

17. An antibody or fragment as claimed in claim 16 together with an anti-cancer agent.

18. The antibody of claim 17, wherein the anti-cancer agent is cyclophosphamide.

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Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
85 90 95

Ile Ser Ser Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln
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115 120 125

Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp
130 135 140

Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn
145 150 155 160

Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu
165 170 175

Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp
180 185 190

Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr
195 200 205

Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser
210 215 220

Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225 230 235

<210> 30
<211> 470
<212> PRT
<213> Artificial

<220>
<223> Synthetic

<400> 30

Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
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Val His Ser Gln Leu Gln Val Gln Glu Ser Gly Pro Gly Leu Val Lys
20 25 30

Pro Ser Glu Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Gly Ser Ile
35 40 45

Ser Asn Ser Tyr Phe Ser Trp Gly Trp Ile Arg Gln Pro Pro Gly Lys
50 55 60

Gly Leu Glu Trp Ile Gly Ser Phe Tyr Tyr Gly Glu Lys Thr Tyr Tyr
65 70 75 80

Asn Pro Ser Leu Lys Ser Arg Ala Thr Ile Ser Ile Asp Thr Ser Lys
85 90 95

Ser Gln Phe Ser Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala
100 105 110

Val Tyr Tyr Cys Pro Arg Gly Pro Thr Met Ile Arg Gly Val Ile Asp
115 120 125

Ser Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys
130 135 140

Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly
145 150 155 160

Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro

165 170 175

Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr
180 185 190

Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val
195 200 205

Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn
210 215 220

Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro
225 230 235 240

Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu
245 250 255

Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp
260 265 270

Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
275 280 285

Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly
290 295 300

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn
305 310 315 320

Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp
325 330 335

Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro
340 345 350

Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu
355 360 365

Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn
370 375 380

Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile
385 390 395 400

Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr
405 410 415

Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys
420 425 430

Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys

435 440 445

Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu
450 455 460

Ser Leu Ser Pro Gly Lys
465 470

<210> 31
<211> 470
<212> PRT
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<220>
<223> Synthetic

<400> 31

Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
1 5 10 15

Val His Ser Gln Leu Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys
20 25 30

Pro Ser Glu Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Gly Ser Ile
35 40 45

Ser Ser Ser Ser Tyr Ser Trp Gly Trp Ile Arg Gln Pro Pro Gly Lys
50 55 60

Gly Leu Glu Trp Ile Gly Ser Phe Tyr Tyr Ser Gly Ile Thr Tyr Tyr
65 70 75 80

Ser Pro Ser Leu Lys Ser Arg Ile Ile Ile Ser Glu Asp Thr Ser Lys
85 90 95

Asn Gln Phe Ser Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala
100 105 110

Val Tyr Tyr Cys Ala Ser Gly Phe Thr Met Ile Arg Gly Ala Leu Asp
115 120 125

Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys
130 135 140

Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly
145 150 155 160

Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro
165 170 175

Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr
180 185 190

Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val
195 200 205

Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn
210 215 220

Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro
225 230 235 240

Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu
245 250 255

Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp
260 265 270

Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
275 280 285

Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly
290 295 300

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn
305 310 315 320

Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp
325 330 335

Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro
340 345 350

Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu
355 360 365

Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn
370 375 380

Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile
385 390 395 400

Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr
405 410 415

Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys
420 425 430

Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys
435 440 445

Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu
450 455 460

Ser Leu Ser Pro Gly Lys
465 470

<210> 32
<211> 236
<212> PRT
<213> Artificial

<220>
<223> Synthetic

<400> 32

Ala Ala Thr Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr
1 5 10 15

Ala Thr Gly Val His Ser Glu Ile Val Leu Thr Gln Ser Pro Ala Thr
20 25 30

Leu Ser Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser
35 40 45

Gln Ser Val Arg Ser Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln
50 55 60

Ala Pro Arg Leu Leu Ile Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile
65 70 75 80

Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
85 90 95

Ile Ser Ser Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln
100 105 110

Arg Ser Asn Trp Pro Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile
115 120 125

Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp
130 135 140

Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn
145 150 155 160

Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu
165 170 175

Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp
180 185 190

Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr
195 200 205

Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser

210 215 220

Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225 230 235

<210> 33
<211> 121
<212> PRT
<213> Homo sapiens

<400> 33

Gln Leu Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
1 5 10 15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Gly Ser Ile Ser Ser Ser
20 25 30

Ser Tyr Ser Trp Gly Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu
35 40 45

Trp Ile Gly Ser Phe Tyr Tyr Ser Gly Ile Thr Tyr Tyr Ser Pro Ser
50 55 60

Leu Lys Ser Arg Ile Ile Ile Ser Glu Asp Thr Ser Lys Asn Gln Phe
65 70 75 80

Ser Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr
85 90 95

Cys Ala Ser Gly Phe Thr Met Ile Arg Gly Ala Leu Asp Tyr Trp Gly
100 105 110

Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 34
<211> 363
<212> DNA
<213> Homo sapiens

<400> 34
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acctgcactg tctctggtgg ctccatcagc agtagtagtt actcctgggg ctggatccgc 120

cagccccag ggaagggcct ggagtggatt gggagtttct attacagtgg gatcacctac 180

tacagcccgt ccctcaagag tcgaattatc atatccgaag acacgtccaa gaaccagttc 240

tccctgaagc tgagttctgt gaccgccgca gacacggctg tgtattactg tgcgagcggg 300

tttactatga ttcggggagc ccttgactac tggggccagg gaaccctggt caccgtctcc 360

tca 363

<210> 35

<211> 107
<212> PRT
<213> Homo sapiens

<400> 35

Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Arg Ser Phe
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
35 40 45

Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
65 70 75 80

Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Arg Ser Asn Trp Pro Pro
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 36
<211> 321
<212> DNA
<213> Homo sapiens

<400> 36
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ctctcctgca gggccagtca gagtgttaga agtttcttag cctggtacca acagaaacct 120

ggccaggctc ccaggctcct catctatgat gcatccaaca gggccactgg catcccagcc 180

aggttcagtg gcagtgggtc tgggacagac ttcactctca ccatcagcag cctagagcct 240

gaagattttg cagtttatta ctgtcagcag cgtagcaact ggcctccgac gttcggccaa 300

gggaccaagg tggaaatcaa a 321

<210> 37
<211> 451
<212> PRT
<213> Artificial

<220>
<223> Synthetic

<400> 37

Gln Leu Gln Val Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
1 5 10 15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Gly Ser Ile Ser Asn Ser

20 25 30

Tyr Phe Ser Trp Gly Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu
35 40 45

Trp Ile Gly Ser Phe Tyr Tyr Gly Glu Lys Thr Tyr Tyr Asn Pro Ser
50 55 60

Leu Lys Ser Arg Ala Thr Ile Ser Ile Asp Thr Ser Lys Ser Gln Phe
65 70 75 80

Ser Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr
85 90 95

Cys Pro Arg Gly Pro Thr Met Ile Arg Gly Val Ile Asp Ser Trp Gly
100 105 110

Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
115 120 125

Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala
130 135 140

Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
145 150 155 160

Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
165 170 175

Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
180 185 190

Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His
195 200 205

Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys
210 215 220

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
225 230 235 240

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
245 250 255

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
260 265 270

Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
275 280 285

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr

290 295 300

Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
305 310 315 320

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
325 330 335

Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
340 345 350

Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser
355 360 365

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
370 375 380

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
385 390 395 400

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
405 410 415

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
420 425 430

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
435 440 445

Pro Gly Lys
450

<210> 38
<211> 1413
<212> DNA
<213> Artificial

<220>
<223> Synthetic

<400> 38
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ctgcaggtgc aggagtcggg cccaggactg gtgaagcctt cggagaccct gtccctcacc 120

tgcactgtct ctggtggctc catcagcaac agttatttct cctggggctg gatccgccag 180

cccccaggga agggactgga gtggattggg agtttctatt atggtgaaaa aacctactac 240

aacccgtccc tcaagagccg agccaccata tccattgaca cgtccaagag ccagttctcc 300

ctgaagctga gctctgtgac cgccgcagac acggctgtgt attactgtcc gagagggcct 360

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<210> 39
<211> 4702
<212> DNA
<213> Mus musculus

<400> 39
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gaatacacca ccagcagtcc cgacctgttg ttggtcatta tccaagtgac gggtgtcagc 840

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gtctagtaga tcaaataaat cc 4702

<210> 40
<211> 4704
<212> DNA
<213> Homo sapiens

<400> 40

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Leu Pro Gln Leu Cys Lys Phe Cys Asp Val Arg Leu Ser Thr Cys Asp
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Asn Gln Lys Ser Cys Met Ser Asn Cys Ser Ile Thr Ala Ile Cys Glu
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Lys Pro His Glu Val Cys Val Ala Val Trp Arg Lys Asn Asp Lys Asn
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