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(54) Title: BINDING COMPOSITIONS; RELATED REAGENTS

(57) Abstract: Isolated and/or recombinant binding compositions, reagents related thereto are provided. Methods of using said reagents and diagnostic kits are also provided.

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BINDING COMPOSITIONS; RELATED REAGENTS

FIELD OF THE INVENTION

The present invention generally relates to compositions related to TGF Beta proteins. In particular, it provides purified binding compositions, and related reagents useful, e.g., in the diagnosis, treatment, and prevention of cell proliferative, autoimmune/inflammatory, cardiovascular, neurological, and developmental disorders, and in the assessment of the effects of exogenous compounds on the expression of nucleic acid and amino acid sequences of such proteins.

BACKGROUND OF THE INVENTION

The first member of the transforming growth factor–beta (TGF-Beta) superfamily of secreted polypeptide factors, TGF-Beta 1, was discovered approximately twenty years ago. Since then, the family has grown considerably and now comprises over thirty vertebrate members and a dozen or so structurally and functionally related proteins in invertebrates such as worms and flies (see, e.g., Attisano & Lee-Hoeflich, 2001 Gen. Biol. 2, review 3010.1; Moustakas, et al., 2001 J. Cell Sci. 114:4359; Wrana, 2000 Cell 100:189; Attisano & Wrana, TGF-beta Pathway, Science's STKE (Connections Map, as seen March 2002), http:// stke.sciencemag.org/cgi/cm/CMP_9876). The TGF Beta family members control many cellular functions, and their activity is critical for regulating numerous developmental and homeostatic processes. Mutations in TGF-Beta family ligands are responsible for a number of human diseases, including, e.g., hereditary chondrodysplasia and persistent mullerian duct syndrome (Massague, et al., 2000 Cell 103: 295). In addition, TGF-Beta itself plays an important role in cancer progression by functioning both as an antiproliferative factor and as a tumor promoter, and numerous components of the signal transduction pathway are tumor suppressors that are functionally mutated in cancer (de Caestecker, et al., 2000 J. Natl. Cancer Inst. 92:1388). Work in vertebrates, worms, and flies have revealed a conserved TGF-Beta signaling pathway.

The specific member TGF Beta 1 is known to be involved in many cellular processes such as cell proliferation and differentiation, migration, differentiation, apoptosis, embryonic development, extracellular matrix formation, bone development, wound healing, hematopoiesis, and immune and inflammatory responses (see, e.g., Roberts, A. B., and Sporn, M. B. (1990) Peptide Growth Factors and Their Receptors, pp. 419-472, Springer-Verlag, Heidelberg, Germany; Massagué, J. (1990) Annu. Rev. Cell Biol. 6, 597-641).

As one of the most powerful and widely distributed pro-fibrogenic mediators, transforming growth factor Beta1 (TGF-Beta1), also plays a predominant role during fibrogenesis. In liver, for example, TGF-Beta1 transforms hepatic stellate cells (HSCs) into myofibroblasts, which are the main source of extracellular matrix proteins and particularly of procollagen I and III (see, e.g., Williams & Knapton (1996) Hepatology 23:1268-1275; Border & Noble (1994) N Engl J Med 1:1286-1292; Friedmann SL. (1999) Semin Liver Dis ;19:129-140; Roberts, et al. (1986) Proc Natl Acad Sci USA 83:4167-4171; Gebhardt R. (2002) Planta Med 68:289-296). The accumulation of excessive extra-cellular matrix is associated with various fibrotic diseases. Thus, there is a need to control agents such as TGF Beta 1 to prevent its deleterious effects in such disease states.

The discovery of new TGF Beta 1 binding compositions satisfies a need in the art by providing compositions that are useful in the diagnosis, prevention, and treatment of cell proliferative, immune/inflammatory, cardiovascular, neurological, fibrotic, and developmental disorders.

SUMMARY OF THE INVENTION

The present invention is based in part upon the discovery of binding compositions specific and/or selective to TGF Beta 1. The invention provides an isolated, and/or recombinant binding composition which specifically and/or selectively binds TGF Beta 1 isoform over TGF Beta 2 and/or TGF Beta 3 and which neutralizes TGF Beta 1 comprising at least one binding site comprising: at least four contiguous amino acids from QQWNGNPPA (SEQ ID NO: 24)(VL CDR3); at least four contiguous amino acids from QQWDSNPPA (SEQ ID NO: 27)(VL CDR3); at least five contiguous amino acids from YIYPYNGDTGYNQKFKS (SEQ ID NO: 14), wherein one of said at least five contiguous amino acids is D (VH CDR2); or at least five contiguous amino acids from GYYWFAY (SEQ ID NO: 15) (VH CDR3); or an isolated and/or recombinant binding composition which specifically and/or selectively binds human TGF Beta 1 isoform over TGF Beta 2 and/or TGF Beta 3 and which neutralizes TGF Beta 1 comprising at least one antibody binding site comprising: at least six contiguous amino acids from LQYASSPYT (SEQ ID NO: 30)(VL CDR3); at least five contiguous amino acids from GYTFTDYTMH (SEQ ID NO: 19)(VH CDR1); at least eight contiguous amino acids from LITPFYGDAIYNQKFKG (SEQ ID NO: 20)(VH CDR2); or at least seven contiguous amino acids from GGLRRGPPFAY (SEQ ID NO: 21)(VH CDR3). In other embodiments, the binding composition further comprises an additional binding site selected from the following: RASSSVSYMH (SEQ ID NO: 22)(1021 VL CDR1); ATSNLAS (SEQ ID NO: 23)(1021 VL CDR2); GYTFTDYNMH (SEQ ID NO: 13)(1021 VH CDR1); RASQEISGYLS (SEQ ID NO: 28)(3821 VL CDR1); ATSSLDS (SEQ ID NO: 29)(3821 VL CDR2); and GYTFTDYTMH (SEQ ID NO: 19)(3821 VH CDR1); at least three said binding sites; at least four said binding sites; at least five said binding sites; at least six said binding sites; at least seven said binding sites; at least eight said binding sites; at least nine said binding sites; at least ten said binding sites; at least eleven said binding sites; at least twelve said binding sites; at least thirteen said binding sites; at least fourteen said binding sites; at least fifteen said binding sites; at least sixteen said binding sites; at least seventeen said binding sites; or at least eighteen said binding sites; wherein the binding site of the binding composition: is specifically immunoreactive with a polypeptide of human TGF Beta-1 is specifically immunoreactive with a polypeptide of

murine TGF Beta-1 is raised against a purified or recombinantly produced human TGF Beta-1 protein or fragment thereof; is in a monoclonal antibody, Fab, Fv, scFv, F(ab)2, or a variable domain of an antibody; has at least one, two, or three conservative substitutions; or is in a human or a humanized antibody framework; wherein the binding composition: is an antibody molecule; is a monoclonal antibody molecule; is a diabody molecule; is a triabody molecule; is a tetrabody molecule; is a minibody molecule; is a monoclonal antiserum; is detectably labeled; is lyophilized; is sterile; or is in a buffered composition; wherein the binding composition: is a monoclonal antibody, wherein said monoclonal antibody comprises at least one sequence at a CDR1, CDR2, or CDR3 of the light chain variable region (LCVR); or at a CDR1, CDR2, or CDR3 of the heavy chain variable region (HCVR) selected from: GYTFTDYNMH (SEQ ID NO: 13)(1021 VH CDR1); RASSSVSYMH (SEQ ID NO: 22)(1021 VL CDR1); YIYPYNGDTGYNQKFKS (SEQ ID NO: 14)(1021 VH CDR2); ATSNLAS (SEQ ID NO: 23) (1021 VL CDR2); GYYWFAY (SEQ ID NO: 15) (1021 VH CDR3); QQWNGNPPA (SEQ ID NO: 24)(1021 VL CDR3); or QQWDSNPPA (SEQ ID NO: 27)(2471 VL CDR3); wherein said CDR2 of the light chain variable region (LCVR) is ATSNLAS (SEQ ID NO: 23) (1021 VL CDR2); wherein said CDR2 of the heavy chain variable region (HCVR) is YIYPYNGDTGYNQKFKS (SEQ ID NO: 14)(1021 VH CDR2); wherein said CDR1 of the light chain variable region (LCVR) is RASSSVSYMH (SEQ ID NO: 22)(1021 VL CDR1); wherein said CDR1 of the heavy chain variable region (HCVR) is GYTFTDYNMH (SEQ ID NO: 13)(1021 VH CDR1); wherein said CDR3 of the light chain variable region (LCVR) is QQWNGNPPA (SEQ ID NO: 24)(1021 VL CDR3); wherein said CDR3 of the heavy chain variable region (HCVR) is GYYWFAY (SEQ ID NO: 15) (1021 VH CDR3); wherein said light chain variable region comprises SEQ ID NO: X (QIVLTQSPAIMSASPGEKVTMTCRASSSVSYMHWYQQKPGSSPKPWIYATSNLA SGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWNGNPPAFGGGTKLEIKRA); wherein a monoclonal antibody of the invention is: is humanized; comprises of human or humanized constant regions; is a Fab fragment; is an Fv fragment; is a scFv fragment; is a F(ab)2 fragment; is detectably labeled; is lyophilized; is encoded in an isolated nucleic acid molecule; is encoded in an isolated nucleic acid molecule that is operably linked in an expression vector; is encoded in an isolated nucleic acid molecule that is operably

linked in an expression vector that is incorporated into a host cell; is a chimeric antibody; is conjugated to another chemical moiety; is sterile; or is a pharmaceutical composition; wherein the CDR1, CDR2, and CDR3 of the heavy variable region and the CDR1, CDR2, and CDR3 the light chain variable region are embedded within a human or humanized framework. A binding composition of the invention that is a monoclonal antibody that is: a Fab fragment; is an Fv fragment; is an scFv fragment; is a F(ab)2 fragment; is fused to a human constant region; is conjugated to another chemical moiety; comprises a heavy chain constant region selected from: IgG1, IgG2, IgG3, IgG4, IgA, IgE, IgM, and IgD; comprises a human light chain constant region; is detectably labeled; is lyophilized; is a fusion protein; is sterile; or comprises a pharmaceutical composition.

A binding composition of the invention that is a monoclonal antibody that comprises at least one sequence at a CDR1, CDR2, or CDR3 of the light chain variable region (LCVR); or at a CDR1, CDR2, or CDR3 of the heavy chain variable region (HCVR) selected from: RASQEISGYLS (SEQ ID NO: 28)(3821 VL CDR1), ATSSLDS (SEQ ID NO: 29)(3821 VL CDR2), LQYASSPYT (SEQ ID NO: 30)(3821 VL CDR3), GYTFTDYTMH (SEQ ID NO: 19)(3821 VH CDR1), LITPFYGDAIYNQKFKG (SEQ ID NO: 20)(3821 VH CDR2), and GGLRRGPPFAY (SEQ ID NO: 21)(3821 VH CDR3); wherein said CDR2 of the light chain variable region (LCVR) is ATSSLDS (SEQ ID NO: 29) (3821 VL CDR2); wherein said CDR2 of the heavy chain variable region (HCVR) is LITPFYGDAIYNQKFKG (SEQ ID NO: 20)(3821 VH CDR2); wherein said CDR1 of the light chain variable region (LCVR) is RASQEISGYLS (SEQ ID NO: 28) (3821 VL CDR1); wherein said CDR1 of the heavy chain variable region (HCVR) is GYTFTDYTMH (SEQ ID NO: 19)(3821 VH CDR1); wherein said CDR3 of the light chain variable region (LCVR) is LQYASSPYT (SEQ ID NO: 30)(3821 VL CDR3); wherein said CDR3 of the heavy chain variable region (HCVR) is GGLRRGPPFAY (SEQ ID NO: 21)(3821 VH CDR3); wherein said light chain variable region comprises residues 21-129 of SEQ ID NO: 10.

(DVQITQSPSSLSASLGERVSLTCRASQEISGYLSWLQQKPDGTIKRLIYATSSLDSGVPKRFSGSRSG SDYSLTISSPESEDFVDYYCLQYASSPYTFGGGTKLEIKRA); wherein said heavy chain variable region comprises residues 29-140 of SEQ ID NO: 12

(AALMRPGVSVKISCKGSGYTFTDYTMHWVKQSHAKSLEWIGLITPFYGDAIYNQKFKGKATMTV DKSSSTAYMELARLTSDDSAIYYCTRGGLRRGPPFAYWGQGTLVTVSA); that is: humanized,

fused to a human constant region, a Fab fragment, an Fv fragment, a scFv fragment, a F(ab)2 fragment, detectably labeled, lyophilized, a chimeric antibody, conjugated to another chemical moiety, sterile, a pharmaceutical composition, or used in the manufacture of a medicament for administration to a mammal for the treatment of a fibrotic condition; wherein said CDR1, CDR2, and CDR3 of the heavy variable region and the CDR1, CDR2, and CDR3 the light chain variable region are linked within a human or humanized framework; wherein the binding composition is a monoclonal antibody that: is a Fab fragment, is an Fv fragment, is an scFv fragment, is a F(ab)2 fragment, is fused to a human constant region, is conjugated to another chemical moiety, comprises a heavy chain constant region selected from: IgG1, IgG2, IgG3, IgG4, IgA, IgE, IgM, and IgD; comprises a human light chain constant region, is detectably labeled, is lyophilized, is a fusion protein, is sterile, or comprises a pharmaceutical composition.

Other kit embodiments include a kit comprising a described binding composition, and: a compartment comprising a binding composition of the invention; and/or instructions for use or disposal of reagents in the kit. In many versions, the kit is capable of making a qualitative or quantitative analysis.

Other embodiments include: a method of using the binding composition of the invention, comprising contacting said binding composition with a biological sample comprising an antigen, thereby forming a TGF Beta 1 binding composition:antigen complex; wherein said biological sample is from a human, and wherein said binding composition is an antibody.

In other embodiments, the invention provides a method of modulating the physiology or development of a mammal comprising administering a binding composition of the invention.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

I. General

It is to be understood that this invention is not limited to the particular compositions, methods, and techniques described herein, as such compositions, methods, and techniques may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which is only limited by the appended claims.

As used herein, including the appended claims, singular forms of words such as "a," "an," and "the" include, e.g., their corresponding plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "an organism" includes, e.g., one or more different organisms, reference to "a cell" includes, e.g., one or more of such cells, and reference to "a method" include, e.g., reference to equivalent steps and methods known to a person of ordinary skill in the art, and so forth.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by a person of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used to practice or test the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references discussed herein are provided solely for their disclosure before the filing date of the present application. Nothing herein is to be construed as an admission that the invention is not entitled to antedate any such disclosure by virtue of its prior invention. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety for the teachings for which they are cited (as the context clearly dictates), including all figures, drawings, pictures, graphs, hyperlinks, and other form of browser-executable code.

II. Definitions

A "**binding composition**" is a molecular entity with selective and/or specific binding affinity for at least one other molecular entity or binding partner. Typically, the association will be in a naturally physiologically relevant interaction, either covalently or non-covalent, and may include members of a multi-protein complex, including, without

limitation, carrier compounds, dimerization or multimerization partners. A binding composition can be naturally derived (e.g., isolated and/or purified) or synthetically produced, either in whole or in part. Typically, a binding composition has at least one region such as, by way of non-limiting example, a surface area, a shape (such as, e.g., a cavity, cleft, crevice, or protrusion), a molecular arrangement, or a spatial configuration, that specifically and/or selectively "fits with," "binds to," or is "complementary with" a particular spatial and/or polar organization of an area or region on a binding partner. Thus, for example, when a binding composition is sufficiently proximate to a potential binding partner, the binding composition and partner will specifically and/or selectively bind each other. Non-limiting examples of a binding composition paired with a binding partner include: antigen-antibody, hapten-binding site, biotin-avidin, hormone-hormone receptor, receptor-ligand, and enzyme-substrate. Non-limiting examples of antibody binding compositions include: antibodies, diabodies, triabodies, tetrabodies, minibodies, Fab fragments (including, such as, e.g., dimeric or trimeric Fabs), Fv fragments, scFv fragments, F(ab)2 fragments, etc. (see, e.g., Hudson & Souriau 2003 Nature Medicine 9:129-34 for non-limiting examples of antibody binding compositions encompassed by the invention).

A "**binding site**" is a specific region, area, or configuration of a molecular entity that takes part in the specific and/or selective binding with another molecular entity. A non-limiting example of a binding site is the contiguous amino acid sequence comprising a complementary determining region (CDR) of an antibody. Another non-limiting example is a binding site formed from the three-dimensional configuration and spatial organization of the amino acid sequences comprising the six CDR loops of the light and heavy variable chains at the rim of the eight-stranded beta barrel of a Fab fragment (see, e.g., Chothia & Lesk, 1987 *J. Mol. Biol.* 196:901-17).

Binding Composotion:TGF Beta 1Complex

The term "**binding composition: TGF Beta 1 complex**", as used herein, refers to a complex of a binding composition and a TGF Beta 1 protein that is formed by specific and/or selective binding of the binding composition to the TGF Beta 1 protein. In a preferred embodiment, the TGF Beta 1 referred to throughout is a primate TGF Beta 1 protein. In a more preferred embodiment, the TGF Beta 1 referred to throughout is a human TGF Beta 1 protein. Specific binding of the binding composition means that the

binding composition has a binding site that recognizes a region of the TGF Beta 1 protein, typically in its native active conformation, but possibly in a denatured conformation, e.g., a Western blot. For example, antibodies raised to a TGF Beta 1 protein and recognizing an epitope of the TGF Beta 1 protein are capable of forming a binding composition:TGF Beta 1 complex by specific binding. Typically, the formation of a binding composition:TGF Beta 1 protein complex allows the measurement of TGF Beta 1 protein in a biological sample, e.g., a mixture with other proteins and biologics.

The term "**specific binding**" as used herein refers to the situation in which one member of a specific binding pair will not show any significant binding to molecules other than its specific binding partner(s). The term is also applicable where e.g. an antigen-binding domain is specific for a particular epitope which is carried by a number of antigens, in which case the specific binding member carrying the antigen-binding domain will be able to bind to the various antigens carrying the epitope. Accordingly, a binding composition specific for TGF Beta 1 "will not show any significant binding to molecules other than its specific binding partner(s)" that is, TGF Beta 1.

The phrases "**specifically binds**" or "**specifically immunoreactive with**", with respect to a binding composition of the invention, refers to a binding reaction that is determinative of the presence of the binding composition in the presence of a heterogeneous population of proteins and other biological components. Thus, under designated immunoassay conditions, the specified binding composition binds a specific protein and does not significantly bind other proteins present in the sample. Specific binding to a binding composition under such conditions may require a binding composition that is selected for its specificity for a particular protein. For example, a binding composition, such as an antibody, raised to the human TGF Beta 1 amino acid sequence depicted in SEQ ID NO:X can be selected to obtain antibodies specifically immunoreactive with that TGF Beta 1 protein and not with other proteins. Such a binding composition could differentiate proteins highly homologous to the human TGF Beta 1 protein, e.g., such as, for example, the other human TGF Beta isoforms (TGF Beta 2, or TGF Beta 3).

A binding composition of the invention preferably neutralizes TGF Beta 1 and has a dissociation constant (Kd) for TGF Beta 1 preferably of less than about: 100nM, 95nM, 90nM, 85nM, 80nM, 75nM, 70nM, 65nM, 60nM, 55nM, 50nM, 45nM, 40nM, 35nM,

30nM, 25nM, 20nM, 15nM, 14nM, 13nM, 12nM, 11nM, or 10nM; more preferably less than about: 10nM, 9.9nM, 9.8nM, 9.7nM, 9.6nM, 9.5nM, 9.4nM, 9.3nM, 9.2nM, 9.1nM 9.0nM, 8.9nM, 8.8nM, 8.7nM, 8.6nM, 8.5nM, 8.4nM, 8.3nM, 8.2nM, 8.1nM 8.0nM, 7.9nM, 7.8nM, 7.7nM, 7.6nM, 7.5nM, 7.4nM, 7.3nM, 7.2nM, 7.1nM 7.0nM, 6.9nM, 6.8nM, 6.7nM, 6.6nM, 6.5nM, 6.4nM, 6.3nM, 6.2nM, 6.1nM, 6.0nM, 5.9nM, 5.8nM, 5.7nM, 5.6nM, 5.5nM, 5.4nM, 5.3nM, 5.2nM, 5.1nM or 5.0nM; even more preferably of less than about 5.0nM, 4.9nM, 4.8nM, 4.7nM, 4.6nM, 4.5nM, 4.4nM, 4.3nM, 4.2nM, 4.1nM 4.0nM, 3.9nM, 3.8nM, 3.7nM, 3.6nM, 3.5nM, 3.4nM, 3.3nM, 3.2nM, 3.1nM 3.0nM 2.9nM, 2.8nM, 2.7nM, 2.6nM, 2.5nM, 2.4nM, 2.3nM, 2.2nM, 2.1nM 2.0nM, 1.9nM, 1.8nM, 1.7nM, 1.6nM, 1.5nM, 1.4nM, 1.3nM, 1.2nM, 1.1nM, 1.0nM, 0.9nM, 0.8nM, 0.7nM, 0.6nM, 0.55nM, 0.5nM, 0.45nM, 0.4nM, 0.35nM, 0.3nM, 0.25nM, 0.2nM, 0.15nM, 0.1nM, 0.09nM, 0.08nM, 0.07nM, 0.06nM, 0.05nM, 0.04nM, 0.03nM,0.025nM, 0.02nM, 0.019nM, 0.018nM, 0.017nM, 0.016nM, 0.015nM, 0.014nM, 0.013nM, 0.012nM, 0.011nM, or 0.010nM. The affinity constant (Kd) of a binding composition of the invention can be determined using any art method, for example, by BIACore™, adapting the method of Karlsson et al., 1991 J. Immunol. Methods 145, 299-240.

An antibody binding composition preferably neutralizes TGF Beta 1 and has a dissociation constant (Kd) for TGF Beta 1 of preferably less than about: 100nM, 95nM, 90nM, 85nM, 80nM, 75nM, 70nM, 65nM, 60nM, 55nM, 50nM, 45nM, 40nM, 35nM, 30nM, 25nM, 20nM, 15nM, 14nM, 13nM, 12nM, 11nM, or 10nM; more preferably less than about: 10nM, 9.9nM, 9.8nM, 9.7nM, 9.6nM, 9.5nM, 9.4nM, 9.3nM, 9.2nM, 9.1nM 9.0nM, 8.9nM, 8.8nM, 8.7nM, 8.6nM, 8.5nM, 8.4nM, 8.3nM, 8.2nM, 8.1nM 8.0nM, 7.9nM, 7.8nM, 7.7nM, 7.6nM, 7.5nM, 7.4nM, 7.3nM, 7.2nM, 7.1nM 7.0nM, 6.9nM, 6.8nM, 6.7nM, 6.6nM, 6.5nM, 6.4nM, 6.3nM, 6.2nM, 6.1nM, 6.0nM, 5.9nM, 5.8nM, 5.7nM, 5.6nM, 5.5nM, 5.4nM, 5.3nM, 5.2nM, 5.1nM or 5.0nM; even more preferably of less than about 5.0nM, 4.9nM, 4.8nM, 4.7nM, 4.6nM, 4.5nM, 4.4nM, 4.3nM, 4.2nM, 4.1nM 4.0nM, 3.9nM, 3.8nM, 3.7nM, 3.6nM, 3.5nM, 3.4nM, 3.3nM, 3.2nM, 3.1nM 3.0nM 2.9nM, 2.8nM, 2.7nM, 2.6nM, 2.5nM, 2.4nM, 2.3nM, 2.2nM, 2.1nM 2.0nM, 1.9nM, 1.8nM, 1.7nM, 1.6nM, 1.5nM, 1.4nM, 1.3nM, 1.2nM, 1.1nM, 1.0nM, 0.9nM, 0.8nM, 0.7nM, 0.6nM, 0.55nM, 0.5nM, 0.45nM, 0.4nM, 0.35nM, 0.3nM, 0.25nM, 0.2nM, 0.15nM, 0.1nM, 0.09nM, 0.08nM, 0.07nM, 0.06nM, 0.05nM, 0.04nM,

0.03nM,0.025nM, 0.02nM, 0.019nM, 0.018nM, 0.017nM, 0.016nM, 0.015nM, 0.014nM, 0.013nM, 0.012nM, 0.011nM, or 0.010nM. Preferably, an antibody binding composition specifically and/or selectively binds TGF Beta 1 as compared to TGF Beta 2 and/or TGF Beta 3, more preferably, an antibody binding composition specifically and/or selectively binds human TGF Beta 1 as compared to human TGF Beta 2 and/or human TGF Beta 3. Preferably, such an antibody has less than about 20% cross-reactivity with TGF Beta 2 and/or TGF Beta 3 (as measured by the ratio of the dissociation constants), more preferably less than about 15% cross-reactivity, and even more preferably has less than about 10% cross-reactivity. Further, an antibody binding composition preferably recognizes the active but not the latent form of TGF Beta 1, more preferably the active but not the latent form of human TGF Beta 1. A preferred property desired for such an antibody to be effective in the amelioration of fibrotic diseases are similar to those of a binding composition of the invention against TGF Beta 1 as described herein.

Neutralization

The term "**neutralize**" or "**antagonize**" with respect to a binding composition of the invention refers to the situation in which the specific and/or selective binding of a binding composition of the invention to another molecular entity results in the abrogation or inhibition of the biological effector function of the other molecular entity bound with the binding composition. With respect to TGF Beta 1, the term "neutralize" or "antagonize" in reference to a binding composition of the invention is intended to refer to a binding composition whose binding to or interaction with TGF Beta 1 results in inhibition of a biological activity induced by TGF Beta 1. Inhibition of TGF Beta 1 biological activity can be assessed by measuring one or more *in vitro* or *in vivo* indicators of TGF Beta 1 biological activity including, but not limited to, inhibition of receptor binding, inhibition of fibrosis, inhibition of chemotaxis, or inhibition of signal transduction in a TGF Beta 1 binding assay (see, e.g., EP 0 945 464 for non-limiting examples of encompassed neutralization assays). Indicators of TGF Beta 1 biological activity can be assessed by any known *in vitro* or *in vivo* assay known in the art. In a non-limiting embodiment, the ability of a binding composition of the invention to neutralize or antagonize TGF Beta 1 activity is assessed by use of the assay as described in Example 3 herein.

The neutralizing activity of a binding composition, such as, for example, an antibody described herein can be tested by any method known in the art. In one non-limiting example, testing can be carried out in a modification of an assay for TGF as described by Randall et al (1993) J. Immunol Methods 164, 61-67. This assay is based on the ability of TGF Beta 1 and TGF Beta 2 to inhibit the interleukin-5 (IL-5) induced proliferation of the erythroleukaemia cell line TF1 and on the ability to reverse the TGF Beta inhibition with TGF Beta specific binding compositions. The assay is reported to be rapid, reproducible and sensitive to less than 500 fg/ml of TGF-beta 1, and 5-10 pg/ml TGF-beta 2. The assay is also reported to be 100-1000-fold less sensitive to other inhibitory molecules such as interferon-beta, interferon-gamma and TNF-alpha. The assay is also reported to be capable of being made specific for TGF-beta 1 or TGF-beta 2 by including specific neutralizing antibodies for TGF-beta 1 or TGF-beta 2 and to recognize all the readily available recombinant molecular species of these molecules as well as the natural proteins produced from human and bovine platelets and to detect TGF-beta in serum samples.

Other assays, as reported herein or as known in the art, are also encompassed. For example, Ueberham, et al., (Hepatology 2003;37(5):1067-78) describe a tetracycline-regulated gene expression system in a double-transgenic mouse model of liver fibrosis where the expression of transforming growth factor beta1 (TGF-Beta 1) is regulated by the addition or removal of doxycycline hydrochloride to drinking water thus permitting switching TGF-Beta 1 expression on or off at will. Increasing TGF-Beta 1 expression in the liver of such animals leads to fibrotic disease states that are reversible by switching off TGF Beta 1 expression — even after liver mass has been reduced 59%. Use of this model permits one to evaluate the effects of binding compositions of the invention on inhibiting TGF Beta 1 biological functions by comparing a binding composition's effects with the effects of switching off TGF Beta 1 expression.

Antibodies

Antibody binding compositions of the invention include, e.g., without limitation, polyclonal, monoclonal, multispecific, human, humanized, or chimeric antibodies, single chain antibodies, Fab fragments, F(ab') fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies (including, e.g., anti-Id antibodies to antibodies of the invention), and an epitope-binding fragment of any of the above.

The term "**antibody**," as used herein refers to immunoglobulin compositions and immunologically active portions of immunoglobulin compositions, e.g., a binding composition molecule that contains a binding site that specifically and/or selectively binds an antigen. An immunoglobulin composition of the invention can be of any type (e.g., IgG, IgE, IgM, IgD, IgA, and IgY), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2) or a subclass of an immunoglobulin molecule. Preferably an antibody is a human antigen-binding antibody fragment of the present invention such as, e.g., without limitation, Fab, Fab' and F(ab')2, Fd, 6 single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv), and fragments comprising either a VL or VH domain. Antigen-binding antibody fragments, including single-chain antibodies, may comprise the variable region(s) alone or in combination with the entirety or a portion of the following: a hinge region, a CH1, a CH2, or a CH3 domain or combinations thereof. Also included in the invention is, e.g., without limitation, an antigen-binding fragment that also can comprise any combination of variable region(s) with a hinge region, e.g., such as a CH1, CH2, or a CH3 domain or combinations thereof. An antibody of the invention may be from any animal origin including birds and mammals. Preferably, the antibodies are human, murine (e.g., mouse and rat), donkey, rabbit, goat, guinea pig, camel, horse, or chicken.

As used herein, the phrase "**human antibodies**" includes, e.g., without limitation, antibodies having an amino acid sequence of a human immunoglobulin including, e.g., without limitation, an antibody isolated from a human immunoglobulin library or from an animal transgenic for one or more human immunoglobulins and that do not express endogenous immunoglobulins, as described herein or, as taught, e.g., in U.S. Patent No. 5,939,598.

A binding composition may be monospecific, bispecific, trispecific or of greater multispecificity. Multispecific antibodies may be specific for different epitopes of a target protein, polypeptide (or fragment thereof) or may be specific for both a TGF Beta 1 as well as for a heterologous epitope, such as a heterologous TGF Beta isoform or solid support material (see, e.g., WO 2093/17715; WO 92/08802; WO 91/00360; WO 92/05793; Tutt, et al. (1991) J. Immunol. 147:60-69; U.S. Patent Nos. 4,474,893; 4,714,681; 4,925,648; 5,573,920; or 5,601,819; or Kostelny, et al. (1992) J. Immunol. 148:1547-1553 .

A binding composition may be described or specified in terms of an epitope(s) or portion(s) of a TGF Beta 1 protein (or fragment thereof) that it recognizes or selectively and/or specifically binds. An epitope(s) or polypeptide portion(s) may be specified as described herein, e.g., by N-terminal and C-terminal positions, by size in contiguous amino acid residues, or as listed in an accompanying Table and/or Figure as described herein. Additionally, an antibody that specifically binds an epitope, polypeptide, protein, or fragment of a polypeptide or protein, may also be specifically excluded from this invention. For instance, Applicants reserve the right to proviso out any antibody that specifically binds an epitope, polypeptide, protein, or fragment of a polypeptide or protein. Accordingly, the present invention can encompass a first (or other) antibody that specifically binds a polypeptide or protein, or fragment thereof, and, at the same time, it can exclude a second (or other) antibody that may also selectively bind the same protein or polypeptide, or fragment thereof, e.g., by binding a different epitope.

Antibodies of the present invention may also be described or specified in terms of their cross-reactivity. Antibodies that do not bind any other analog, ortholog, paralog, or homolog of a target protein, polypeptide (or fragment thereof) are included.

Further encompassed by the present invention is an antibody that selectively binds a polypeptide, which is encoded by a polynucleotide that stably hybridizes, under stringent hybridization conditions (as described herein), to a TGF Beta polynucleotide sequence.

A binding composition may also be characterized or specified in terms of its binding affinity to a protein or polypeptide (fragment thereof), or epitope. In one embodiment, a preferred binding affinity of a binding composition, e.g., an antibody or antibody binding fragment, includes, e.g., a binding affinity that demonstrates a dissociation constant or Kd of less than about: 5 X 10^{-2}M, 10^{-2}M, 5 X 10^{-3}M, 10^{-3}M, 5 X 10^{-4}M, 10^{-4}M, 5 X 10^{-5}M, 10^{-5}M, 5 X 10^{-6}M, 10^{-6}M, 5 X 10^{-7}M, 10^{-7}M, 5 X 10^{-8}M, 10^{-8}M, 5 X 10^{-9}M, 10^{-9}M, 5 X 10^{-10}M, 10^{-10}M, 5 X 10^{-11}M, 10^{-11}M, 5 X 10^{-12}M, 10^{-12}M, 5 X 10^{-13}M, 10^{-13}M, 5 X 10^{-14}M, 10^{-14}M, 5 X 10^{-15}M, or 10^{-15}M.

The invention also encompasses antibodies that competitively inhibit binding of a binding composition to an epitope of a TGF Beta 1 as determined by any known art method for determining competitive binding, e.g., the immunoassays described herein. In preferred embodiments, the antibody competitively inhibits binding to the epitope by at

least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 60%, or at least 50%.

Antibodies of the present invention may act as antagonists of TGF Beta 1 (or fragment thereof). For example, an antibody or binding composition of present invention can disrupt, e.g., an interaction, either partially or completely, of TGF Beta 1 with its cognate receptor/ligand. Preferably, antibodies of the present invention bind an antigenic epitope of TGF Beta 1, or a portion thereof.

Likewise encompassed by the invention, are antibodies that bind a ligand and prevent it binding to a receptor. Similarly encompassed are ligand-binding antibodies that inhibit receptor activation without inhibiting receptor binding. Alternatively, ligand-binding antibodies that activate a receptor are also included.

Antibodies of the present invention may be used, e.g., without limitation, to purify, detect, or target a TGF Beta 1 (or fragment thereof) for, e.g., *in vitro* and/or *in vivo* diagnostic and therapeutic methods. For example, the antibodies have use in immunoassays for qualitatively and/or quantitatively measuring levels of TGF Beta 1 (or fragment thereof) of the present invention in a biological sample (see, e.g., Harlow, et al., Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, cur. ed.).

As discussed in more detail herein, a binding composition of the present invention may be used either alone or in combination with other compositions. Furthermore, a binding composition such as an antibody may be recombinantly fused to a heterologous polypeptide at the N- or C-terminus, or chemically conjugated (including covalently and non-covalently conjugations) to a polypeptide or other compositions. For example, antibodies of the present invention may be recombinantly fused or conjugated to molecules useful as labels in detection assays and effector molecules such as heterologous polypeptides, drugs, radionuclides, or toxins (see, e.g., WO 92/08495; WO 91/14438; WO 89/12624; U.S. Patent No. 5,314,995; and EP 396,387).

A binding composition of the invention includes, e.g., derivatives that are modified, e.g., by the covalent attachment of any type of molecule to, e.g., an antibody such that the covalent attachment does not prevent the antibody from generating an anti-idiotypic response. For example, but not by way of limitation, an antibody derivative includes, e.g., antibodies that have been modified, e.g., by glycosylation, acetylation,

pegylation, phosphylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc.

Any of numerous chemical modifications may be carried out by known techniques, including, e.g., but not limited to, specific chemical cleavage, acetylation, formulation, metabolic synthesis of tunicamycin, etc. Additionally, a derivative may contain one or more non-classical amino acids. A binding composition may be generated by any suitable known art method.

Various adjuvants may be used to increase an immunological response depending on the host species, these include, e.g., without limitation, Freund's (complete and incomplete), mineral gels such as e.g., aluminum hydroxide, surface active substances such as e.g., lysolecithin, plutonic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as, e.g., BCG (bacille Calmette-Guerin) and corynebacterium parvum. Such adjuvants are known in the art.

Monoclonal antibodies can be prepared using a variety of art known techniques including, e.g., the use of hybridoma, recombinant, and phage display technologies, or combinations thereof. For example, monoclonal antibodies can be produced using hybridoma techniques including those known in the art and taught, e.g., in Harlow, et al., Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, current edition); Hammerling, et al., in: Monoclonal Antibodies and T-Cell Hybridomas 563-68 1 (Elsevier, N.Y., 1981).

The term "**monoclonal antibody**" as used herein is not limited to antibodies produced through hybridoma technology. The term "monoclonal antibody" refers to an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced.

Methods for producing and screening for specific antibodies using hybridoma technology are routine and known in the art and are discussed herein. In a non-limiting example, mice are immunized with a polypeptide of the invention or a cell expressing such a polypeptide. Once an immune response is detected, e.g., antibodies specific for the antigen are detected in the mouse serum, the mouse spleen is harvested, and splenocytes isolated. The splenocytes are then fused by known techniques to any suitable myeloma cells; e.g., SP20 cells (available from the ATCC).

Hybridomas are then selected and cloned by limited dilution. The hybridoma clones are then assayed by art known methods to discover cells that secrete antibodies that bind a binding composition polypeptide (or fragment thereof). Ascites fluid, which generally contains high levels of antibodies, can be generated by immunizing mice with positive hybridoma clones.

Accordingly, the present invention provides methods of generating monoclonal antibodies as well as antibodies produced by the method comprising culturing a hybridoma cell secreting an antibody of the invention wherein, preferably, the hybridoma is generated by fusing splenocytes isolated from a mouse immunized with an antigen of the invention with myeloma cells and then screening the hybridomas resulting from the fusion for hybridoma clones that secrete an antibody able to bind a binding composition polypeptide.

Antibody fragments that recognize specific epitopes may be generated by known techniques. For example, Fab and F(ab')2 fragments of the invention may be produced by proteolytic cleavage of immunoglobulin molecules, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')2 fragments). F(ab')2 fragments contain the variable region, the light chain constant region and the CH1 domain of the heavy chain. For example, a binding composition can also be generated using various phage display methods known in the art in which functional antibody domains are displayed on the surface of phage particles, which carry a polynucleotide sequence encoding them.

In a particular embodiment, a phage display method is used to display antigen-binding domains expressed from a repertoire or combinatorial antibody library (e.g., human or murine). Phage that express an antigen binding domain that binds an antigen of interest can be selected or identified with antigen, e.g., using labeled antigen or antigen bound or captured to a solid surface or bead. Typically, phage used in these methods are filamentous phage including, e.g., fd and M 13 binding domains expressed from phage with Fab, Fv or disulfide stabilized Fv antibody domains recombinantly fused to either the phage gene III or gene VIII protein. Examples of phage display methods contemplated for use include, e.g., those of Brinkman, et al., J. Immunol. Methods 182:41-50 (1995); Ames, et al., J. Immunol. Methods184:177-186 (1995); Kettleborough, et al., Eur. J. Immunol. 24:952-958 (1994); Persicet, et al., Gene 187 9-18 (1997); Burton,

et al., Advances in Immunology 57:191-280(1994); PCT application No. PCT/GB91/01134; WO 90/02809; WO 91/10737; WO 92/01047; WO 9208619; WO 93/1 1236; WO 95/15982; WO95/20401; and U.S. Patent Nos. 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5.427.908; 5,516,637; 5,780,225; 5,658,727; 5,733,743 and 5,969,108.

After phage selection, antibody coding regions from a phage are isolated and used to generate whole antibodies, including human antibodies, or any other desired antigen binding fragment, and expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria, e.g., as described herein and in the literature. For example, techniques to recombinantly produce Fab, Fab' and F(ab')2 fragments can also be employed using art known methods such as, e.g., WO 92122324; Mullinax, et al., BioTechniques 12(6):864-869 (1992); and Sawai, et al., AJRI 34:26-34 (1995); and Better, et al., Science 240:1041-1043 (1988). Examples of producing single-chain Fvs and antibodies include, e.g., U.S. Patents 4,946,778 and 5,258,498; Huston, et al., Methods in Enzymology 203:46-88 (1991); Shu, et al., Proc. Natl. Acad. Sci. USA 90:7995-7999(1993); and Skerra, et al., Science 240: 1038-1040 (1988). For some uses, including *in vivo* use of antibodies in humans and *in vitro* detection assays, it may be preferable to use chimera, humanized, or human antibodies.

A **chimeric antibody** is a molecule in which different portions of the antibody are derived from different animal species, such as antibodies having a variable region derived from a murine monoclonal antibody and a human immunoglobulin constant region. Methods for producing chimeric antibodies are known in the art (see, e.g., Morrison, Science 229: 1202(1985); Oi, et al., BioTechniques 4:214 (1986); Gillies, et al., (1989) J. Immunol. Methods 125: 191-202; U.S. Patent Nos. 5,807,715; 4,816,567; and 4,816397.

Humanized antibodies are antibody molecules from non-human species that bind a desired antigen having one or more complementarity determining regions (CDRs) from a non-human species and a framework region from a human immunoglobulin molecule. Often, framework residues of the human framework regions are substituted with a corresponding residue from a CDR donor antibody to alter, preferably improve, antigen binding. These framework substitutions are identified by any known art method, e.g., by (1) modeling the interactions of a CDR and framework residues to identify framework residues important for antigen binding and/or (2) by sequence comparison to identify

unusual framework residues at particular positions (see, e.g., U.S. Patent No. 5,585,089, Riechmann, et al., Nature 332:323 (1988). Antibodies can be humanized using a variety of known techniques including, e.g., CDR-grafting (see, e.g., EP 239,400; WO 91/09967; U.S. Patent Nos. 5,225,539; 5,530,101; and 5,585,089), veneering or resurfacing (see, e.g., EP 592,106; EP 519,596; Padlan, Molecular Immunology 28(4/5):489-498 (1991); Studnicka, et al., Protein Engineering 7(6):805-814 (1994); Roguska, et al., Proc. Natl. Acad. Sci. USA 91:969-973 (1994)), and chain shuffling (see, e.g., U.S. Patent No. 5,565,332).

Completely human antibodies are particularly desirable for therapeutic treatment of human patients. Human antibodies can be made using a variety of known methods including, e.g., phage display methods described herein using antibody libraries derived from human immunoglobulin sequences (see, e.g., U.S. Patent Nos. 4,444,887 and 4,716,111; and WO 98/46645, WO 98/150433, WO 00/58513104 WO 98/124893, WO 98/116654, WO 96/134096, WO 96/133735, and WO 91/10741).

Human antibodies can also be produced using transgenic mice that are incapable of expressing functional endogenous immunoglobulins, but which can express human immunoglobulin genes. Generally, human heavy and light chain immunoglobulin gene complexes may be introduced randomly or by homologous recombination into mouse embryonic stem cells. Alternatively, human variable regions, constant regions, and diversity regions may be introduced into mouse embryonic stem cells in addition to the human heavy and light chain genes. The mouse heavy and light chain immunoglobulin genes may be rendered non-functional (separately or simultaneously) with the introduction of human immunoglobulin loci by homologous recombination. In particular, homozygous deletion of the JH region prevents endogenous antibody production. The modified embryonic stem cells are expanded and microinjected into blastocysts to produce chimeric mice. The chimeric mice are then bred to produce homozygous offspring that express human antibodies. The transgenic mice are immunized in the normal fashion with a selected antigen, e.g., all or a portion of a (or fragment thereof) polypeptide of the invention. Monoclonal antibodies directed against the antigen can be obtained from an immunized, transgenic mouse using conventional hybridoma technology. The human immunoglobulin transgenes harbored by the transgenic mice rearrange during B cell differentiation, and subsequently undergo class switching and

somatic mutation. Thus, it is possible to produce therapeutically useful IgG, IgA, IgM, and IgE antibodies.

For an overview of the technology for producing human antibodies, see, e.g., Lonberg and Huszar, Int. Rev. Immunol. 13:65-93 (1995). A more detailed discussion on producing human antibodies and human monoclonal antibodies including protocols can be found, e.g., in WO 98/24893; WO 92/01047; WO 96/34096; WO 96133735; European Patent No. 0 598 877; U.S. Patent Nos. 5,413,923; 5,625,126; 5,633,425; 5,569,825; 5,661,016; 5,545,806; 5,814,318; 5,885,793; 5,916,771; and 5,939,598. In addition, commercial companies such as, e.g., Abgenix, Inc. (Freemont, CA) and Genpharm (San Jose, CA) can be hired to produce human antibodies.

Completely human antibodies that recognize a selected epitope can be generated by "guided selection." In this method, a selected non-human monoclonal antibody, e.g., a mouse antibody, is used to guide the selection of a completely human antibody recognizing the same epitope (see, e.g., Jespers, et al. (1988) BioTechnology 12:899-903).

Further, antibodies of the invention can, in turn, be used to generate anti-idiotype antibodies that "mimic" a polypeptide (or fragment thereof) of the invention using known techniques (see, e.g., Greenspan & Bona, FASEB J. 7(5):437-444; (1989) and Nissinoff, J. (1991) Immunol. 147(8):2429-2438).

For example, antibodies that bind and competitively inhibit polypeptide multimerization and/or competitively inhibit binding of a polypeptide of the invention to a ligand can be used to generate anti-idiotypes that "mimic" the polypeptide multimerization and/or binding domain and, as a consequence, bind to and neutralize a polypeptide and/or its ligand. Such neutralizing anti-idiotypes, or Fab fragments of such anti-idiotypes, can be used in therapeutic regimens to neutralize a polypeptide ligand. For example, such anti-idiotypic antibodies can be used to bind a polypeptide of the invention (or fragment thereof) and/or to bind its ligand/receptor, and thereby block its biological activity.

Polynucleotides Encoding Antibodies.

The invention further provides a nucleic acid molecule comprising a polynucleotide sequence encoding a binding composition of the invention and/or a fragment thereof.

The invention also encompasses polynucleotides that hybridize under stringent or lower stringency hybridization conditions, e.g., as defined herein, to a polynucleotide that encodes a binding composition, preferably, that specifically and/or selectively binds a mature polypeptide or protein of the invention, preferably, a binding composition that binds to TGF Beta 1.

The polynucleotides may be obtained, and the nucleotide sequence of the polynucleotides determined, by any known art method. For example, if the nucleotide sequence of the antibody is known (as here for the TGF Betas), a polynucleotide encoding the antibody may be assembled from chemically synthesized oligonucleotides (e.g., as described in Kutmeier, et al., (1994) BioTechniques 17:242), which, briefly described, involves synthesizing overlapping oligonucleotides containing portions of the sequence encoding the antibody, annealing, and ligating those oligonucleotides, then, amplifying the ligated oligonucleotides using a polymerase chain reaction.

Alternatively, a polynucleotide encoding an antibody can be generated from nucleic acid of any suitable source. If a clone containing a nucleic acid molecule encoding a particular antibody is not available, but, however, the sequence of the antibody molecule is known, then a nucleic acid encoding the immunoglobulin may be chemically synthesized or obtained from a suitable source. For example the source may be an antibody cDNA library, or a cDNA library generated from poly A+ RNA, isolated from any tissue or cell expressing the antibody of interest, such as, e.g., a hybridoma cells selected to express an antibody of the invention by PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of a polynucleotide sequence of interest or by cloning using an oligonucleotide probe specific for the particular gene sequence to identify, e.g., a cDNA clone from a cDNA library that encodes the antibody, a nucleic acid molecule for the antibody can be generated.

Amplified nucleic acids may be cloned into replicable cloning vectors using any known art method. Once the nucleotide and corresponding amino acid sequence of the antibody are determined, the nucleotide sequence of the antibody may be manipulated using any known art method, e.g., recombinant DNA techniques, site directed mutagenesis, PCR, etc. to generate antibodies having a different amino acid sequence to create amino acid substitutions, deletions, and/or insertions (see, e.g., Sambrook, et al.,

and Ausubel, et al., eds., cur. ed., Current Protocols in Molecular Biology, John Wiley & Sons, NY).

In a specific embodiment, the amino acid sequence of the heavy and/or light chain variable domains may be inspected to identify the sequences of complementarity determining regions (CDRs) by known methods, e.g., by comparing known amino acid sequences of other heavy and chain light variable regions to determine regions of sequence hypervariability. Using routine recombinant DNA techniques, one or more CDRs may be inserted within framework regions, e.g., into human framework regions to humanize a non-human antibody as here for the mAbs 1021, 2471, and 3821. The framework regions may be naturally occurring or consensus framework regions, and are preferably human framework regions (for a listing of human framework regions see, e.g., Chothia, et al. (1998) J. Mol. Biol. 278: 457-479).

Preferably, the polynucleotide generated by the combination of the framework regions and CDRs encodes an antibody that specifically and/or selectively binds TGF Beta 1 (or fragment thereof). Preferably, as discussed herein, one or more amino acid substitutions may be made within the framework regions to improve binding of the antibody to its antigen.

Additionally, such methods may be used to make amino acid substitutions or deletions of one or more variable-region, cysteine residues participating in an intrachain disulfide bond to generate antibody molecules lacking one or more intrachain disulfide bonds. Other alterations to the polynucleotide are encompassed by the present invention and within the skill of an ordinary artisan, e.g., such as a molecular biologist.

In addition, "chimeric antibody" techniques can be used by, e.g., splicing genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity (see, e.g., Morrison, et al., Proc. Natl. Acad. Sci. 81:851-855 (1984); Neuberger, et al., Nature 312: 604-608 (1984); Takeda, et al., Nature 314:452-454 (1985)). A chimeric antibody, e.g., humanized antibodies, is a molecule in which different portions are derived from different animal species, such as those having a variable region, derived from a murine mAb and constant region from a human immunoglobulin, e.g., humanized antibodies.

Alternatively, techniques can be adapted to produce single chain antibodies (see, e.g., U.S. Patent No. 4,946,778; Bird, Science 242:423-42 (1988); Huston, et al., Proc.

Natl. Acad. Sci. USA 85:5879-5883 (1988); and Ward, et al., Nature 334:544-54 (1989)). Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an ammo acid bridge, resulting in a single chain polypeptide. Techniques for the assembly of functional Fv fragments in *E. coli* may also be used (Skerra, et al. (1988) Science 242: 1038- 1041).

Polynucleotide and Polypeptide Fragments

The present invention also encompasses fragments of a binding composition polynucleotide. A binding composition polynucleotide "**fragment**" encompasses a short polynucleotide of a nucleic acid molecule, which is a portion of a sequence described in a Table herein or a portion of a polynucleotide sequence of a SEQ ID NO:.

Polynucleotide fragments of the invention encompass a polynucleotide sequence that is preferably at least about 15 nucleotides, more preferably at least about: 20, 21, 22, 24, 26, or 29 nucleotides, favorably at least about: 30, 32, 34, 36, 38, or 39 nucleotides, and even more preferably, at least about: 40, 42, 44, 46, 48, or 49 nucleotides, desirably at least about: 50, 52, 54, 56, 58, or 59 nucleotides, particularly at least about 75 nucleotides, or at least about 150 nucleotides in length.

A polynucleotide fragment "**at least 20 nucleotides in length**," e.g., is intended to include, e.g., 20 or contiguous bases from the cDNA sequence contained in a Table herein or a portion of a polynucleotide sequence of a SEQ ID NO:.

In this context, "**at least about**" includes, e.g., a specifically recited value (e.g., 20nt), and a value that is larger or smaller by one or more nucleotides (e.g., 5, 4, 3, 2, or 1), at either terminus or at both termini. Preferably, these fragments encode a polypeptide possessing biological activity as defined herein, e.g., immunogenicity, or antigenicity. More preferably, a polynucleotide fragment can be used as a probe or primer as discussed herein. Furthermore, the present invention also encompasses a polynucleotide that stably hybridizes to a polynucleotide fragment described herein under either stringent or lowered stringency hybridization conditions. Additionally incorporated are polypeptides encoded by a polynucleotide fragment or a hybridized polynucleotide stably bound to a polynucleotide fragment of the invention.

In the present invention, a "**polypeptide fragment or segment**" encompasses an amino acid sequence that is a portion of a sequence in a Table herein or a portion of a polynucleotide sequence of a SEQ ID NO:. Protein and/or polypeptide fragments or

segments may be "free-standing," or they may comprise part of a larger polypeptide or protein, of which the fragment or segment forms a portion or region, e.g., a single continuous region of a SEQ ID NO: herein connected in a fusion protein.

Preferably, a polypeptide segment can have a length of contiguous amino acids that is at least about: 7, 8, 9, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 110, 120, 130, 140, or 150 contiguous amino acids in length. In this context "**about**" includes, e.g., the specifically recited ranges or values described herein, and it also encompasses values that differ from these recited values by several amino acid residues (e.g., plus or minus 5, plus or minus 4, plus or minus 3, plus or minus 2, or; plus or minus 1 amino acid residues), at either or both ends of the fragment. Further, a polynucleotide encoding a polypeptide such a fragment is also encompassed by the invention.

Moreover, the invention encompasses proteins or polypeptides comprising a plurality of said amino acid segments or fragments, e.g., nonoverlapping, segments of a specified length. Typically, a plurality will be at least two, more usually at least three, and preferably at least: four, five, six, seven, eight, nine, or more. While minimum lengths of a segment are provided, maximum lengths of various sizes are also encompassed for any specific plurality of segments, e.g., a plurality of three segments *in toto* could have one segment of length 7 contiguous amino acids, and two additional non-overlapping segments, each of which has a length of 12. Features of one of the different genes should not be taken to limit those of another of the genes.

Also preferred are polypeptide fragments or segments (and their corresponding polynucleotide fragments) that characterize structural or functional domains, such as, fragments, or combinations thereof, that comprise e.g., alpha-helix, and alpha-helix forming regions, beta-sheet, and beta-sheet-forming regions, turn, and turn-forming regions, coil, and coil-forming regions, hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, loop regions, hairpin domains, beta-alpa-beta motifs, helix bundles, alpha/beta barrels, up and down beta barrels, jelly roll or swiss roll motifs, transmembrane domains, surface-forming regions, substrate binding regions, transmembrane regions, linkers, immunogenic regions, epitopic regions, and high antigenic index regions. Polypeptide fragments of a sequence herein

falling within conserved domains are specifically encompassed by the present invention. Moreover, polynucleotides encoding these domains are also encompassed.

Other preferred polypeptide segments are biologically active fragments. Biologically active fragments are those exhibiting activity similar, but not necessarily identical, to an activity of a binding composition polypeptide (or fragment thereof). The biological activity of the fragments may include, e.g., an improved desired activity, or a decreased undesirable activity. Polynucleotides encoding these polypeptide fragments are also encompassed by the invention.

Preferably, the polynucleotide fragments encode a polypeptide that demonstrates a functional activity. The phrase "**functional activity**" encompasses a polypeptide segment that can accomplish one or more known functional activities. Such functional activities include, e.g., without limitation, biological activity, antigenicity [ability to bind (or compete with a polypeptide for binding) to an antibody binding composition to a polypeptide of the invention], immunogenicity (ability to generate antibody that binds to a polypeptide of the invention), ability to form multimers with a polypeptide of the invention, and the ability to bind to a receptor or ligand of a polypeptide of the invention.

The functional activity of a polypeptide (including fragments, variants, derivatives, and analogs thereof) can be assayed by various methods. For example, where one is assaying for the ability to bind or compete with a full-length polypeptide of the invention for binding to an antibody of a polypeptide of the invention, various immunoassays known in the art can be used, including, e.g., without limitation, competitive and non-competitive assay systems using techniques such as radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoradiometric assays, gel diffusion precipitation reactions, immunodiffusion assays, in situ immunoassays (using colloidal gold, enzyme or radioisotope labels, for example), western blots, precipitation reactions, agglutination assays (e.g., gel agglutination assays, hemagglutination assays, complement fixation assays, immunofluorescence assays, protein A assays, and immunoelectrophoresis assays, etc.)

In another embodiment, antibody binding is accomplished by detecting a label on the primary antibody. In another embodiment, the primary antibody is detected by detecting binding of a secondary antibody or reagent to the primary antibody. In a further

embodiment, the secondary antibody is labeled. Many means are known in the art for detecting binding in an immunoassay and are within the scope of the present invention.

In another embodiment, where a ligand is identified, or the ability of a polypeptide fragment, variant or derivative of the invention to multimerize is being evaluated, binding can be assayed, e.g., by using reducing and non-reducing gel chromatography, protein affinity chromatography, and affinity blotting (see generally, Phizicky, et al. (1995) Microbial. Rev. 59:94-123). In another embodiment, physiological correlates of binding of a polypeptide to its substrates (signal transduction) can be assayed with common techniques.

In addition, assays described herein (see, e.g., the "Examples" section of the application), or otherwise known in the art, can routinely be applied to measure the ability of a binding composition of the invention (its fragments, variants derivatives and analogs thereof) to modulate a related biological activity (either *in vitro* or *in vivo*) of TGF Beta 1.

Methods of Producing Antibodies

An antibody binding composition of the invention can be produced by any known art method, in particular, by chemical synthesis or preferably, by recombinant expression techniques.

Recombinant expression of a binding composition of the invention, or fragment, derivative or analog thereof, (e.g., a heavy or light chain of an antibody of the invention or a single chain antibody of the invention), requires construction of an expression vector containing a polynucleotide sequence that encodes the antibody. Once a polynucleotide sequence encoding an antibody molecule or a heavy or light chain of an antibody, or portion thereof (preferably containing the heavy or light chain variable domain), of the invention has been obtained, the vector for the production of the antibody molecule may be produced by known recombinant DNA technology techniques.

Methods known in the art can be used to construct expression vectors containing antibody coding sequences and appropriate transcriptional and translational control signals. These methods include, e.g., without limitation, *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. The invention, thus, provides replicable vectors comprising a nucleotide sequence encoding an antibody molecule of the invention, or a heavy or light chain thereof, or a heavy or light chain variable domain, operably-linked to a promoter. Such vectors may include, e.g., the

nucleotide sequence encoding the constant region of the antibody molecule (see, e.g., WO 86/05807; WO 89/01036; or U.S. Patent No. 5,122,464) and the variable domain of the antibody may be cloned into such a vector for expression of the entire heavy or light chain.

Generally speaking, an expression vector is transferred to a host cell by conventional techniques and the transfected cells are then cultured to produce an antibody. Thus, the invention includes, e.g., host cells containing a polynucleotide encoding an antibody of the invention, or a heavy or light chain thereof, or a single chain antibody of the invention, operably linked to a heterologous promoter. In preferred embodiments for the expression of double-chained antibodies, vectors encoding both the heavy and light chains may be co-expressed in a host cell for expression of the entire immunoglobulin molecule, as detailed herein or known in the art.

A variety of host-expression vector systems may be utilized to express antibody molecules of the invention. Such host-expression systems represent vehicles by which any coding sequence of interest may be produced and subsequently purified. However, when transformed or transfected with an appropriate nucleotide coding sequence, host-expression system cells may also represent an antibody molecule of the invention *in situ*. These cells include, e.g., without limitation, microorganisms such as bacteria (e.g., *E. coli, B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA, or cosmid DNA expression vectors containing antibody coding sequences; yeast (e.g., *Saccharomyces, Pichia*) transformed with recombinant yeast expression vectors containing antibody coding sequences; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing antibody coding sequences; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing antibody coding sequences; or mammalian cell systems (e.g., COS, CHO, BHK, 293, 3T3 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter; the vaccinia virus 7.5K promoter).

Preferably, bacterial cells such as *Escherichia coli*, and more preferably, eukaryotic cells, especially for the expression of whole recombinant antibody molecule,

are used for the expression of a recombinant antibody molecule. For example, mammalian cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus is an effective expression system for antibodies (Foecking, et al. (1986) Gene 45:101; Cockett, et al. (1990) Bio/Technology 8:2).

In bacterial systems, a number of expression vectors may be advantageously selected depending upon the intended use of the expressed antibody molecule. For example, when a large quantity of protein is to be produced, like, e.g., for the generation of pharmaceutical compositions of an antibody molecule, then vectors that direct the expression of high levels of fusion protein products, which are readily purified may be desirable. Such vectors include, e.g., without limitation, the *E. coli* expression vector pUR278 (Ruther, et al., EMBO J. 2: 1791 (1983)), in which the antibody coding sequence may be ligated individually into the vector in frame with the lac Z coding region so that a fusion protein is produced; pIN vectors (Inouye and Inouye, Nucleic Acids Res. 13:3 101-3 109 (1985); Van Heeke and Schuster, J. Biol. Chem. 24:5503-5509 (1989)); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST).

In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption and binding to matrix glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include, e.g., thrombin, or factor Xa protease cleavage sites so that the cloned target gene product can be released from a GST moiety. One insect system used as a vector to express a foreign gene, is the *Autographa californica* nuclear polyhedrosis virus (AcNPV) system. The AcNPV virus grows in *Spodopteru frugiperda* cells. The antibody coding sequence may be cloned individually into non-essential regions (e.g., the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (e.g., the polyhedrin promoter).

In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the antibody coding sequence of interest may be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome using *in vitro* or *in vivo* recombination. Insertion in a non-essential region of the viral genome (e.g., region E1 or

E3) results in a recombinant virus that is viable and capable of expressing the antibody molecule in infected hosts (see, e.g., Logan and Shenk, (1984) Proc. Natl. Acad. Sci. USA 8 1:355-359). Specific initiation signals may be required for efficient translation of inserted antibody coding sequences. These signals include, e.g., the ATG initiation codon, and adjacent sequences. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure proper translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic.

The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see, e.g., Bittner, et al., Methods in Enzymol. 153:5 l-544 (1987)). In addition, a host cell strain may be chosen that modulates the expression of the inserted sequence, or modifies and processes the gene product in the specific fashion desired. Such modifications (e.g., glycosylation) and processing (e.g., cleavage) of protein products may be important for the function of a protein.

Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of a foreign protein that is expressed. To this end, eukaryotic host cells that possess the cellular machinery for proper: processing of the primary transcript, glycosylation, and phosphorylation may be used. Such mammalian host cells include, e.g., without limitation, CHO, VERY, BHK, Hela, COS, MDCK, 293, 3T3, W138, and, in particular, breast cancer cell lines such as, e.g., BT483, Hs578T, HTB2, BT20 and T47D, and normal mammary gland cell line such as, e.g., CRL7030 and Hs578Bst.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines that stably express an antibody molecule may be engineered. Rather than using expression vectors that contain viral origins of replication, host cells can be transformed with controlled by appropriate expression control elements (e.g., promoter, a polynucleotide sequence enhancer sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker.

After introducing foreign polynucleotide sequence, engineered cells can grow for l - 2 days in an enriched media, before switching to a selective media. A selectable marker

in a recombinant plasmid confers resistance to selection and allows cells to stably integrate the plasmid into their chromosomes and to form foci that can be subsequently cloned and expanded into cell lines. This method can be used to engineer cell lines that express an antibody molecule. Such engineered cell lines are particularly useful in screening and evaluating compounds that interact either directly or indirectly with an antibody molecule of the invention.

A number of selection systems can be used, including, e.g., without limitation, herpes simplex virus thymidine kinase (Wigler, et al., Cell 11:223 (1977)) in the cells, hypoxanthine-guanine phosphoribosyltransferase in hgprt- cells (Szybalska and Szybalski, Proc. Natl. Acad. Sci. USA 48:202 (1992)), or adenine phosphoribosyltransferase in hgprt-cells (Lowy, et al., Cell 22:817 (1980)). Also, anti-metabolite resistance can be used as the basis of selection for the following genes: dhfr—which confers resistance to methotrexate (Wigler, et al., Natl. Acad. Sci. USA 77:357 (1980); O'Hare, et al., Proc. Natl. Acad. Sci. USA 78:1527 (1981)); gpt—which confers resistance to mycophenolic acid (Mulligan and Berg, Proc. Natl. Acad. Sci. USA 78:2072 (1981)); neo—which confers resistance to the aminoglycoside G-20418 (Clinical Pharmacy 12:488-505; Wu and Wu, Biotherapy 3:87-95 (1991)); Tolstoshev, Ann. Rev. Pharmacol. Toxicol. 32:573-596 (1993); Mulligan, Science 260:926-932 (1993); and Morgan and Anderson, Ann. Rev. Biochem. 62: 19 1-2 17 (1993); May, 1993, TJB TECH 1 l(5): 155-215); and hygro—which confers resistance to hygromycin (Santerre, et al., Gene 30: 147 (1984)).

Known art methods can be routinely applied to select a desired recombinant clone (see, e.g., in Ausubel, et al. (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, NY (1993); Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990); and in Chapters 12 and 13, Dracopoli, et al. (eds.), Current Protocols in Human Genetics, John Wiley & Sons, NY (1994); Colberre-Garapin, et al., J. Mol. Biol. 150: 1 (198 1).

Expression levels of an antibody molecule can be increased by vector amplification (see, e.g., Bebbington and Hentschel, Vol. 3 (Academic Press, New York, 1987)). When a marker in the vector-system expressing antibody is amplifiable, increasing the level of inhibitor present in the host cell culture will increase the number of copies of the marker gene. Since the amplified region is associated with the antibody

gene, production of the antibody also increases (Crouse, et al., Mol. Cell. Biol. 3:257 (1983)). The host cell may be co-transfected with two expression vectors of the invention, the first vector encoding a heavy chain derived polypeptide and the second vector encoding a light chain derived polypeptide. The two vectors may contain identical selectable markers that enable equal expression of heavy and light chain polypeptides. Alternatively, a single vector may be used that encodes, and is capable of expressing, both heavy and light chain polypeptides. In such situations, the light chain should be placed before the heavy chain to avoid an excess of toxic free heavy chain (Proudfoot, Nature 322:52 (1986); Kohler, Proc. Natl. Acad. Sci. USA 77:2197 (1980)).

Coding sequences for heavy and light chains may comprise cDNA or genomic DNA. Once an antibody molecule of the invention has been (produced by, e.g., an animal, chemically synthesized, or recombinantly expressed), it may be purified by any known method, e.g., by chromatography (e.g., ion exchange, affinity, particularly by affinity for the specific antigen after Protein A, and sizing column chromatography), centrifugation, differential solubility, or any other technique for protein purification. In addition, a binding composition or fragments thereof can be fused to heterologous polypeptide sequences to facilitate purification using any art known method or one described herein.

The present invention encompasses antibodies recombinantly fused or chemically conjugated (including both covalent and non-covalent conjugations) to a polypeptide (or portion thereof, preferably comprising at least: 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100 contiguous amino acids of the polypeptide) to generate fusion proteins. The fusion does not necessarily need to be direct, but may occur through linker sequences.

Antibodies fused or conjugated to a polypeptide may also be used in *in vitro* immunoassays and in purification methods using known art methods (see e.g., Harbor, et al., *supra*, and WO 9312 1232; EP 439,095; Naramura et al. (1994) Immunol. Lett. 39:9 l-99; U.S. Patent No. 5,474,981; Gillies, et al. (1992) Proc. Natl. Acad. Sci. USA 89:1428-1432; Fell, et al. (1991) J. Immunol. 146: 2446-2452).

The present invention further includes compositions comprising a polypeptide (or fragment thereof) fused or conjugated to an antibody domain other than a variable region. For example, a polypeptide of the invention (or fragment thereof) may be fused or conjugated to an antibody Fc region, or portion thereof. The antibody portion that is

fused to a polypeptide of the invention (or fragment thereof) may comprise a constant region, a hinge region, a CH1 domain, a CH2 domain, and/or a CH3 domain or any combination of whole domains or portions thereof. A polypeptide (or fragment thereof) may also be fused or conjugated to an antibody portion described herein to form multimers. For example, Fc portions fused to a polypeptide of the invention (or fragment thereof) can form dimers through disulfide bonding between the Fc portions. Higher multimeric forms can be made by fusing the polypeptides to portions of IgA and IgM.

Methods for fusing or conjugating a polypeptide of the invention (or fragment thereof) to an antibody portion are known (see, e.g., U.S. Patent Nos. 5,336,603; 5,622,929; 5,359,046; 5,349,053; 5,447,851; 5,112,946; EP 307,434; EP 367,166; WO 96/04388; WO9106,570; Ashkenazi, et al. (1991) Proc. Natl. Acad. Sci. USA 88: 10535-10539; Zheng, et al. (1995) J. Immunol. 154:5590-5600; and Vie, et al. (1992) Proc. Natl. Acad. Sci. USA 89: 11337- 11341).

As discussed herein, a polypeptide, polypeptide fragment, may be fused or conjugated to an antibody portion described herein or known in the art to increase the *in vivo* half-life. Further, a polypeptide, polypeptide fragment, may be fused or conjugated to an antibody portion to facilitate purification. One example uses chimeric proteins comprising the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or ligh1 chains of mammalian immunoglobulins. (see, e.g., EP 394,827; Traunecker, et al. (1988) Nature 33 1:84-86).

A polypeptide, polypeptide fragment, fused or conjugated to an antibody having disulfide-linked dimeric structures (due to the IgG) may also be more efficient in binding and neutralizing other molecules, than the monomeric secreted protein or protein fragment alone (see, e.g., Fountoulakis, et al. (1995) 3. Biochem. 270: 3958-3964).

In many cases, the Fc part of a fusion protein is beneficial in therapy and diagnosis, and thus can result in, e.g., improved pharmacokinetic properties (see, e.g., EP A232, 262). Alternatively, deleting the Fc part after the fusion protein has been expressed, detected, and purified, can be favored. For example, the Fc portion may hinder therapy and diagnosis if the fusion protein is used as an antigen for immunizations. In drug discovery, e.g., human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5 (see,

e.g., Bennett, et al. (1995) J. Molecular Recognition 8:52-58; Johanson, et al. (1995) J. Biol. Chem. 270:9459-9471).

Moreover, a binding composition (or fragment thereof) can be fused to marker sequences, such as a peptide to facilitate purification. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., Chatsworth, CA), among others, many of which are commercially available. Hexa-histidine provides for convenient purification of a fusion protein (Gentz, et al. (1989) Proc. Natl. Acad. Sci. USA 86:821-824). Other peptide tags useful for purification include, e.g., the "HA" tag, which corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson, et al. (1984) Cell 37:767) and the "flag" tag.

The present invention further encompasses antibodies or fragments thereof conjugated to a diagnostic or therapeutic agent. The antibodies can be used diagnostically to, for-example, monitor the development or progression of a tumor as part of a clinical testing procedure to determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling the antibody to a detectable substance. Examples of detectable substances include, e.g., various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, radioactive materials, positron emitting metals using various positron emission tomographies, and nonradioactive paramagnetic metal ions. The detectable substance may be coupled or conjugated either directly to the antibody (or fragment thereof) or indirectly, through an intermediate (such as, e.g., an art known linker) using established techniques (see, e.g., U.S. Patent No. 4,741,900 for metal ions that can be conjugated to antibodies for use as diagnostics according to the present invention). Examples of suitable enzymes include, e.g., without limitation, horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include, e.g., without limit, streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include, e.g., without limitation, umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride, or phycoerythrin; an example of a luminescent material includes, e.g., without limitation, luminol; examples of bioluminescent materials include, e.g., without limitation, luciferase, luciferin, and aequorin; and examples of a suitable radioactive material includes, e.g., I^{125}, I^{131}, I^{111} or Tc^{99}.

Further, an antibody of the invention (or fragment thereof) may be conjugated to a moiety such as a cytotoxin, e.g., a cytostatic or cytocidal agent, a therapeutic agent or a radioactive metal ion, e.g., an alpha-emitter such as, e.g., Bi^{213}. A cytotoxin or cytotoxic agent can include, e.g., any agent that is detrimental to a cell such as, e.g., without limitation, paclitaxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, l-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof.

Therapeutic agents include, e.g., without limitation, anti-metabolites (such as, e.g., methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (such as, e.g., mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclothosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (such as, e.g., daunorubicin (formerly, daunomycin) and doxorubicin), antibiotics (such as, e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (such as, e.g., vincristine and vinblastine).

A conjugate of the invention can be used to modify a given biological response, a therapeutic agent or drug moiety is not to be construed as being limited to typically classical chemical therapeutic agents. For example, a drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, e.g., a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheriatoxin; or a protein such as, e.g., tumor necrosis factor, a-interferon, B-interferon, nerve growthfactor, platelet derived growth factor, tissue plasminogen activator, an apoptotic agent (such as, e.g., TNF-alpha, TNF-beta, AIM I (see, e.g., WO 97/33899), AIM II (see, e.g., WO 97/34911), Fas Ligand (see, e.g., Takahashi, et al., Int. Immun., 6: 1567-1574 (1994)), VEGI (see, e.g., WO 99/23105), a thrombotic agent or an anti-angiogenic agent, e.g., angiostatin or endostatin; or, biological response modifiers such as, e.g., lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophage colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors.

Antibodies can also be attached to solid supports, which are particularly useful for immunoassays or purification of the target antigen. Such solid supports include, e.g., without limitation, glass, cellulose, poly-acrylamide, nylon, polystyrene, polyvinyl chloride, or polypropylene. Techniques for conjugating a therapeutic moiety to an antibody are known, see, e.g., Amon, et al., "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in Monoclonal Antibodies And Cancer Therapy, Reisfeld, et al. (eds.), pp. 243-56 (Alan R. Liss, Inc.1985); Hellstrom, et al., "Antibodies For Drug Delivery", in Controlled Drug Delivery (2nd Ed.), Robinson, et al. (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in Monoclonal Antibodies '84: Biological And Clinical Applications, Pinchera, et al. (eds.), pp. 475-506 (1985); "Analysis, Results, and Future Prospective of the Therapeutic Use Of Radiolabeled Antibody in Cancer Therapy", in Monoclonal Antibodies for Cancer Detection and Therapy, Baldwin, et al. (eds.), pp. 303-16 (Academic Press 1985), and Thorpe, et al., "The Preparation and Cytotoxic Properties of Antibody-Toxin Conjugates," Immunol. Rev. 62: 119-58 (1982).

Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described U.S. Patent No. 4,676,980. An antibody, with or without a therapeutic moiety conjugated to it, administered alone, or in combination with cytotoxic factor(s) and/or cytokine(s) can be used as a therapeutic.

B. Immunophenotyping

An antibody (or fragment thereof) of the invention may be utilized for immunophenotyping of cell lines and biological samples. The translation product of a TGF Beta 1 (or fragment thereof) may be useful as a cell specific marker, or more specifically, as a cellular or tissue specific marker (which is differentially expressed at various stages of differentiation and/or maturation of particular cell types).

Monoclonal antibodies directed against a specific epitope, or combination of epitopes, permit screening of cell populations expressing such a marker. Various techniques can be used using an antibody of the invention (or fragment thereof) to screen for cells expressing a marker(s) including, e.g., without limitation, magnetic separation using antibody-coated magnetic beads, "panning" with antibody attached to a solid matrix (i.e., plate), and flow cytometry (see, e.g., U.S. Patent 5,985,660; or Morrison, et al. (1999) Cell 96737-49).

These techniques permit screening of cell populations such as, e.g., might be found with hematological malignancies (e.g., minimal residual disease (MRD) in acute leukemic patients) and "non-self" cells in transplantations to prevent Graft-versus-Host Disease (GVHD). Alternatively, these techniques permit screening of hematopoietic stem and progenitor cells, which are capable of undergoing proliferation and/or differentiation, as might be found, e.g., in human umbilical cord blood.

C. Immunoassays

A particular protein can be measured by a variety of immunoassay methods including, e.g., without limitation, competitive and non-competitive assay systems using techniques such as, e.g., without limitation, western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, and protein A immunoassays. For a review of immunological and immunoassay procedures in general, see Stites and Terr (eds.) (1991) Basic and Clinical Immunology (7th ed.). Moreover, the immunoassays of the present invention can be performed in many configurations, which are reviewed extensively in Maggio (ed.) (1980) Enzyme Immunoassay CRC Press, Boca Raton, Florida; Tijan (1985) "Practice and Theory of Enzyme Immunoassays," Laboratory Techniques in Biochemistry and Molecular Biology, Elsevier Science Publishers B.V., Amsterdam; and Harlow and Lane Antibodies, A Laboratory Manual, *supra*. See also Chan (ed.) (1987) Immunoassay: A Practical Guide Academic Press, Orlando, FL; Price and Newman (eds.) (1991) Principles and Practice of Immunoassays Stockton Press, NY; and Ngo (ed.) (1988) Non-isotopic Immunoassays Plenum Press, NY.

Immunoassays for measurement can be performed by a variety of methods known to those skilled in the art. In brief, immunoassays to measure the protein can be either competitive or noncompetitive binding assays. In competitive binding assays, the sample to be analyzed competes with a labeled analyte for specific binding sites on a capture agent bound to a solid surface. Preferably, the capture agent is an antibody specifically reactive with a TGF Beta 1 protein as described herein. The concentration of labeled analyte bound to the capture agent is inversely proportional to the amount of free analyte present in the sample.

In a competitive binding immunoassay, the target protein present in the sample competes with labeled protein for binding to a specific binding composition, for example, a binding composition, such as an antibody, that is specifically and/or selectively reactive with the target protein. The binding composition may be bound to a solid surface to effect separation of bound-labeled protein from the unbound-labeled protein. Alternately, the competitive binding assay may be conducted in liquid phase and a variety of techniques known in the art may be used to separate the bound-labeled protein from the unbound-labeled protein. Following separation, the amount of bound labeled protein is determined. The amount of protein present in the sample is inversely proportional to the amount of labeled protein binding.

Alternatively, a homogeneous immunoassay may be performed in which a separation step is not needed. In these immunoassays, the label on the protein is altered by the binding of the protein to its specific binding composition. This alteration in the labeled protein results in a decrease or increase in the signal emitted by label, so that measurement of the label at the end of the immunoassay allows for detection or quantitation of the protein.

Competitive assays are also particularly useful, where the cells are contacted and incubated with a labeled binding partner or antibody having known binding affinity to the protein, such as ^{125}I-antibody, and a test sample whose binding affinity to the binding composition is being measured. The bound and free-labeled binding compositions are then separated to assess the degree of protein binding. The amount of test compound bound is inversely proportional to the amount of labeled binding partner binding to the known source. Any one of numerous techniques can be used to separate bound from free protein to assess the degree of protein binding. This separation step could typically involve a procedure such as adhesion to filters followed by washing, adhesion to plastic followed by washing, or centrifugation of the cell membranes. Viable cells could also be used to screen for the effects of drugs on a TGF beta protein mediated function (e.g., second messenger levels, such as, e.g., cell proliferation; inositol phosphate pool changes, transcription using a luciferase-type assay; and others). Some detection methods allow for elimination of a separation step, e.g., a proximity-sensitive detection system.

Qualitative or quantitative analysis of proteins may also be determined by a variety of noncompetitive immunoassay methods. For example, a two-site, solid phase

sandwich immunoassay may be used. In this type of assay, a binding composition for the protein, for example an antibody, is attached to a solid support. A second protein-binding composition, which may also be an antibody, and which binds the protein at a different site, is labeled. After binding at both sites on the protein has occurred, the unbound-labeled binding composition is removed and the amount of labeled binding composition bound to the solid phase is measured. The amount of labeled binding composition bound is directly proportional to the amount of protein in the sample.

Western blot analysis can be used to determine the presence of proteins in a sample. Electrophoresis is carried out, for example, on a tissue sample suspected of containing the protein. Following electrophoresis to separate the proteins, and transfer of the proteins to a suitable solid support, e.g., a nitrocellulose filter, the solid support is incubated with an antibody reactive with the protein. This antibody may be labeled, or alternatively may be detected by subsequent incubation with a second labeled antibody that binds the primary antibody.

The immunoassay formats described above employ labeled assay components. The label may be coupled directly or indirectly to the desired component of the assay according to methods well known in the art. A variety of labels and methods may be used. Traditionally, a radioactive label incorporating ^{3}H, ^{125}I, ^{35}S, ^{14}C, or ^{32}P was used. Non-radioactive labels include proteins, which bind to labeled antibodies, fluorophores, chemiluminescent agents, enzymes, and antibodies, which can serve as specific-binding pair members for a labeled protein. The choice of label depends on sensitivity required, ease of conjugation with the compound, stability requirements, and available instrumentation. For a review of various labeling or signal producing systems, which may be used, see U.S. Patent No. 4,391,904.

Antibody binding compositions reactive with a particular protein can also be measured by a variety of immunoassay methods. For a review of immunological and immunoassay procedures applicable to the measurement of antibodies by immunoassay techniques, see Stites and Terr (eds.) Basic and Clinical Immunology (7th ed.) *supra*; Maggio (ed.) Enzyme Immunoassay, supra; and Harlow and Lane Antibodies, A Laboratory Manual, *supra*.

In brief, immunoassays to measure antisera reactive with the targeted protein can be either competitive or noncompetitive binding assays. In competitive binding assays,

the sample analyte competes with a labeled analyte for specific binding sites on a capture agent bound to a solid surface. Preferably, the capture agent is a purified recombinant protein. Other sources of proteins, including isolated or partially purified naturally occurring protein, may also be used. Noncompetitive assays include sandwich assays, in which the sample analyte is bound between two analyte-specific binding reagents. One of the binding compositions is used as a capture agent and is bound to a solid surface. The second binding composition is labeled and is used to measure or detect the resultant complex by visual or instrument means. A number of combinations of capture agent and labeled binding composition can be used. A variety of different immunoassay formats, separation techniques, and labels can be used similar to those described above for the measurement of a protein.

Exemplary immunoassays (without limitation) are described herein. Immunoprecipitation protocols generally comprise lysing a population of cells in a lysis buffer such as RIPA buffer (1% NP-40 or Triton X- 100, 1% sodiumdeoxycholate, 0.1% SDS, 0.15 M NaCl, 0.01 M sodium phosphate at pH 7.2, 1%Trasylol) supplemented with protein phosphatase and/or protease inhibitors (e.g., EDTA, PMSF, aprotinin, sodium vanadate), adding the antibody of interest to the cell lysate, incubating for a period of time (e.g., 1-4 hours) at 4°C, adding protein A and/or protein G sepharose beads to the cell lysate, incubating for about an hour or more at 4°C, washing the beads in lysis buffer, and resuspending the beads in SDS/sample buffer.

The ability of the antibody of interest to immunoprecipitate a particular antigen can be assessed by, e.g., Western blot analysis. One of skill in the art would be knowledgeable as to the parameters are modifiable to increase binding of an antibody to an antigen and to decrease background (e.g., by pre-clearing the cell lysate with sepharose beads). Further discussion of immunoprecipitation protocols can be found in, e.g., Ausubel et al, eds., 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York.

Western blot analysis generally comprises preparing a protein sample, electrophoresis of the sample through polyacrylamide gel (e.g., 8%-20% SDS-PAGE depending on the molecular weight of the antigen), transferring the protein sample from the polyacrylamide gel to a membrane such as nitrocellulose, PVDF, or nylon, then blocking the membrane in blocking solution (e.g., PBS with 3% BSA or non-fat milk),

washing the membrane in washing buffer (e.g., PBS-Tween 20), blocking the membrane with primary antibody (the antibody of interest) diluted in blocking buffer, washing the membrane in washing buffer, blocking the membrane with a secondary antibody (that recognizes the primary antibody, e.g., an anti-human antibody) conjugated to an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase) or radioactive molecule (e.g., ^{32}P or ^{125}I) diluted in blocking buffer, then washing the membrane in wash buffer, and detecting the presence of the antigen. An ordinary artisan would know what parameters to modify to increase signal and reduce background (see, e.g., Ausubel et al, eds., 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York for such teachings.)

An ELISA assay comprises preparing an antigen, coating the well of a 96 well microtiterplate with the antigen, adding the antibody of interest conjugated to a detectable compound such as an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase) to the well and incubating for a period of time, and detecting the presence of the antigen. In ELISAs, the antibody of interest does not have to be conjugated to a detectable compound; instead, a second antibody (which recognizes the antibody of interest) conjugated to a detectable compound may be added to the well. Further, instead of coating the well with the antigen, the antibody may be coated to the well. In this case, a second antibody conjugated to a detectable compound may be added following the addition of the antigen of interest to the coated well. An ordinary artisan can determine without undue experimentation what parameters to adjust, e.g., to increase signal as well as what other variations for an ELISA should be used (see, e.g., Ausubel, et al., eds., 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York).

The binding affinity of an antibody to an antigen and the off-rate of an antibody-antigen interaction can be determined by, e.g., using a competitive binding assay. One non-limiting example is a radioimmunoassay comprising incubating labeled antigen (e.g., using ^{3}H or ^{125}I) with an antibody of interest in the presence of increasing amounts of unlabeled antigen, and then detecting the amount of antibody bound to the labeled antigen. The affinity of the antibody of interest for a particular antigen and the binding off-rates can be determined from the data by, e.g., Scatchard plot analysis. Competition with a second antibody can also be determined using, e.g., radioimmunoassays. In this

case, the antigen is incubated with antibody of interest conjugated to a labeled compound (e.g., ^{3}H or ^{125}I) in the presence of increasing amounts of an unlabeled second antibody.

Therapeutic Uses

The present invention further encompasses binding composition-based therapies that involve administering an antibody binding composition to an animal, preferably a mammal, most preferably a primate (e.g., a human), to modulate, treat, inhibit, effect, or ameliorate one or more of the disclosed diseases, disorders, or conditions described herein. For example, antibodies specific for human TGF Beta 1 have been shown to be effective in animal models for the treatment of fibrotic diseases and other diseases such as rheumatoid arthritis where the TGF receptor (TGFR) is overexpressed. Antibodies against TGFR have been shown to be effective in the treatment of glomerulonephritis (W.A Border et al. Nature 346, 371-374, 1990); neural scarring (A. Logan et al. Eur. J. Neurosci. 6, 355-363, 1994); dermal scarring (M. Shah et al. Lancet 339, 213-214 1992; M.Shah et al. J. Cell Science 107, 1137-1157, 1994; M. Shah et al. 108, 985-1002, 1995); lung fibrosis (S.N. Giri et al. Thorax 48, 959-966, 1993); arterial injury (Y.G. Wolf, L.M. Rasmussen & E. Ruoslahti J. Clin. Invest. 93, 1172-1178, 1994), and rheumatoid arthritis (Wahl et al J. Exp. Medicine 177, 225-230, 1993). Consequently, binding compositions of the invention would also be effective in the amelioration of conditions, states, or diseases, such as, e.g., fibrotic diseases and immune/inflammatory conditions associated with TGF Beta 1.

Therapeutic binding compositions of the invention include, e.g., without limitation, antibodies of the invention (including fragments, analogs and derivatives thereof as described herein) and nucleic acid molecules encoding antibodies of the invention (including fragments, analogs and derivatives thereof and anti-idiotypic antibodies as described herein). An antibody of the invention can be used to modulate, treat, inhibit, ameliorate, or prevent diseases, disorders, or conditions associated with aberrant expression and/or activity of a TGF Beta 1 polypeptide (or fragment thereof), including, e.g., without limitation, any one or more of the diseases, disorders, syndromes or conditions described herein. The treatment, amelioration, and/or prevention of diseases, disorders, or conditions associated with aberrant expression and/or activity of a TGF Beta 1 polypeptide includes, e.g., without limitation, ameliorating symptoms associated with those diseases, disorders, or conditions.

Binding compositions of the invention, such as, e.g., antibodies may be provided in pharmaceutically acceptable compositions as known in the art or as described herein. A summary of the ways in which an antibody binding composition of the present invention may be used therapeutically includes, e.g., without limitation, binding a TGF Beta 1 polypeptide (or a fragment thereof) locally or systemically in the body or by direct cytotoxicity of the antibody, e.g. as mediated by complement (CDC) or by effector cells (ADCC). Some of these approaches are described in more detail herein. Using teachings provided herein, one of ordinary skill in the art will know how to use an antibody or binding composition of the present invention for diagnostic, monitoring, or therapeutic purposes without undue experimentation.

An antibody of this invention may be advantageously utilized in combination with other monoclonal or chimeric antibodies, or with lymphokines or hematopoietic growth factors (such as, e.g., IL-2, IL-3, and IL-7), e.g., that serve to increase the number or activity of effector cells that interact with the antibody. An antibody of the invention may be administered alone or in combination with other types of treatments (e.g., radiation therapy, chemotherapy, hormonal therapy, immunotherapy, and anti-tumor agents).

Generally, administration of products of a species origin or species reactivity (in the case of antibodies) that is the same species as that of the patient is preferred. Thus, in a preferred embodiment, human antibodies, fragments derivatives, analogs, or nucleic acid molecules, are administered to a human patient for therapy or prophylaxis.

It is preferable to use high affinity and/or potent *in vivo* inhibiting and/or neutralizing antibodies against TGF Beta 1 polypeptides (fragments or regions thereof), for both immunoassays directed to and treatment, inhibition, amelioration or prevention therapy of syndromes, diseases, conditions, or disorders related to TGF Beta 1. Such antibodies, fragments, regions, or portions will preferably have an affinity for human TGF Beta 1. Additionally preferred binding affinities for a binding composition of the invention, e.g., such as an antibody, include, e.g., those with a dissociation constant or Kd less than about: 5×10^{-2}M, 10^{-2}M, 5×10^{-3}M, 10^{-3}M, 5×10^{-4}M, 10^{-4}M, 5×10^{-5}M, 10^{-5}M, 5×10^{-6}M, 10^{-6}M, 5×10^{-7}M, 10^{-7}M, 5×10^{-8}M, 10^{-8}M, 5×10^{-9}M, 10^{-9}M, 5×10^{-10}M, 10^{-10}M, 5×10^{-11}M, 10^{-11}M, 5×10^{-12}M, 10^{-12}M, 5×10^{-13}M, 10^{-13}M, 5×10^{-14}M, 10^{-14}M, 5×10^{-15}M, or 10^{-15}M.

Physical Variants

The invention also encompasses polypeptides having substantial amino acid sequence similarity with an amino acid sequence described herein. Amino acid sequence similarity, or sequence identity, is determined by optimizing residue matches. This changes when considering conservative substitutions as matches. Conservative substitutions typically include substitutions within the following groups: glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid; asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine. See also Needleham, et al. (1970) J. Mol. Biol. 48:443-453; Sankoff, et al. (1983) Time Warps, String Edits, and Macromolecules: The Theory and Practice of Sequence Comparison Chapter One, Addison-Wesley, Reading, MA; and software packages from IntelliGenetics, Mountain View, CA; and the University of Wisconsin Genetics Computer Group, Madison, WI.

The invention encompasses, but is not limited to, polypeptides that are functionally related to a polypeptide encoded by the specific sequence identifiers of the present application. Functionally related polypeptides include any polypeptide sharing a functional characteristic with a binding composition of the present invention (e.g., the ability to selectively and/or specifically bind TGF Beta 1). Such functionally related polypeptides include, without limitation, additions or substitutions of amino acid residues within the amino acid sequence encoded by the sequences described herein; particularly, those that result in a silent change, thus producing a functionally equivalent polypeptide. Amino acid substitutions may be made based on similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphiphatic nature of the residues involved.

For example, nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan, and methionine; polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine; positively charged (basic) amino acids include arginine, lysine, and histidine; and negatively charged (acidic) amino acids include aspartic acid and glutamic acid.

Furthermore, non-classical amino acids or chemical amino acid analogs may be substituted or added into a polypeptide sequence. Non-classical amino acids include, e.g., without limitation, D-isomers of the common amino acids; 2,4-diaminobutyric acid; a-amino isobutyric acid, 4-aminobutyric acid, Abu, 2-amino butyric acid, g-Abu, e-Ahx, 6-amino hexanoic acid, Aid, 2-amino isobutyric acid, 3-amino propionic acid, ornithine,

norleucine, norvaline, hydroxyproline, sarcosine, citrulline, homocitrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, b-alanine, fluoro-amino acids, designer amino acids such as b-methyl amino acids, Ca-methyl amino acids, Na-methyl amino acids, and amino acid analogs in general. Furthermore, the amino acid can be either dextrorotary (D) or levorotary (L).

While random mutations can be made to a nucleic acid molecule (using well known random mutagenesis techniques) and the resulting polypeptides can be tested for activity, site-directed mutations of coding sequences can be engineered (using well known site-directed mutagenesis techniques) to generate mutant with increased function (e.g., increased or decreased binding affinity).

To design functionally related and functionally variant polypeptides, it is useful to distinguish between conserved and variable amino residues using the homology comparison tables provided herein. To preserve LP function, it is preferable that conserved residues remain unaltered and that the conformational folding of the LP functional sites be preserved. Preferably, alterations of non-conserved residues are carried out with conservative alterations (e.g., a basic amino acid is replaced by a different basic amino acid). To produce altered function variants, it is preferred to make non-conservative changes at variable and or conserved residues. Deletions at conserved and variable residues can also be used to create altered function variants.

Although site-specific mutation sites are predetermined, mutants need not be site-specific. Protein mutagenesis can be conducted by making amino acid insertions or deletions. Substitutions, deletions, insertions, or any combinations may be generated to arrive at a final construct. Insertions include amino- or carboxyl- terminal fusions, e.g. epitope tags. Random mutagenesis can be conducted at a target codon and the expressed mutants can then be screened for the desired activity. Methods for making substitution mutations at predetermined sites in DNA having a known sequence are well known in the art (e.g., by M13 primer mutagenesis or polymerase chain reaction (PCR) techniques; see also, Sambrook, et al. (cur. ed.) and Ausubel, et al. (cur. ed., and Supplements). The mutations in the DNA normally should not place coding sequences out of reading frames and preferably do not create complementary regions that could hybridize to produce secondary mRNA structure such as loops or hairpins.

Additions of peptide moieties to facilitate handling are familiar and routine art techniques. Moreover, a binding composition (including any fragment thereof, and specifically an epitope) can be combined with parts of the constant domain of an immunoglobulin e.g., (IgA, IgE, IgG, IgM) portions thereof (CH 1, CH2, CH3), and any combination thereof including both entire domains and portions thereof), resulting in a chimeric polypeptide. Such fusion proteins can facilitate purification and often are useful to increase the *in vivo* half-life of the protein. For example, this has been demonstrated for chimeric proteins comprising the first two domains of a human CD4 polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins (EP 394,827, Traunecker, et al. (1988) Nature, 331:84-86). Fusion proteins with disulfide-linked dimeric structures (due to the IgG domain) can also be more efficient in binding and neutralizing other molecules than a monomeric secreted protein or sole protein fragment (Fountoulakis, et al. (1995) J. Biochem.15 270:3958-3964). Enhanced delivery of an antigen across an epithelial barrier to the immune system has been demonstrated for antigens (e.g., insulin) conjugated to an FcRn binding partner such as IgG or Fc fragments (see, e.g., WO 96/22024 and WO 99/104813). IgG fusion proteins that have a disulfide-linked dimeric structure due to the IgG portion disulfide bonds have also been found more efficient in binding and neutralizing other molecules than monomeric polypeptides or fragments thereof alone (Fountoulakis, et al. (1995) J. Biochem. 270:3958-3964).

Additionally, a fusion protein can comprise various portions of the constant region of an immunoglobulin molecule together with a human protein (or part thereof) EP-A-O 464 533 (Canadian counterpart 2045869). In many cases, the Fc part in a fusion protein is beneficial in therapy and diagnosis, and thus, can result in, e.g., improved pharmacokinetic properties (EP-A 0232 262.). Alternatively, deleting the Fc part after the fusion protein has been expressed, detected, and purified, may be desired. For example, the Fc portion may hinder therapy and/or diagnosis if the fusion protein is used as an immunogen for immunizations. In drug discovery for example, human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to identify hIL-5 antagonists (Bennett, et al. (1995) I. Molecular Recognition 8:52-58; and Johanson, et al. (1995) J. Biol. Chem. 270:9459-9471).

Furthermore, new constructs may be made by combining similar functional domains from other proteins. For example, protein-binding or other segments may be "swapped" between different new fusion polypeptides or fragments (see, e.g., Cunningham, et al. (1989) Science 243:1330-1336; and O'Dowd, et al. (1988) J. Biol. Chem. 263:15985-15992). Thus, new chimeric polypeptides exhibiting new combinations of specificities will result from the functional linkage of protein-binding specificities and other functional domains.

Moreover, a binding composition can be fused to a marker sequence, such as a peptide, to facilitate purification. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as, e.g., the tag provided in a pQE vector (QIAGEN, Inc., Chatsworth, CA, 91311), which provides for convenient purification of the fusion protein (Gentz, et al. (1989) Proc. Natl. Acad. Sci. USA 86:821-824). Another useful peptide-purification tag is the "HA" tag, which corresponds to an epitope derived from an influenza hemagglutinin protein (Wilson, et al. (1984) Cell 37:767).

Additionally, fusion constructions may be generated through the techniques of gene-shuffling, motif-shuffling, exon shuffling, and/or codon shuffling (collectively referred to as "**DNA shuffling**").

"**Substantially pure**" refers to nucleic acid or protein or polypeptide that are removed from their natural environment and are isolated and/or separated from other contaminating proteins, nucleic acids, and other biologicals. Purity, or "**isolation**" may be assayed by standard methods, and will ordinarily be at least about 50% pure, more ordinarily at least about 60% pure, generally at least about 70% pure, more generally at least about 80% pure, often at least about 85% pure, more often at least about 90% pure, preferably at least about 95% pure, more preferably at least about 98% pure, and in most preferred embodiments, at least 99% pure. Similar concepts apply, e.g., to binding compositions, e.g., such as antibodies of the invention. For example, it may be desirable to purify an polypeptide from recombinant cell proteins or polypeptides. Typical exemplary suitable purification procedures include, e.g., without limitation, fractionation on an ion-exchange column; ethanol precipitation; reversed-phase HPLC; chromatography on silica or cation-exchange resins (such as, e.g., DEAE); chromatofocusing; SDS-PAGE; ammonium sulfate precipitation; gel filtration (using, e.g., Sephadex G-75); protein A Sepharose columns (e.g., to remove contaminants such as

IgG); and metal chelating columns (e.g., to bind epitope-tagged forms of a binding composition polypeptide). Various art known methods of protein purification may be employed (see, e.g., Deutscher, (1990) Methods in Enzymology 182: 83-9 and Scopes, (1982) Protein Purification: Principles and Practice, Springer-Verlag, NY.) Typically, the purification method selected depends, e.g., on the nature of the production process used and the particular LP polypeptide produced.

In another example, a chimeric protein comprising a heterologous moiety, which can be recognized by another molecule, can be purified using a commercially available affinity matrix. Such moieties include, without limit, glutathione S-transferase (GST), maltose binding protein (MBP), thioredoxin (Trx), calmodulin binding peptide (CBP), 6-His, FLAG, *c-myc*, and hemagglutinin (HA). The GST, MBP, Trx, CBP, and 6-His moieties enable purification of their cognate fusion proteins on immobilized glutathione, maltose, phenylarsine oxide, calmodulin, and metal-chelate resins, respectively. The FLAG, *c-myc*, and hemagglutinin (HA) moieties enable immunoaffinity purification of fusion proteins using commercially available monoclonal and polyclonal antibodies that specifically recognize these epitope tags.

"**Solubility**" of a protein or polypeptide is reflected by sedimentation measured in Svedberg units, which are a measure of the sedimentation velocity of a molecule under particular conditions. The determination of the sedimentation velocity was classically performed in an analytical ultracentrifuge, but is typically now performed in a standard ultracentrifuge (see, Freifelder (1982) Physical Biochemistry (2d ed.) W.H. Freeman & Co., San Francisco, CA; and Cantor and Schimmel (1980) Biophysical Chemistry parts 1-3, W.H. Freeman & Co., San Francisco, CA). As a crude determination, a sample containing a putatively soluble polypeptide is spun in a standard full sized ultracentrifuge at about 50K rpm for about 10 minutes, and soluble molecules will remain in the supernatant. A soluble particle or polypeptide will typically be less than about 30S, more typically less than about 15S, usually less than about 10S, more usually less than about 6S, and, in particular embodiments, preferably less than about 4S, and more preferably less than about 3S. Solubility of a polypeptide or fragment depends upon the environment and the polypeptide. Many parameters affect polypeptide solubility, including temperature, electrolyte environment, size and molecular characteristics of the polypeptide, and nature of the solvent. Typically, the temperature at which the

polypeptide is used ranges from about 4° C to about 65° C. Usually the temperature at use is greater than about 18° C and more usually greater than about 22° C. For diagnostic purposes, the temperature will usually be about room temperature or warmer, but less than the denaturation temperature of components in the assay. For therapeutic purposes, the temperature will usually be body temperature, typically about 37° C for humans, though under certain situations the temperature may be raised or lowered in situ or *in vitro*.

The size and structure of the polypeptide should generally be in a substantially stable state, and usually not in a denatured state. The polypeptide may be associated with other polypeptides in a quaternary structure, e.g., to confer solubility, or associated with lipids or detergents in a manner which approximates natural lipid bilayer interactions.

The solvent will usually be a biologically compatible buffer, of a type used for preservation of biological activities, and will usually approximate a physiological solvent. Usually the solvent will have a neutral pH, typically between about 5 and 10, and preferably about 7.5. On some occasions, a detergent will be added, typically a mild non-denaturing one, e.g., CHS (cholesteryl hemisuccinate) or CHAPS (3-[3-cholamidopropyl)-dimethylammonio]-1-propane sulfonate), or a low enough concentration as to avoid significant disruption of structural or physiological properties of the protein.

Variants

The present invention encompasses variants of a polynucleotide sequence disclosed in a SEQ ID NO:, or the complementary strand thereto. The present invention also encompasses variants of a polypeptide sequence disclosed in a SEQ ID NO:. The term "variant" refers to a polynucleotide or polypeptide differing from a polynucleotide sequence or a polypeptide of the present invention, but retaining essential properties thereof. Generally, variants are closely similar overall in structural and/or sequence identity, and, in many regions, identical to a polynucleotide or polypeptide of the present invention.

The present invention encompasses nucleic acid molecules that comprise, or alternatively consist of, a polynucleotide sequence that is at least: 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to, e.g., a polynucleotide coding sequence of a SEQ ID NO: (or a strand complementary thereto); a nucleotide sequence encoding a polypeptide

of a SEQ ID NO:; and/or polynucleotide fragments of any of these nucleic acid molecules (e.g., a fragment as defined herein). Polynucleotides, that stably hybridize to a polynucleotide fragment (as defined herein) under stringent hybridization conditions or lower stringency conditions, are also encompassed by the invention, as are polypeptides (or fragments thereof) encoded by these polynucleotides.

The present invention is also directed to polypeptides that comprise, or alternatively consist of, an amino acid sequence that is at least: 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, e.g., a polypeptide sequence of SEQ ID NO: Y (or fragments thereof); a polypeptide sequence encoded by a cDNA contained in a deposited clone, and/or a polypeptide fragment of any of these polypeptides (e.g., those fragments as defined herein). A polynucleotide sequence having at least some "percentage identity," (e.g., 95%) to another polynucleotide sequence, means that the sequence being compared (e.g., the test sequence) may vary from another sequence (e.g. the referent sequence) by a certain number of nucleotide differences (e.g., a test sequence with 95% sequence identity to a reference sequence can have up to five point mutations per each 100 contiguous nucleotides of the referent sequence). In other words, for a test sequence to exhibit at least 95% identity to a referent sequence, up to 5% of the nucleotides in the referent may differ, e.g., be deleted or substituted with another nucleotide, or a number of nucleotides (up to 5% of the total number of nucleotides in the reference sequence) may be inserted into the reference sequence. The test sequence may be: an entire polynucleotide sequence, e.g., as shown in Tables 1-18, the ORF (open reading frame), or any fragment, segment, or portion thereof (as described herein). As a practical matter, determining if a particular nucleic acid molecule or polynucleotide sequence exhibits at least about: 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to a binding composition polynucleotide sequence can be accomplished using known computer programs.

Typically, in such a sequence comparison, one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are input into a computer, subsequent coordinates are designated, if necessary, and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percentage sequence identity for a test sequence(s) relative to the reference sequence, based on the parameters of a designated program.

Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith and Waterman (1981) Adv. Appl. Math. 2:482, by the homology alignment algorithm of Needlman and Wunsch (1970) J. Mol. Biol. 48:443, by the search for similarity method of Pearson and Lipman (1988) Proc. Nat'l Acad. Sci. USA 85:2444, by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by visual inspection (see generally, Ausubel, et al. supra).

A typical method for determining , a best overall match (also referred to as a global sequence alignment) between a test and a referent sequence can be determined using , e.g., the FASTDB computer program based on the algorithm of Brutlag, et al. (1990) Comp. App. Biosci. 6: 237-245. In a FASTDB sequence alignment, the test and referent sequences are, e.g., both DNA sequences. An RNA sequence can be compared by converting U's to T's. The result of a global sequence alignment is given in terms of a percentage identity.

Typical parameters used in a FASTDB alignment of DNA sequences to calculate percent identity are, e.g., Matrix=Unitary, k-tuple=4, Mismatch Penalty=l, Joining Penalty=30, Randomization Group Length=0, Cutoff Score=1, Gap Penalty=5, Gap Size Penalty 0.05, Window Size=500, or the length of the referent nucleotide sequence, whichever is shorter. If the referent sequence is shorter than the test sequence because of 5' or 3' deletions, not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for 5' and 3' truncations of the subject sequence when calculating percent identity. For referent sequences truncated at the 5' or 3' ends, relative to the test sequence, the percentage identity is corrected by calculating the number of bases of the test sequence that are 5' and 3' of the subject sequence, which are not matched/aligned, as a percentage of the total bases of the test sequence. Whether a nucleotide is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percentage identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percentage identity score. The corrected score is what is used for the purposes of sequence identity for the present invention. Ordinarily, bases outside the 5' and 3' bases of the subject sequence, as displayed by the FASTDB

alignment, which are not matched/aligned with the test sequence, are calculated for the purposes of manually adjusting the percent identity score.

For example, a 90 base referent sequence is aligned to a 100 base test sequence to determine percentage identity. The deletions occur at the 5' end of the referent sequence and therefore, the FASTDB alignment does not show a matched/alignment of the first 10 bases at the 5' end. The 10 unpaired bases represent 10% of the sequence (number of bases at the 5' and 3' ends not matched/total number of bases in the test sequence) so 10% is subtracted from the percentage identity score calculated by the FASTDB program. If the remaining 90 bases were perfectly matched the final percentage identity would be 90%.

In another example, a 90 base referent sequence is compared with a 100 base test sequence. This time the deletions are internal deletions so that there are no bases on the 5' or 3' of the subject sequence, which are not matched/aligned with the test. In this case, the percentage identity calculated by FASTDB is not manually corrected. Again, only bases 5' and 3' of the subject sequence that are not matched/aligned with the test sequence are manually corrected. No other manual corrections are to made for the purposes of the present invention.

Especially preferred are polynucleotide variants containing alterations, which produce silent substitutions, additions, or deletions, but do not alter the properties or activities of the encoded polypeptide. Nucleotide variants produced by silent substitutions due to the degeneracy of the genetic code are preferred. Polynucleotide variants can be produced for a variety of reasons, e.g., to optimize codon expression for a particular host (change codons in the human mRNA to those preferred by a bacterial host such as *E. coli*).

A further indication that two nucleic acid sequences of polypeptides are substantially identical is that the polypeptide encoded by the first nucleic acid is immunologically cross reactive with the polypeptide encoded by the second nucleic acid, as described herein. Thus, a polypeptide is typically substantially identical to a second polypeptide, for example, where the two peptides differ only by conservative substitutions. Another indication that two nucleic acid sequences are substantially identical is that the two molecules hybridize to each other under stringent conditions, as described below.

A polypeptide exhibiting or having at least about, e.g., 95% "sequence identity" to another amino acid sequence may include, e.g., up to five amino acid alterations per each 100 amino acid stretch of the test amino acid sequence. In other words, a first amino acid sequence that is at least 95% identical to a second amino acid sequence, can have up to 5% of its total number of amino acid residues different from the second sequence, e.g., by insertion, deletion, or substitution of an amino acid residue.

Alterations in amino residues of a polypeptide sequence may occur, e.g., at the amino or carboxy terminal positions or anywhere between these terminal positions, interspersed either individually among residues in the sequence or in one or more contiguous amino residue sections, portions, or fragments within the sequence.

As a practical matter, whether any particular polypeptide sequence exhibits at least about: 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% similarity to another sequence, for example, such as shown in a Table herein or to an amino acid sequence encoded by a cDNA contained in a deposited clone, can be determined conventionally by using known methods in the art, e.g., a computer algorithm such as ClustalW.

A preferred method for determining the best overall match (also called a global sequence alignment) between two sequences (either nucleotide or amino acid sequences) uses the FASTDB algorithm of Brutlag, et al. (1990) Comp. App. Biosci. 6:237-245. The result of such a global sequence alignment is given as a percentage of sequence identity, e.g., with 100% representing complete sequence identity.

Typical FASTDB parameters for amino acid alignments are, e.g.,: Matrix=PAM 0, k-tuple=2, Mismatch Penalty=1, Joining Penalty=20, Randomization Group Length=0, Cutoff Score=1, Window Size=sequence length, Gap Penalty=5 Gap Size Penalty=0.05, Window Size=500 or the length of the subject amino acid sequence, whichever is shorter.

If the subject sequence is shorter than the test sequence due to N- or C-terminal deletions, not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for N-and C-terminal truncations of the subject sequence when calculating global percent identity. For subject sequences truncated at the N- and C-termini, relative to the test sequence, the percent identity is corrected by calculating the number of residues of the test sequence that are N- and C-terminal of the subject sequence, which are not matched/aligned with a corresponding subject residue, as a percent of the total bases of the test sequence.

Whether a residue is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percentage identity score. This final percentage identity score is what is used for the purposes of the present invention.

Only residues to the N- and C-termini of the subject sequence, which are not matched/aligned with the test sequence, are considered for the purposes of manually adjusting the percent identity score. That is, only test residue positions outside the farthest N- and C-terminal residues of the subject sequence. For example, a 90 amino acid residue subject sequence is aligned with a 100-residue test sequence to determine percent identity. The deletion occurs at the N-terminus of the subject sequence and therefore, the FASTDB alignment does not show a matching/alignment of the first 10 residues at the N-terminus. The unpaired residues represent 10% of the sequence (number of residues at the N- and C-termini not matched/total number of residues in the test sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 residues were perfectly matched the final percent identity would be 90%.

In another example, a 90-residue subject sequence is compared with a 100-residue test sequence. This time the deletions are internal deletions so there are no residues at the N- or C-termini of the subject sequence, which are not matched/aligned with the test. In this case, the percent identity calculated by FASTDB is not manually corrected. Once again, only residue positions outside the N- and C-terminal ends of the subject sequence, as displayed in the FASTDB alignment, which are not matched/aligned with the test sequence are manually corrected for. No other manual corrections are to made for the purposes of the present invention.

Variants encompassed by the present invention may contain alterations in the coding regions, non-coding regions, or both.

Moreover, variants in which 1-2, 1-5, or 5-10 amino acids are substituted, deleted, or added in any combination are also preferred.

Naturally occurring variants encompassed herein are "**allelic variants**," which refer to one of several alternate forms of a gene occupying a given locus on a

chromosome of an organism. Allelic variants can vary at either the polynucleotide and/or polypeptide level and both types of variants are encompassed by the present invention.

Alternatively, non-naturally occurring variants may be produced by mutagenesis techniques or by direct synthesis using known methods of protein engineering and recombinant DNA technology. Such variants may be generated to improve or alter the characteristics of a binding composition polypeptide (or fragment thereof).

For instance, one or more amino acids can be deleted from the N-terminus or C-terminus of a secreted polypeptide of the invention (or fragment thereof) without a substantial loss of biological function. For example, Ron, et al. (1993) J. Biol. Chem. 268: 2984-2988, reported variant KGF proteins having heparin binding activity even after deleting 3, 8, or 27 amino-terminal amino acid residues. Similarly, interferon gamma was shown to exhibit up to ten times increased activity after 8-10 amino acid residues were deleted from the carboxy terminus (Dobeli, et al. (1988) J. Biotechnology 7:199-216)

Moreover, ample evidence demonstrates that polypeptide or polynucleotide variants retain a biological activity similar to that of the naturally occurring protein. For example, Gayle, et al. (1993) J. Biol. Chem 268:22105-22111, conducted extensive mutational analysis of human cytokine IL-1 alpha using random mutagenesis to generate over 3,500 individual IL-1 alpha mutants that averaged (over the entire length of the molecule) 2.5 amino acid changes per variant. Multiple mutations were examined at every possible amino acid position. The results showed that most of the molecule could be altered with little effect on either binding or biological activity. In fact, out of more than 3,500 nucleotide sequences examined, only 23 amino acid sequences produced a protein that differed significantly in activity from the wild-type. Moreover, even if deleting one or more amino acids from the N-terminus or C-terminus of the polypeptide results in modification or loss of one or more biological functions, other biological activities may be retained.

For example, antigenicity and/or immunogenicity can be retained (e.g., the ability of a deletion variant to induce and/or to bind antibodies that recognize a mature form of a polypeptide) when less than the majority of the residues of the secreted form are removed from the N-terminus or C-terminus. Whether a polypeptide lacking N- or C-terminal residues of a protein retains such activities can readily be determined by routine methods such as those described herein or known in the art.

Thus, the invention also encompasses, e.g., polypeptide variants that show biological activity such as, e.g., immunogenicity, or antigenicity. Such variants include, e.g., deletions, insertions, inversions, repeats, and substitutions selected so as have little effect on activity using general rules known in the art. For example, teachings on making phenotypically silent amino acid substitutions are provided, e.g., by Bowie, et al. (1990) Science 247: 1306-1310

One technique compares amino acid sequences in different species to identify the positions of conserved amino acid residues since changes in an amino acid at these positions are more likely to affect a protein function. In contrast, the positions of residues where substitutions are more frequent generally indicate that amino acid residues at these positions are less critical for a protein function. Thus, to a first degree, positions tolerating amino acid substitutions typically may be modified while still maintaining a biological activity of a protein.

A second technique uses genetic engineering to introduce amino acid changes at specific positions of a polypeptide to identify regions critical for a protein function. For example, site directed mutagenesis or alanine-scanning mutagenesis (the introduction of single alanine mutations at every residue in the molecule) can be used. (Cunningham and Wells (1989) Science 244: 1081-1085) A resulting mutein can subsequently be tested for a biological activity.

These two techniques have revealed that proteins are surprisingly tolerant of amino acid substitutions and they generally indicate which amino acid changes are likely to be permissive at certain amino acid positions in a protein. For example, typically, most buried amino acid residues (those within the tertiary structure of the protein) require nonpolar side chains, whereas few features of surface side chains are generally conserved. Moreover, tolerated conservative amino acid substitutions involve replacement of the aliphatic or hydrophobic amino acids Ala, Val, Leu and Ile; replacement of the hydroxyl residues Ser and Thr; replacement of the acidic residues Asp and Glu; replacement of the amide residues Asn and Gln, replacement of the basic residues Lys, Arg, and His; replacement of the aromatic residues Phe, Tyr, and Trp, and replacement of the small-sized amino acids Ala, Ser, Thr, Met, and Gly.

Besides using conservative amino acid substitutions, other variants of the present invention include, e.g., but are restricted to, e.g., (i) substitutions with one or more of the

non-conserved amino acid residues, where the substituted amino acid residues may or may not be one encoded by the genetic code, or (ii) substitution with one or more of amino acid residues having a substituent group, or (iii) fusion of the mature polypeptide with another compound, such as a compound to increase the stability and/or solubility of the polypeptide (e.g., polyethylene glycol), or (iv) fusion of the polypeptide with additional amino acids, such as, e.g., an IgG Fc fusion region peptide, or leader or secretory sequence, or a sequence facilitating purification. All such variants would be within the scope of those skilled in the art of molecular biology given Applicants' teachings herein, e.g., specifying unique polynucleotide and polypeptide sequences.

For example, polypeptide variants containing amino acid substitutions of charged amino acids with other charged or neutral amino acids may produce polypeptides with improved characteristics e.g., such as less aggregation. Aggregation of pharmaceutical formulations both reduces activity and increases clearance due to the aggregate's immunogenic activity (Pinckard, et al. (1967) Clin. Exp. Immunol. 2:331-340; Robbins, et al. (1987) Diabetes 36:838-845; Cleland, et al. (1993) Crit. Rev. Therapeutic Drug Carrier Systems 10:307-377).

A further embodiment of the invention encompasses a protein that comprises an amino acid sequence of the present invention that contains at least one amino acid substitution, but not more than 50 amino acid substitutions, even more preferably, not more than 40 amino acid substitutions, still more preferably, not more than 30 amino acid substitutions, and still even more preferably, not more than 20 amino acid substitutions, nor more than 15 amino acid substitutions.

Of course, in order of ever-increasing preference, it is highly preferable for a peptide or polypeptide to have an amino acid sequence that comprises an amino acid sequence of the present invention, which contains at least: one, but not more than: 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid substitutions.

In specific embodiments, the number of additions, substitutions, and/or deletions in an polypeptide sequence of the present invention or fragments thereof (e.g., a mature form and/or other fragments described herein), is at least: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 10-50, or 50-150; wherein conservative amino acid substitutions are more preferable than non-conservative substitutions.

Uses

The present invention provides reagents that will find use in diagnostic and/or therapeutic applications as described herein, e.g., in the description of kits for diagnosis.

The phrase, "**measuring level of TGF Beta**" is intended to mean herein measuring or estimating (either qualitatively and/or quantitatively) a level of, e.g., a polypeptide (or fragment thereof) in a first sample (e.g., preferably a biological sample) either directly (e.g., by determining or estimating an absolute protein or mRNA level) or relatively (e.g., by comparing to a polypeptide or mRNA level in a second sample). In one embodiment, the level in the first sample is measured or estimated from an individual having, or suspected of having, a disease, syndrome, disorder or condition and comparing that level to a second level, wherein the second level is obtained from an individual not having and/or not being suspected of having a disease, syndrome, disorder or condition. Alternatively, the second level is determined by averaging levels from a population of individuals not having or suspected of having a disease, syndrome, disorder, or condition.

As is appreciated in the art, once a standard level is determined, it can be used repeatedly as a standard for comparison. A "**biological sample**" is intended to mean herein any sample comprising biological material obtained from, using, or employing, e.g., an organism, body fluid, exudates, lavage product, waste product, cell (or part thereof), cell line, organ, biopsy, tissue culture, or other source originating from, or associated with, a living cell, tissue, organ, or organism, which contains, e.g., a polypeptide (or fragment thereof), a protein (or fragment thereof), a mRNA (or fragment thereof), or polynucleotide sequence (or fragment thereof) of the present invention, including, e.g., without limitation, a sample such as from, e.g., hair, skin, blood, saliva, semen, vomit, synovial fluid, amniotic fluid, breast milk, lymph, pulmonary sputum, urine, fecal matter, a lavage product, etc.

As indicated, a biological sample can include, e.g., without limitation, body fluids (e.g., such as semen, lymph, sera, plasma, urine, synovial fluid and spinal fluid) that contain a polypeptide (or fragment thereof), mRNA (or fragment thereof), a protein (or fragment thereof), or polynucleotide (or fragment thereof) of the present invention, by product, or, waste product; and/or other tissue source found to express a polypeptide (or fragment thereof), mRNA (or fragment thereof), or nucleic acid (or fragment thereof), by product, or, waste product; of the present invention. Methods for obtaining biological

samples, e.g., tissue biopsies, body fluids, cells, or waste products from mammals are known in the art. Where the biological sample is to include, e.g., mRNA, a tissue biopsy is a preferred source. The method(s) provided herein may preferably be applied in a diagnostic method and/or a kit in which a polynucleotide and/or a binding composition polypeptide (or fragment thereof) are attached to a solid support.

Use of Binding Compositions

A binding composition can be used to measure protein or polypeptide expression of a recombinant cell, as a means of assessing the quality and/or quantity of transformation of the host cell. Antibodies and other binding compositions directed towards TGF Beta 1 may be used to purify the corresponding molecule. As described herein, antibody purification of TGF Beta 1 components is both possible and practicable. Antibodies and other binding compositions may also be used in a diagnostic fashion to determine whether TGF Beta 1 protein components are present in a tissue sample or cell population using well-known techniques described herein. The ability to attach a binding composition to a TGF Beta 1 protein provides a means to diagnose disorders associated with TGF Beta 1 misregulation. Antibodies and other binding compositions may also be useful as histological markers. It is likely that TGF Beta 1 protein expression is limited to specific tissue types. By directing a probe, such as an antibody to a TGF Beta 1 protein it is possible to use the probe to distinguish tissue and cell types in situ or *in vitro*.

Diagnosis and Imaging Using an Antibody

Labeled antibodies, fragments, derivatives, and analogs thereof that specifically bind TGF Beta 1 can be used for diagnostic purposes to detect, modulate, ameliorate, diagnose, or monitor diseases, disorders, syndromes, and/or conditions associated with aberrant expression and/or activity of TGF Beta 1.

Encompassed herein are methods for detecting aberrant expression and/or activity of a TGF Beta 1 polypeptide (or fragment thereof) by, e.g., comprising assaying TGF Beta 1 in a sample, having one or more antibodies specific to the TGF Beta 1, e.g., a biological sample such as, e.g., cells or fluids, and comparing the level of expression in the sample with a standard level of expression, whereby a significant increase or decrease in the level of expression under study is compared to a standard level of expression to determine if the expression is aberrant.

Antibodies of the invention can be used to assay polypeptide levels in a sample, e.g., using classical immunohistological methods known to those of skill in the art (see e.g., Jalkanen, et al., J. Cell. Biol. 101:976-985 (1985); Jalkanen, et al., J. Cell . Biol. 105:3087-3096 (1987)). Other antibody-based methods typically useful for detecting polypeptide expression include, e.g., immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include, e.g., without limitation, enzyme labels, such as glucose oxidase; radioisotopes, such as, e.g., iodine (^{125}1, ^{121}1), carbon (^{14}C), sulfur (^{35}S), tritium (^{3}H), indium (^{112}In), and technetium (^{99}Tc); luminescent labels, such as luminol; and e.g., fluorescent labels, such as fluorescein and rhodamine, and biotin.

One aspect of the invention is the detection or diagnosis of a, condition, disease, syndrome, or disorder associated with aberrant expression of a binding composition polypeptide (or fragment thereof) in a mammal, preferably a primate, and most preferably a human primate.

In one embodiment, detection or diagnosis comprises administering to a subject (e.g., parentally, subcutaneously, or intraperitoneally) an effective amount of a labeled binding composition that specifically and/or selectively binds a TGF Beta 1 polypeptide (or fragment thereof); detecting the labeled molecule in the subject to determine the amount of labeled molecule in relation to a typical background level to determine that the subject has a particular disease, disorder, condition, syndrome, or state that is associated with an over-expression, under-expression, or miss-expression of TGF Beta 1.

Background levels can be determined by various methods, including, e.g., comparing the amount of detected, labeled-molecule with a standard value previously determined for a particular system. It is understood, that the size of the subject and the imaging system used are important factors in determining the quantity of imaging moiety needed to produce diagnostic images. In the case of using, e.g., a radioisotope moiety in a human subject, the quantity of radioactivity injected normally ranges from about 5 to 20 militaries of ^{99}Tc. The labeled antibody or antibody fragment preferentially accumulates at the location of a cell that contains a specific polypeptide (or fragment thereof) of interest. Applications of the above methods, e.g., for *in vivo* tumor imaging are further described, e.g., in Burchiel, et al. (1982) "Immunopharmacokinetics of Radiolabeled

Antibodies and Their Fragments": Chapter 13 in Tumor Imaging: The Radiochemical Detection of Cancer, Burchiel & Rhodes (eds.) Masson Publishing Inc.).

Depending on several variables, (e.g., including the type of label used and the mode of administration), the time interval for permitting a labeled molecule to preferentially concentrate at a site in a subject and for unbound labeled molecule to be cleared (e.g., to background level) is approximately in the range of 6 to 48 hours, 6 to 24 hours, or 6 to 12 hours. In another embodiment, the time interval (following administration) is approximately in the range of 5 to 20 days or 5 to 10 days.

In another embodiment, monitoring of a disease, condition, syndrome, or state of a disorder is carried out by repeating the method for initial diagnosis, e.g., one month after initial diagnosis, six months after initial diagnosis, one year after initial diagnosis, etc. Presence of the labeled molecule can also be detected in a subject using methods art known for *in vivo* scanning. These methods depend, e.g., upon the type of label used.

Skilled artisans will be able to determine the appropriate method for detecting a particular label. Methods and devices that may be used for diagnostic methods of the invention include, e.g., without limitation, computed topography (CT), whole body scan such as, e.g., position emission tomography (PET), magnetic resonance imaging (MRI), and sonography. In a specific embodiment, a molecule associated with a composition of the invention is labeled with a radioisotope and is detected in a subject using a radiation responsive surgical instrument (see, e.g., U.S. Patent No. 5441,050).

In another embodiment, a molecule is labeled with a fluorescing compound and is detected in the patient using a fluorescence responsive instrument. In another embodiment, a molecule is labeled with a positron emitting metal and is detected in a subject using positron emission-tomography. In yet another embodiment, a molecule is labeled with a paramagnetic label and is detected in a subject using magnetic resonance imaging (MRI).

Diagnostic Uses

In another embodiment, a binding composition (e.g., an antibody) is used to diagnose a disorder, state, condition, syndrome, or disease associated with the expression of TGF Beta 1. In a similar manner, the same binding composition can be used in an assay to monitor a subject being treated for a TGF Beta 1 associated condition. Diagnostic assays include methods that utilize the binding composition and a label to

detect it in a sample, e.g., in a human body fluid or in a cell or tissue extract. Binding compositions, such as, e.g., antibodies, are used with or without modification, and are labeled by covalent or non-covalent attachment of a reporter molecule.

A variety of protocols for measuring TGF Beta 1 (or fragment thereof), including ELISAs, RIAs, and FACS, are known in the art and provide a basis for diagnosing altered or abnormal levels of TGF Beta 1 expression. Normal or standard expression values are established using any art known technique, e.g., by combining a sample comprising an TGF Beta 1 polypeptide (or fragment thereof) with, e.g., antibodies under conditions suitable to form an TGF Beta 1:binding composition (antibody) complex. The amount of a standard complex formed is quantitated by various methods, such as, e.g., photometric means. Amounts of TGF Beta 1 polypeptide (or fragment thereof) expressed in subject, control, and samples (e.g., from biopsied tissue) are then compared with the standard values. Deviation between standard and subject values establishes parameters for correlating a particular disorder, state, condition, syndrome, or disease with a certain level of expression (or lack thereof) for an TGF Beta 1 polypeptide (or fragment thereof).

Once the presence of a disorder, state, condition, syndrome, or disease is established and a treatment protocol is initiated, assays are repeated on a regular basis to monitor the level of TGF Beta 1 expression. The results obtained from successive assays are used to show the efficacy of treatment over a period ranging from several days to months. With respect to disorders of cell proliferation (e.g., a cancer), the presence of an abnormal amount of TGF Beta 1 (either under- or over expressed) in biopsied tissue from a subject may indicate a predisposition for the development of a disorder, state, condition, syndrome, or disease of cell proliferation or it may provide a means for detecting such a disorder, state, condition, syndrome, or disease prior to the appearance of actual clinical symptoms. A more definitive initial detection may allow earlier treatment thereby preventing and/or ameliorating further progression of cell proliferation.

Therapeutic Uses

This invention also provides reagents with useful therapeutic value. A binding composition of the invention, e.g., such as an antibody, is useful in the treatment of conditions associated with abnormal physiology or development, including abnormal proliferation, e.g., cancerous conditions, or degenerative conditions, immune/inflammatory, cardiovascular, neurological, fibrotic, and developmental

disorders. Abnormal proliferation, regeneration, degeneration, and atrophy may be modulated by appropriate therapeutic treatment using a composition(s) provided herein. For example, a disease or disorder associated with abnormal expression or abnormal signaling by TGF Beta 1 is a target for an antagonist of the TGF Beta 1 protein such as a binding composition of the invention. The use of antibodies against TGF Beta for the treatment of disease has been the subject of patent applications for fibrotic disease (W091/04748); dermal scarring (W092/17206); macrophage deficiency diseases (PCT/US93/00998); macrophage pathogen infections (PCT/US93/02017); neural scarring (PCT/US93/03068); vascular disorders (PCT/US93/03795); prevention of cataract (W095/13827) (all of which are incorporated by reference herein for their teachings of diseases that are capable of amelioration by influencing TGF Beta 1 levels).

Other abnormal developmental conditions encompassed herein are known in cell types shown to possess TGF Beta 1 mRNA by northern blot analysis (see, e.g., Berkow (ed.) The Merck Manual of Diagnosis and Therapy, Merck & Co., Rahway, N.J.; Thorn et al. Harrison's Principles of Internal Medicine, McGraw-Hill, N.Y.; and Rich (ed.) Clinical Immunology; Principles and Practice, Mosby, St. Louis (cur. ed.); and below). Developmental or functional abnormalities, (e.g., of the neuronal, immune, or hematopoetic system) cause significant medical abnormalities and conditions which may be susceptible to prevention or treatment using compositions provided herein.

Recombinant and/or isolated binding compositions of the invention, such as, e.g., antibodies, can be purified and administered to a subject for treatment. These reagents can be combined for use with additional active or inert ingredients, e.g., in conventional pharmaceutically acceptable carriers or diluents, e.g., immunogenic adjuvants, along with physiologically innocuous stabilizers and excipients. These combinations can be sterile filtered and placed into dosage forms as by lyophilization in dosage vials or storage in stabilized aqueous preparations. This invention also contemplates use of antibodies or binding fragments thereof, including forms which are not complement binding.

Another therapeutic approach included within the invention involves direct administration of reagents, formulations, or compositions by any conventional administration techniques (such as, e.g., without limit, local injection, inhalation, or systemic administration) to a subject. The reagents, formulations, or compositions included within the bounds and metes of the invention may be targeted by any of the

methods described herein (e.g., polynucleotide delivery techniques). The actual dosage of reagent, formulation, or composition that modulates a disease, disorder, condition, syndrome, etc., depends on many factors, including the size and health of an organism, however one of one of ordinary skill in the art can use the following teachings describing methods and techniques for determining clinical dosages (see, e.g., Spilker (1984) Guide to Clinical Studies and Developing Protocols, Raven Press Books, Ltd., New York, pp. 7-13, 54-60; Spilker (1991) Guide to Clinical Trials, Raven Press, Ltd., New York, pp. 93-101; Craig and Stitzel (eds. 1986) Modern Pharmacology, 2d ed., Little, Brown and Co., Boston, pp. 127-33; Speight (ed. 1987) Avery's Drug Treatment: Principles and Practice of Clinical Pharmacology and Therapeutics, 3d ed., Williams and Wilkins, Baltimore, pp. 50-56; Tallarida, et al. (1988) Principles in General Pharmacology, Springer-Verlag, New York, pp. 18-20; and U.S. Pat. Nos. 4,657,760; 5,206,344; and 5,225,212.). Generally, in the range of about between 0.5 fg/ml and 500 ug/ml inclusive final concentration is administered per day to a human adult in any pharmaceutically acceptable carrier. Furthermore, animal experiments provide reliable guidance for the determination of effective does for human therapy. Interspecies scaling of effective doses can be performed following art known principles (e.g., see, Mordenti and Chappell (1989) "The Use of Interspecies Scaling in Toxicokinetics," in Toxicokinetics and New Drug Development; Yacobi, et al. (eds.) Pergamon Press, NY).

Effective doses can also be extrapolated using dose-response curves derived from *in vitro* or animal-model test systems. For example, for antibodies a dosage is typically 0.1 mg/kg to 100 mg/kg of a recipient's body weight. Preferably, a dosage is between 0.1 mg/kg and 20 mg/kg of a recipient's body weight, more preferably 1 mg/kg to 10 mg/kg of a recipient's body weight. Generally, homo-specific antibodies have a longer half-life than hetero-specific antibodies, (e.g., human antibodies last longer within a human host than antibodies from another species, e.g., such as a mouse, probably, due to the immune response of the host to the foreign composition). Thus, lower dosage of human antibodies and less frequent administration is often possible if the antibodies are administered to a human subject. Furthermore, the dosage and frequency of administration of antibodies of the invention may be reduced by enhancing uptake and tissue penetration (e.g., into the brain) by using modifications such as, e.g., lipidation.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the compositions of the invention and instructions such as, e.g., for disposal (typically, in a form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products).

The quantities of reagents necessary for effective treatment will depend upon many different factors, including means of administration, target site, physiological state of the patient, and other medicaments administered. Thus, treatment dosages should be titrated to optimize safety and efficacy. Typically, dosages used *in vitro* may provide useful guidance in the amounts useful for in situ administration of these reagents. Animal testing of effective doses for treatment of particular disorders will provide further predictive indication of human dosage. Various considerations are described, e.g., in Gilman, et al. (eds.) (1990) Goodman and Gilman's: The Pharmacological Bases of Therapeutics (8th ed.) Pergamon Press; and (1990) Remington's Pharmaceutical Sciences (17th ed.) Mack Publishing Co., Easton, PA. Methods for administration are discussed therein and below, e.g., for oral, intravenous, intraperitoneal, or intramuscular administration, transdermal diffusion, and others. Pharmaceutically acceptable carriers will include water, saline, buffers, and other compounds described, e.g., in the Merck Index, Merck & Co., Rahway, NJ. Dosage ranges would ordinarily be expected to be in amounts lower than 1 mM concentrations, typically less than about 10 μM concentrations, usually less than about 100 nM, preferably less than about 10 pM (picomolar), and most preferably less than about 1 fM (femtomolar), with an appropriate carrier. Slow release formulations, or a slow release apparatus will often be utilized for continuous administration.

Binding compositions may be administered directly to the host to be treated or, depending on the size of the compounds, it may be desirable to conjugate them to carrier proteins such as ovalbumin or serum albumin before their administration. Therapeutic formulations may be administered in any conventional dosage formulation. While it is possible for the active ingredient to be administered alone, it is preferable to present it as a pharmaceutical formulation. Formulations typically comprise at least one active ingredient, as defined above, together with one or more acceptable carriers thereof. Each carrier should be both pharmaceutically and physiologically acceptable in the sense of

being compatible with the other ingredients and not injurious to the patient. Formulations include those suitable for oral, rectal, nasal, or parenteral (including subcutaneous, intramuscular, intravenous and intradermal) administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. See, e.g., Gilman, et al. (eds.) (1990) Goodman and Gilman's: The Pharmacological Bases of Therapeutics (8th ed.) Pergamon Press; and (1990) Remington's Pharmaceutical Sciences (17th ed.) Mack Publishing Co., Easton, PA; Avis, et al. (eds.) (1993) Pharmaceutical Dosage Forms: Parenteral Medications Dekker, NY; Lieberman, et al. (eds.) (1990) Pharmaceutical Dosage Forms: Tablets Dekker, NY; and Lieberman, et al. (eds.) (1990) Pharmaceutical Dosage Forms: Disperse Systems Dekker, NY. The treatment of this invention may be combined with or used in association with other therapeutic agents.

The present invention also provides a pharmaceutical composition. Such a composition comprises, e.g., a therapeutically effective amount of a composition of the invention in a pharmaceutically acceptable carrier. As used herein, the term "**pharmaceutically acceptable carrier**" means a carrier approved by a federal regulatory agency of the United States of America, or a regulatory/administrative agency of a state government of the United States or a carrier that is listed in the U.S. Pharmacopoeia or other pharmacopoeia; which is generally recognized by those in the art for use in an animal, e.g., a mammal, and, more particularly, in a primate, e.g., a human primate.

The term "**carrier**" as used herein refers to a diluent, adjuvant, excipient, or vehicle that is administered with a composition of the invention. A pharmaceutical carrier typically can be a sterile liquid, such as water or oils, (including those of petroleum, animal, vegetable, or synthetic origin, e.g., such as peanut oil, soybean oil, mineral oil, sesame oil and the like). Typically, sterile water is a preferred carrier when a pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions.

Suitable pharmaceutical excipients include, e.g., without limit, starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water,

ethanol and the like. A composition of the invention, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents.

A composition of the invention can be in a solution, suspension, emulsion, tablet, pill, capsule, powder, sustained-release formulation, etc., or it can be formulated as a suppository (with traditional binders, and/or carriers, e.g., such as triglycerides).

Oral formulations encompassed include, e.g., without limit, standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Additional examples of suitable pharmaceutical carriers are described in the current edition of "Remington's Pharmaceutical Sciences" by E.W. Martin. Such formulations will contain a therapeutically effective amount of a composition of the invention, preferably in purified form, together with a suitable amount of carrier to provide for proper administration to a subject. Traditionally, a formulation will suit the mode of administration.

In a preferred embodiment, a composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to, e.g., a human. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include, e.g., a solubilizing agent and a local anesthetic such as lidocaine to promote comfort at the injection site. Generally, ingredients are supplied either separately or mixed in unit dosage form, e.g., as a dry lyophilized powder or water free concentrate in a hermetically sealed container (such as an ampoule or sachet indicating the quantity of active agent). Where a composition is to be administered by infusion, it can be dispensed using an infusion bottle containing sterile pharmaceutical grade water or saline. Where a composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed before administration.

Compositions of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include, e.g., without limit, anionic salts (such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc.,) and cationic salts, (e.g., such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc). The amount of the composition of the invention that will be effective in the modulation treatment, inhibition, amelioration, or prevention of a disease, syndrome,

condition, or disorder associated with aberrant expression and/or activity of a polypeptide (or fragment thereof), or a polynucleotide (or fragment thereof) of the invention can be determined without undue experimentation by the ordinary artisan using standard clinical techniques.

In addition, *in vitro* assays may optionally be employed to help identify optimal dosage ranges. Dosage requirements in a circumstance typically will depend on, e.g., the route of administration, the seriousness of the disease, condition, syndrome, or disorder; and the judgment of the practitioner or clinician.

Another therapeutic approach included within the invention involves direct administration of a composition of the invention by any conventional administration technique (such as, e.g., without limit, local injection, inhalation, or systemic administration), to a subject with e.g., an infectious, a microbial, a bacterial, a viral or a fungal condition. A composition or formulation may also be targeted to a specific cell or a receptor by any method described herein or known in the art.

Immune Activity

The effect of TGF Betas on immune and inflammatory responses includes (i) inhibition of proliferation of all T-cell subsets (ii) inhibitory effects on proliferation and function of B lymphocytes (iii) down-regulation of natural-killer cell activity and the T-cell response (iv) regulation of cytokine production by immune cells (v) regulation of macrophage function and (vi) leukocyte recruitment and activation. Accordingly, a binding composition of the invention can be useful in ameliorating, treating, preventing, modulating, and/or diagnosing a disease, disorder, syndrome, or condition of the immune system, by, e.g., activating or inhibiting the proliferation, differentiation, or mobilization (chemotaxis or directed movement) of an immune cell. Typically, immune cells develop through a process called hematopoiesis, producing myeloid (platelets, red blood cells, neutrophils, and macrophages) and lymphoid (B and T lymphocytes) cells from pluripotent stem cells. The etiology of an immune disease, disorder, syndrome, or condition may be genetic and/or somatic.

A binding composition can be useful in ameliorating, treating, preventing, modulating, and/or diagnosing a disease, disorder, syndrome, and/or a condition of a hematopoietic cell. A binding composition could be used to increase or inhibit the differentiation or proliferation of a hematopoietic cell, including a pluripotent stem cell

such an effect can be implemented to treat, prevent, modulate, or ameliorate a disease, disorder, syndrome, and/or a condition associated with a decrease in a specific type of hematopoietic cell. An example of such an immunological deficiency, disease, disorder, syndrome, and/or condition includes, e.g., without limitation, a blood condition (e.g. agammaglobulinemia, digammaglobulinemia), ataxia telangiectasia, common variable immunodeficiency, Digeorge Syndrome, HIV infection, HTLV-BLV infection, leukocyte adhesion deficiency syndrome, lymphopenia, phagocyte bactericidal dysfunction, severe combined immunodeficiency (SCIDs), Wiskott-Aldrich Disorder, anemia, thrombocytopenia, or hemoglobinuria.

Moreover, a binding composition can be used to modulate hemostatic or thrombolytic activity. For example, increasing hemostatic or thrombolytic activity can treat or prevent a blood coagulation condition such as e.g., afibrinogenemia, a factor deficiency, a blood platelet disease (e.g. thrombocytopenia), or a wound resulting from e.g., trauma, surgery, etc. Alternatively, a composition of the invention can be used to decrease hemostatic or thrombolytic activity or to inhibit or dissolve a clotting condition. Such compositions can be important in a treatment or prevention of a heart condition, e.g., an attack infarction, stroke, or mycardial scarring.

A binding composition may also be useful in ameliorating, treating, preventing, modulating and/or diagnosing an autoimmune disease, disorder, syndrome, and/or condition such as results, e.g., from the inappropriate recognition by a cell of the immune system of the self as a foreign material. Such an inappropriate recognition results in an immune response leading to detrimental effect destruction on the host, e.g., on a host cell, tissue, protein, or moiety, e.g., a carbohydrate side chain. Therefore, administration of a binding composition which inhibits a detrimental immune response, particularly, e.g., a proliferation, differentiation, or chemotaxis of a T-cell, may be effective in detecting, diagnosing, ameliorating, or preventing such an autoimmune disease, disorder, syndrome, and/or condition. Examples of autoimmune conditions that can be affected by the present invention include, e.g., without limit Addison's Disease syndrome hemolytic anemia, anti-phospholipid syndrome, rheumatoid arthritis, dermatitis, allergic encephalomyelitis, glomerulonephritis, Goodpasture's Syndrome, Graves' Disease syndrome, Multiple Sclerosis, Myasthenia Gravis, Neuritis, Ophthalmia, Bullous Pemphigoid, Pemphigus, Polyendocrinopathies, Purpura, Reiter's Disease syndrome, Stiff-Man Syndrome,

Autoimmune Thyroiditis, Systemic Lupus Erythematosus, Autoimmune Pulmonary Inflammation, Guillain-Barre Syndrome, insulin dependent diabetes mellitus, and autoimmune inflammatory eye disease.

Similarly, allergic reactions and conditions, such as asthma (e.g., allergic asthma) or other respiratory problems, may also be ameliorated, treated, modulated or prevented, and/or diagnosed by a binding composition (or fragment thereof), or an agonist or antagonist thereto. Moreover, such inventive compositions can be used to effect, e.g., anaphylaxis, hypersensitivity to an antigenic molecule, or blood group incompatibility.

A binding composition may also be used to modulate, ameliorate, treat, prevent, and/or diagnose organ rejection or graft-versus-host disease (GVHD). Generally speaking, organ rejection occurs by a host's, immune-cell destruction of a transplanted tissue or cell. A similarly destructive immune response is involved in GVHD, however, in this case, transplanted foreign immune cells destroy host tissues and/or cells. Administration of a composition of the invention, which ameliorates or modulates such a deleterious immune response (e.g., a deleterious proliferation, differentiation, or chemotaxis of a T cell), can be effective in modulating, ameliorating, diagnosing, and/or preventing organ rejection or GVHD.

Similarly, a binding composition may also be used to detect, treat, modulate, ameliorate, prevent, and/or diagnose an inflammation, e.g., by inhibiting the proliferation and/or differentiation of a cell involved in an inflammatory response, or an inflammatory condition (either chronic or acute), including, e.g., without limitation, chronic prostatitis, granulomatous prostatitis and malacoplakia, an inflammation associated with an infection (such as, e.g., septic shock, sepsis, or systemic inflammatory response syndrome (SIRS)), ischemia-reperfusion injury, endotoxin lethality, arthritis, complement-mediated hyperacute rejection, nephritis, cytokine or chemokine induced lung injury, inflammatory bowel disease syndrome, Crohn's disease syndrome, or a condition resulting from an over production of a cytokine(s) (e.g., TNF or IL-1 .)

Proliferative Disorders

An binding composition can be used to modulate, ameliorate, treat, prevent, and/or diagnose a hyperproliferative disease, condition, disorder, or syndrome (such as, e.g., a neoplasm) via direct or indirect interactions. For example, such as by initiating the proliferation of cells that, in turn, modulate a hyperproliferative state; or by increasing an

immune response (e.g., by increasing the antigenicity of a protein involved in a hyperproliferative condition); or by causing the proliferation, differentiation, or mobilization of a specific cell type (e.g., a T-cell). A desired effect using a composition of the invention may also be accomplished either by, e.g., enhancing an existing immune response, or by initiating a new immune response. Alternatively, the desired result may be effected either by, e.g., diminishing or blocking an existing immune response, or by preventing the initiation of a new immune response.

Examples of such hyperproliferative states, diseases, disorders, syndromes, and/or conditions include, e.g., without limitation, a neoplasm of the colon, abdomen, bone, breast, digestive system, liver, pancreas, peritoneum, endocrine system (e.g., an adrenal gland, a parathyroid gland, the pituitary, the testicles, the ovary, the thymus, or the thyroid), eye, head, neck, nervous system (central or peripheral), the lymphatic system, pelvis, skin, spleen, thorax, and urogenital system. Similarly, other hyperproliferative conditions, include, e.g., without limit hypergammaglobulinemia, lymphoproliferative conditions, paraproteinemias, purpura, sarcoidosis, Hamartoma, Sezary Syndrome, Waldenstron's Macroglobulinemia, Gaucher's Disease syndrome, histiocytosis, and other hyperproliferative states. For example, TGF-beta1 is an important regulator of the normal and malignant prostate. Prostate cancer cells express high levels of TGF-beta1, which seems to enhance prostate cancer growth and metastasis by stimulating angiogenesis and by inhibiting immune responses directed against tumour cells. Prostate cancer cells frequently lose their TGF-beta receptors and acquire resistance to the anti-proliferative and pro-apoptotic effects of TGF-beta1. Accordingly, high expression of TGF-beta1 and loss of TGF-beta receptor expression have been associated with a particularly bad prognosis in human prostate cancer patients. TGF-Beta1 is also associated with breast cancer.

One preferred embodiment utilizes an binding composition to inhibit aberrant cellular division, through a polynucleotide delivery technique. Thus, the present invention provides a method for treating, preventing, modulating, ameliorating, preventing, inhibiting, and/or diagnosing cell proliferative diseases, disorders, syndromes, and/or conditions described herein by inserting into an abnormally proliferating cell a composition of the present invention, wherein said composition beneficially modulates an

excessive condition of cell proliferation, e.g., by inhibiting transcription and/or translation.

Local administration to an abnormally proliferating cell may be achieved by any art known method or technique discussed herein including, e.g., without limit to transfection, electroporation, microinjection of cells, or in vehicles (such as a liposome, lipofectin, or a naked polynucleotide). By **"cell proliferative condition"** is meant any human or animal disease, syndrome, disorder, condition, or state, affecting any cell, tissue, any site or any combination of organs, tissues, or body parts, which is characterized by a single or multiple local abnormal proliferation of cells, groups of cells, or tissues, whether benign or malignant. Any amount of binding composition may be administered as long as it has a desired effect on the treated cell, e.g., a biologically inhibiting effect on an abnormally proliferating cell.

The present invention also encompasses an antibody-based therapy that involves administering an antibody binding composition to a subject to ameliorate, treat, prevent, modulate, and/or diagnose one or more of the described diseases, disorders, syndromes, and/or conditions discussed herein. Methods for producing antibodies (both polyclonal and monoclonal) are known in the art or described herein. Such antibodies may be provided in a pharmaceutically acceptable formulation as known in the art or described herein. A partial summary of the manner in which an antibody may be used includes, e.g., binding polypeptides (or fragments thereof) of TGF Beta 1 locally or systemically in the body or by direct cytotoxicity of the antibody, e.g. as mediated by complement (CDC) or by effector cells (ADCC). Some of these approaches are described in more detail herein. Supplied with the teachings provided herein, one of ordinary skill in the art will know how to use a binding composition for diagnostic, monitoring, or therapeutic purposes without undue experimentation. In particular, an antibody, fragment, or derivative thereof of the present invention is useful for ameliorating, modulating, treating, preventing, and/or diagnosing a subject having or developing a cell proliferative and/or a cell differentiation disease, syndrome, disorder, state and/or condition as described herein. Bringing about an effect on such a condition can include, e.g., administering a single or multiple dose of an Antibody, or its fragment, derivative, or conjugate thereof.

An antibody may be advantageously utilized in combination with other monoclonal or chimeric antibodies, or with lymphokines or hematopoietic growth factors

which, e.g., serve to increase the number or activity of effector cells that interact with an antibody. Preferably a high affinity and/or potent *in vivo* inhibiting and/or neutralizing antibody that selectively and/or specifically binds TGF Beta 1 polypeptide (or fragment thereof), or an agonist or antagonist thereto, will be used against a composition of the present invention for an immunoassay on to effect a disease, disorder, syndrome, and/or condition which is associated with expression of a TGF Beta 1 polypeptide (or fragment thereof) or an agonist or antagonist thereto.

An antibody, fragments thereof, or regions thereof, preferably will have a binding affinity for the TGF Beta 1 protein or fragment thereof that will be, e.g., with a dissociation constant or Kd less than about 5X10^{-}6M, 10^{-}6M, 5X10^{-}7M, 10^{-}7M, 5X10^{-}8M, 10^{-}8M, 5X10^{-}9M, 10^{-}9M, 5X10^{-}10M, 10^{-}10M, 5X10^{-}11M, 10^{-}11M, 5X10^{-}12M, 10^{-}12M, 5X10^{-}13M, 10^{-}13M, 5X10^{-}14M, 10^{-}14M, 5X10^{-}15M, or 10^{-}15M.

Cardiovascular Conditions

A binding composition may be used to, modulate, ameliorate, effect, treat, prevent, and/or diagnose a cardiovascular disease, disorder, syndrome, and/or condition. As described herein, including, e.g., without limitation, cardiovascular abnormalities, such as arterio-arterial fistula, arteriovenous fistula, cerebral arteriovenous malformations, congenital heart defects, pulmonary atresia, and Scimitar Syndrome peripheral artery disease, syndrome, such as limb ischemia.

Additional cardiovascular disorders encompass, e.g., congenital heart defects which include, e.g., aortic coarctation, car triatriatum, coronary vessel anomalies, crisscross heart, dextrocardia, patent ductus arteriosus, Ebstein's anomaly, Eisenmenger complex, hypoplastic left heart syndrome, levocardia, tetralogy of fallot, transposition of great vessels, double outlet right ventricle, tricuspid atresia, persistent truncus arteriosus, and heart septal defects, such as e.g., aortopulmonary septal defect, endocardial cushion defects, Lutembacher's Syndrome, trilogy of Fallot, and ventricular heart septal defects.

Further cardiovascular conditions include, e.g., heart disease syndrome, such as, e.g., arrhythmias, carcinoid heart disease syndrome, high cardiac output, low cardiac output, cardiac tamponade, endocarditis (including bacterial endocarditis), heart aneurysm, cardiac arrest, congestive heart failure, congestive cardiomyopathy, paroxysmal dyspnea, cardiac edema, heart hypertrophy, congestive cardiomyopathy, left ventricular hypertrophy, right ventricular hypertrophy, post-infarction heart rupture,

ventricular septal rupture, heart valve disease, myocardial disease, myocardial ischemia, pericardial effusion, pericarditis (including constrictive and tuberculous pericarditis), pneumopericardium, post-pericardiotomy syndrome, pulmonary heart disease syndrome, rheumatic heart disease syndrome, ventricular dysfunction, hyperemia, cardiovascular pregnancy complications, Scimitar Syndrome, cardiovascular syphilis, and cardiovascular tuberculosis.

Further cardiovascular disorders include, e.g., arrhythmias including, e.g., sinus arrhythmia, atrial fibrillation, atrial flutter, bradycardia, extra systole, Adams-Stokes Syndrome, bundle-branch block, sinoatrial block, long QT syndrome, parasystole, Lown-Ganong-Levine Syndrome, Mahaim-type pre-excitation syndrome, Wolff-Parkinson-White syndrome, sick sinus syndrome, and ventricular fibrillation tachycardias.

Tachycardias encompassed with the cardiovascular condition described herein include, e.g., paroxysmal tachycardia, supraventricular tachycardia, accelerated idioventricular rhythm, atrioventricular nodal re-entry tachycardia, ectopic atrial tachycardia, ectopic junctional tachycardia, sinoatrial nodal re-entry tachycardia, sinus tachycardia, Torsades de Pointes Syndrome, and ventricular tachycardia.

Additional cardiovascular disorders include, e.g., heart valve disease such as, e.g., aortic valve insufficiency, aortic valve stenosis, heart murmurs, aortic valve prolapse, mitral valve prolapse, tricuspid valve prolapse, mitral valve insufficiency, mitral valve stenosis, pulmonary atresia, pulmonary valve insufficiency, pulmonary valve stenosis, tricuspid atresia, tricuspid valve insufficiency, and tricuspid valve stenosis.

Myocardial conditions associated with cardiovascular disease include, e.g., myocardial diseases such as, e.g., alcoholic cardiomyopathy, congestive cardiomyopathy, hypertrophic cardiomyopathy, aortic subvalvular stenosis, pulmonary subvalvular stenosis, restrictive cardiomyopathy, Chagas cardiomyopathy, endocardial fibroelastosis, endomyocardial fibrosis, Kearns Syndrome, myocardial reperfusion injury, and myocarditis.

Cardiovascular conditions include, e.g., myocardial ischemias such as, e.g., coronary disease syndrome, such as e.g., angina pectoris, coronary aneurysm, coronary arteriosclerosis, coronary thrombosis, coronary vasispasm, myocardial infarction, and myocardial stunning.

Cardiovascular diseases also encompassed herein include, e.g., vascular diseases such as e.g., aneurysms, angiodysplasia, angiomatosis, bacillary angiomatosis, Hippel-Lindau Disease syndrome, Klippel-Trenaunay-Weber Syndrome, Sturge-Weber Syndrome, angioneurotic edema, aortic disease, Takayasu's Arteritis, aortitis, Leriche's Syndrome, arterial occlusive disease, arteritis, enarteritis, polyarteritis nodosa, cerebrovascular disease, diabetic angiopathies, diabetic retinopathy, embolism, thrombosis, erythromeialgia, hemorrhoids, hepatic veno-occlusive disease syndrome, hypertension, hypotension, ischemia, peripheral vascular diseases, phlebitis, pulmonary veno-occlusive disease syndrome, Raynaud's disease syndrome, CREST syndrome, retinal vein occlusion, Scimitar syndrome, superior vena cava syndrome, telangiectasia, ataxia telangiectasia, hereditary hemorrhagic telangiectasia, varicocele, varicose veins, varicose ulcer, vasculitis, and venous insufficiency.

Cardiovascular conditions further include, e.g., aneurysms such as, e.g., dissecting aneurysms, false aneurysms, infected aneurysms, ruptured aneurysms, aortic aneurysms, cerebral aneurysms, coronary aneurysms, heart aneurysms, and iliac aneurysms.

Arterial occlusive cardiovascular conditions include, e.g., arteriosclerosis, intermittent claudication, carotid stenosis, fibromuscular dysplasias, mesenteric vascular occlusion, Moyamoya disease syndrome, renal artery obstruction, retinal artery occlusion, and thromboangiitis obliterans.

Cerebrovascular cardiovascular conditions include, e.g., carotid artery disease, cerebral amyloid angiopathy, cerebral aneurysm, cerebral anoxia, cerebral arteriosclerosis, cerebral arteriovenous malformation, cerebral artery disease, cerebral embolism and thrombosis, carotid artery thrombosis, sinus thrombosis, Wallenberg's syndrome, cerebral hemorrhage, epidural hematoma, subdural hematoma, subarachnoid hemorrhage, cerebral infarction, cerebral ischemia (including transient cerebral ischemia), subclavian steal syndrome, periventricular leukomalacia, vascular headache, cluster headache, migraine, and vertebrobasilar insufficiency.,

Embolic cardiovascular conditions include, e.g., air embolisms, amniotic fluid embolisms, cholesterol embolisms, blue toe syndrome, fat embolisms, pulmonary embolisms, and thromboembolisms.

Thrombotic cardiovascular conditions include, e.g., coronary thrombosis, hepatic vein thrombosis, retinal vein occlusion, carotid artery thrombosis, sinus thrombosis,

Wallenberg's syndrome, and thrombophlebitis. Ischemic conditions include, e.g., cerebral ischemia, ischemic colitis, compartment syndromes, anterior compartment syndrome, myocardial ischemia, reperfusion injuries, and peripheral limb ischemia.

Vasculitic conditions include, e.g., aortitis, arteritis, Behcet's Syndrome, Churg-Strauss Syndrome, mucocutaneous lymph node syndrome, thromboangiitis obliterans, hypersensitivity vasculitis, Schoenlein-Henoch purpura, allergic cutaneous vasculitis, and Wegener's granulomatosis.

A binding composition can be beneficial in ameliorating critical limb ischemia and coronary disease. A binding composition may be administered using any art known method, described herein A binding composition may administered as part of a therapeutic composition or formulation, as described in detail herein. Methods of delivering a binding composition are also described herein.

Anti-Hemopoietic Activity

The naturally occurring balance between endogenous stimulators and inhibitors of angiogenesis is one in which inhibitory influences typically predominate (see, e.g., Rastinejad, et al., Cell 56345-355 (1989)). When neovascularization occurs under normal physiological conditions, such as wound healing, organ regeneration, embryonic development, and female reproductive processes, angiogenesis is stringently regulated, and delimited spatially and temporally. In pathological angiogenesis such as, e.g., during solid tumor formation, these regulatory controls fail and unregulated angiogenesis can become pathologic by sustaining progression of many neoplastic and non-neoplastic diseases.

A number of serious diseases are dominated by abnormal neovascularization (including, e.g., solid tumor growth and metastases, arthritis, some types of eye conditions, and psoriasis; see, e.g., reviews by Moses, et al., Biotech. 9630-634 (1991); Folkman, et al., N. Engl. J. Med., 333: 1757-1763 (1995); Auerbach, et al., J. Microvasc. Res. 29:401-4 11 (1985); Folkman, "Advances in Cancer Research," eds. Klein and Weinhouse, Academic Press, New York, pp. 175-203 (1985); Patz, Am. J. Opthalmol. 94:7 15-743 (1982); and Folkman, et al., Science 221:7 19-725 (1983). Moreover, TGF beta 1 and its receptors ALK-5 and ALK-1, are implied in the vascular maturation phase of angiogenesis (see, e.g., Bull Acad Natl Med. 2000;184 (3):537-44). In a number of pathological conditions, angiogenesis contributes to a disease-state, e.g., for example,

significant data have accumulated suggesting that solid tumor formation is dependent on angiogenesis (see, e.g., Folkman and Klagsbrun, Science 235:442-447 (1987)). In another embodiment of the invention, administration of a binding composition provides for the treatment, amelioration, modulation, diagnosis, and/or inhibition of a disease, disorder, syndrome, and/or condition associated with neovascularization.

Malignant and metastatic conditions that can be effected in a desired fashion using a binding composition include, e.g., without limitation, a malignancy, solid tumor, and a cancer as described herein or as otherwise known in the art (for a review of such disorders, syndromes, etc. see, e.g., Fishman, et al., Medicine, 2d Ed., J. B. Lippincott Co., Philadelphia (1985)). Thus, the present invention provides a method of ameliorating, modulating, treating, preventing, and/or diagnosing an angiogenesis-related disease and/or disorder, comprising administering to a subject in need thereof a beneficially effective amount of a binding composition. For example, cancers that may be so affected using a composition of the invention includes, e.g., without limit a solid tumor, including e.g., prostate, lung, breast, ovarian, stomach, pancreas, larynx, esophagus, testes, liver, parotid, biliary tract, colon, rectum, cervix, uterus, endometrium, kidney, bladder, thyroid cancer; primary tumors and metastases; melanomas; glioblastoma; Kaposi's sarcoma; leiomyosarcoma; non-small cell lung cancer; colorectal cancer; advanced malignancies; and blood born tumors such as e.g., leukemia.

Moreover, a binding composition may be delivered topically, to treat or prevent cancers such as, e.g., skin cancer, head and neck tumors, breast tumors, and Kaposi's sarcoma. Within yet another aspect, a binding composition may be utilized to treat superficial forms of bladder cancer by, e.g., intravesical administration into the tumor, or near the tumor site; via injection or a catheter. Of course, the appropriate mode of administration will vary according to the cancer to be treated. Other modes of delivery are discussed herein.

A binding composition may also be useful in modulating, ameliorating, treating, preventing, and/or diagnosing another disease, disorder, syndrome, and/or condition, besides a cell proliferative condition (e.g., a cancer) that is assisted by abnormal angiogenic activity. Such close group conditions include, e.g., without limitation, benign tumors, e.g., such as hemangiomas, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas; atherosclerotic plaques; ocular angiogenic diseases, e.g., diabetic

retinopathy, retinopathy of prematurity, macular degeneration, cornea graft rejection, neovascular glaucoma, retrolental fibroplasia, rubeosis, retinoblastoma, uvietis and Pterygia (abnormal blood vessel growth) of the eye; rheumatoid arthritis; psoriasis; delayed wound healing; endometriosis; vasculogenesis; granulations; hypertrophic scars (keloids); nonunion fractures; scleroderma; trachoma; vascular adhesions; myocardial angiogenesis; coronary collaterals; cerebral collaterals; arteriovenous malformations; ischemic limb angiogenesis; Osler-Webber Syndrome; plaque neovascularization; telangiectasia; hemophiliac joints; angiofibroma; fibromuscular dysplasia; wound granulation; Crohn's disease; and atherosclerosis.

As noted above, the present invention also provides methods for ameliorating, treating, preventing, and/or diagnosing neovascular diseases of the eye, including e.g., corneal graft neovascularization, neovascular glaucoma, proliferative diabetic retinopathy, retrolental fibroplasia and macular degeneration. Moreover, ocular diseases, disorders, syndromes, and/or conditions associated with neovascularization that can be modulated ameliorated, treated, prevented, and/or diagnosed with a binding composition include, e.g., without limit; neovascular glaucoma, diabetic retinopathy, retinoblastoma, retrolental fibroplasia, uveitis, retinopathy of premature macular degeneration, corneal graft neovascularization, as well as other inflammatory eye diseases, ocular tumors, and diseases associated with choroidal or iris neovascularization (see, e.g., reviews by Waltman, et al., (1978) Am. J. Ophthal. 8.51704-710 and Gartner, et al., (1978) Sun. Ophthd. 22:291-3 12). Thus, within one aspect of the present invention methods are provided for treating or preventing neovascular diseases of the eye such as corneal neovascularization (including corneal graft neovascularization), comprising administering to a patient a therapeutically effective amount of a binding composition to the cornea, such that the formation of blood vessels is inhibited or delayed. Briefly, the cornea is a tissue that normally lacks blood vessels. In certain pathological conditions however, capillaries may extend into the cornea from the pericorneal vascular plexus of the limbus. When the cornea becomes vascularized, it also becomes clouded, resulting in a decline in the patient's visual acuity. Visual loss may become complete if the cornea completely opacifies. A wide variety of diseases, disorders, syndromes, and/or conditions can result in corneal neovascularization, including e.g., corneal infections (e.g., trachoma, herpes simplex keratitis, leishmaniasis and onchocerciasis), immunological processes (e.g., graft

rejection and Stevens-Johnson's syndrome), alkali burns, trauma, inflammation (of any cause), toxic and nutritional deficiency states, and as a complication of using contact lenses.

Within another aspect, methods are provided for treating or preventing neovascular glaucoma, comprising administering to a patient a therapeutically effective amount of a binding composition to the eye, such that the formation of blood vessels is inhibited. In one embodiment, the composition may be administered topically to the eye to treat or prevent early forms of neovascular glaucoma. Within other embodiments, the composition may be implanted by injection into the region of the anterior chamber angle. Within other embodiments, the composition may also be placed in any location such that the composition is continuously released into the aqueous humor. Within another aspect, methods are provided for treating or preventing proliferative diabetic retinopathy, comprising administering to a patient a therapeutically effective amount of a binding composition to the eyes, such that the formation of blood vessels is inhibited.

Additional, diseases, disorders, syndromes, and/or conditions that can be modulated, ameliorated, treated, prevented, and/or diagnosed with a binding composition include, e.g., without limitation, hemangioma, arthritis, psoriasis, angiofibroma, atherosclerotic plaques, delayed wound healing, granulations, hemophilic joints, hypertrophic scars, nonunion fractures, Osler-Weber syndrome, pyogenic granuloma, scleroderma, trachoma, and vascular adhesions.

Moreover, diseases, disorders, states, syndromes, and/or conditions that can be modulated, ameliorated, treated, prevented, and/or diagnosed with a binding composition include, e.g., without limitation, solid tumors, blood born tumors such as leukemias, tumor metastasis, Kaposi's sarcoma, benign tumors (e.g., hemangiomas), acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas, rheumatoid arthritis, psoriasis, ocular angiogenic diseases, e.g., diabetic retinopathy, retinopathy of prematurity, macular degeneration, corneal graft rejection, neovascular glaucoma, retrolental fibroplasia, rubeosis, retinoblastoma, and uvietis, delayed wound healing, endometriosis, vasculogenesis, granulations, hypertrophic scars (keloids), nonunion fractures, scleroderma, trachoma, vascular adhesions, myocardial angiogenesis, coronary collaterals, cerebral collaterals, arteriovenous malformations, ischemic limb angiogenesis, Osler-Webber Syndrome, plaque neovascularization, telangiectasia, hemophiliac joints,

angiofibroma fibromuscular dysplasia, wound granulation, Crohn's disease, syndrome, atherosclerosis, birth-control inhibition of vascularization necessary for embryo implantation during the control of menstruation, and diseases that have angiogenesis as a pathologic consequence such as, e.g., cat scratch disease (Rochele minalia quintosa), ulcers (*Helicobacter pylori*), Bartonellosis and bacillary angiomatosis.

Diseases at the Cellular Level

Diseases associated with increased cell survival or the inhibition of apoptosis that could be modulated, ameliorated, treated, prevented, and/or diagnosed by a binding composition include, e.g., cancers (such as, e.g., follicular lymphomas, carcinomas with p53 mutations, and hormone-dependent tumors, including, e.g., but without limit, colon cancer, cardiac tumors, pancreatic cancer, melanoma, retinoblastoma, glioblastoma, lung cancer, intestinal cancer, testicular cancer, stomach cancer, neuroblastoma, myxoma, myoma, lymphoma, endothelioma, osteoblastoma, osteoclastoma, osteosarcoma, chondrosarcoma, adenoma, breast cancer, prostate cancer, Kaposi's sarcoma and ovarian cancer); autoimmune conditions (such as, e.g., multiple sclerosis, Sjogren's syndrome, Hashimoto's thyroiditis, biliary cirrhosis, Behcet's disease syndrome, Crohn's disease syndrome, polymyositis, systemic lupus erythematosus, immune-related glomerulonephritis, and rheumatoid arthritis); viral infections (such as, e.g., herpes viruses, pox viruses, and adenoviruses); inflammation; graft v. host disease syndrome, acute graft rejection, and chronic graft rejection.

In preferred embodiments, a binding composition is used to inhibit growth, progression, and/or metastases of cancers such as, in particular, those listed herein. Additional diseases, states, syndromes, or conditions associated with increased cell survival that could be modulated, ameliorated, treated, prevented, or diagnosed by a binding composition include, e.g., without limitation, progression, and/or metastases of malignancies and related disorders such as leukemia including acute leukemias (such as, e.g., acute lymphocytic leukemia, acute myelocytic leukemia, including myeloblastic, promyelocytic, myelomonocytic, monocytic, and erythroleukemia) and chronic leukemias (e.g., chronic myelocytic, chronic granulocytic, leukemia, and chronic lymphocytic leukemia)), polycythemia Vera, lymphomas (e.g., Hodgkin's disease, and non-Hodgkin's disease), multiple myeloma, Waldenstrom's macroglobulinemia, heavy chain disease, syndrome, and solid tumors including, e.g., without limitation, sarcomas and carcinomas

(such as, e.g., fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, menangioma, melanoma, neuroblastoma, and retinoblastoma).

Diseases associated with increased apoptosis that could be modulated, ameliorated, treated, prevented, and/or diagnosed by a binding composition include, e.g., AIDS, conditions (such as, e.g., Alzheimer's disease syndrome, Parkinson's disease syndrome, Amyotrophic lateral sclerosis, Retinitis pigmentosa, Cerebellar degeneration and brain tumor, or prion associated disease); autoimmune conditions (such as, e.g., multiple sclerosis, Sjogren's syndrome, Hashimoto's thyroiditis, biliary cirrhosis, Behcet's disease syndrome, Crohn's disease syndrome, polymyositis, systemic lupus erythematosus, immune-related glomerulonephritis, and rheumatoid arthritis); myelodysplastic syndromes (such as aplastic anemia), graft v. host disease syndrome; ischemic injury (such as that caused by myocardial infarction, stroke and reperfusion injury); liver injury (such as, e.g., hepatitis related liver injury, ischemia reperfusion injury, cholestosis (bile duct injury), and liver cancer); toxin-induced liver disease (such as, e.g., that caused by alcohol), septic shock, cachexia, and anorexia.

Moreover, a binding composition could be used to prevent and heal damage to the lungs due to various pathological states, such as, e.g., stimulating proliferation and differentiation to promote repair of alveoli and bronchiolar epithelium. For example, emphysema, inhalation injuries, that (e.g., from smoke inhalation) and burns, which cause necrosis of the bronchiolar epithelium and alveoli could be effectively ameliorated,

treated, prevented, and/or diagnosed using a polynucleotide or polypeptide of the invention (or fragment thereof), or an agonist or antagonist thereto.

Also, a binding composition could be used to stimulate the proliferation of and differentiation of type II pneumocytes, to help treat or prevent hyaline membrane diseases, such as e.g., infant respiratory distress syndrome and bronchopulmonary displasia, (in premature infants). A binding composition could stimulate the proliferation and/or differentiation of a hepatocyte and, thus, could be used to alleviate or treat a liver condition such as e.g., fulminant liver failure (caused, e.g., by cirrhosis), liver damage caused by viral hepatitis and toxic substances (e.g., acetaminophen, carbon tetrachloride, and other known hepatotoxins).

Neurological Diseases

Nervous system diseases, disorders, syndromes, states, and/or conditions that can be modulated, ameliorated, treated, prevented, and/or diagnosed with a binding composition include, e.g., without limitation, nervous system injuries diseases, disorders, states, syndromes, and/or conditions that result in either a disconnection or misconnection of an axon or dendrite; a diminution or degeneration of a cell (or part of a cell) of the nervous system (such as, e.g., without limitation, neurons, astrocytes, microglia, macroglia, oligodendroglia, Schwann cells, and ependymal cells); demyelination or improper mylenation; neural cell dysfunction (such as, e.g., failure of neurotransmitter release or uptake); or interference with mylenization. For example, TGF-beta 1 mRNA and protein were recently found to increase in animal brains after experimental lesions that cause local deafferentation or neuron death. Elevations of TGF-beta 1 mRNA after lesions are prominent in microglia but are also observed in neurons and astrocytes. Moreover, TGF-beta 1 mRNA autoinduces its own mRNA in the brain. These responses provide models for studying the increases of TGF-beta 1 protein observed in beta A/amyloid-containing extracellular plaques of Alzheimer's disease (AD) and Down's syndrome (DS) and in brain cells of AIDS victims. Involvement of TGF-beta 1 in these human brain disorders has been discussed in relation to the potent effects of TGF-beta 1 on wound healing and inflammatory responses in peripheral tissues. It is hypothesized that TGF-beta 1 has an organizing role in responses to neurodegeneration and brain injury that are similar to those observed in non-neural tissues. (see, e.g., Finch, et al., J Cell Biochem. 1993 Dec;53(4):314-22).

Nervous system lesions that may be modulated, ameliorated, treated, prevented, and/or diagnosed in a subject using a binding composition of the invention, include, e.g., without limitation, the following lesions of either the central (including spinal cord and brain) or peripheral nervous system: (1) ischemic lesions, in which a lack of oxygen in a portion of the nervous system results in neuronal injury or death, including e.g., cerebral infarction (or ischemia), or spinal cord infarction (or ischemia); (2) traumatic lesions, including, e.g., lesions caused by physical injury or associated with surgery (e.g., lesions that sever a portion of the nervous system), or compression injuries; (3) malignant lesions, in which a portion of the nervous system is comprised by malignant tissue, which is either a nervous system associated malignancy or a malignancy derived from non-nervous-system tissue; (4) infectious lesions, in which a portion of the nervous system is comprised because of infection (e.g., by an abscess or associated with infection by human immunodeficiency virus, herpes zoster, or herpes simplex virus or with Lyme disease, syndrome, tuberculosis, syphilis); (5) degenerative lesions, in which a portion of the nervous system is comprised because of a degenerative process including, without limit, degeneration associated with Parkinson's disease syndrome, Alzheimer's disease syndrome, Huntington's chorea, or Amyotrophic lateral sclerosis (ALS); (6) lesions associated with a nutritional condition, in which a portion of the nervous system is comprised by a nutritional disorder (or a disorder of metabolism including, without limit, vitamin B 12 deficiency, folic acid deficiency, Wernicke disease, syndrome, tobacco-alcohol amblyopia, Marchiafava-Bignami disease (primary degeneration of the corpus callosum), and alcoholic cerebellar degeneration; (7) neurological lesions associated with systemic diseases including, e.g., without limitation, diabetes (diabetic neuropathy, Bell's palsy), systemic lupus erythematosus, carcinoma, or sarcoidosis; (8) lesions caused by toxic substances including e.g., alcohol, lead, or a neurotoxin; and (9) demyelinating lesions in which a portion of the nervous system is comprised by a demyelinating cause (including, e.g., without limitation, multiple sclerosis, human immunodeficiency virus-associated myelopathy, transverse myelopathy or various etiologies, progressive multifocal leukoencephalopathy, and central pontine myelinolysis).

In specific embodiments, motor neuron diseases, disorders, syndromes, and/or conditions that may be modulated, ameliorated, treated, prevented, and/or diagnosed using a binding composition include, e.g., without limitation, infarction, infection,

exposure to toxin, trauma, surgical damage, degenerative disease or malignancy (that may affect motor neurons as well as other components of the nervous system), as well as conditions that selectively affect neurons such as, e.g., without limitation, Amyotrophic lateral sclerosis progressive spinal muscular atrophy, progressive bulbar palsy, primary lateral sclerosis, infantile and juvenile muscular atrophy, progressive bulbar paralysis of childhood (Fazio-Londe syndrome), poliomyelitis post polio syndrome, and Hereditary Motorsensory Neuropathy (Charcot-Marie-Tooth Disease).

Chemotaxis

A binding composition may have an effect on a chemotaxis activity. TGF-β1 has been shown to be chemotactic for fibroblasts; it induces the synthesis of matrix proteins and glycoproteins and inhibits collagen degradation by induction of protease inhibitors and reduction of metalloproteases (see, e.g., Ward & Hunninghake, Am. J. Respir. Crit. Care Med. 1998; 157: S123-S129). Briefly, chemotactic molecules can attract or mobilize (but may also repeal) cells (e.g., monocytes, fibroblasts, neutrophils, T-cells, mast cells, eosinophils, epithelial and/or endothelial cells) or cell processes (e.g., filopodia, psuedopodia, lamellapodia, dendrites, axons, etc.) to a particular site (e.g., such as inflammation, infection, site of hyperproliferation, the floor plate of the developing spinal cord, etc.). In some instances, such mobilized cells can then fight off and/or modulate a particular trauma, abnormality, condition, syndrome, or disease. A binding composition may have an effect on a chemotactic activity of a cell (such as, e.g., an attractive or repulsive effect).

A chemotactic molecule can be used to modulate, ameliorate, treat, prevent, and/or diagnose inflammation, infection, hyperproliferative diseases, disorders, syndromes, and/or conditions, or an immune system disorder by increasing the number of cells targeted to a particular location in the body. For example, a chemotactic molecule can be used to attract an immune cell to an injured location in a subject. A binding composition that had an effect on a chemotactant could also attract a fibroblast, which can be used to modulate, ameliorate, and/or treat a wound. It is also contemplated that a binding composition may inhibit a chemotactic activity to modulate, ameliorate, treat, prevent, and/or diagnose a disease, disorder, syndrome, and/or a condition.

Kits

This invention also contemplates use of binding compositions in a variety of diagnostic kits and methods for detecting the presence of TGF Beta 1. Typically, the kit will have a compartment containing either a defined TGF Beta 1 or a binding composition, which recognizes one or the other, e.g., binding partner fragments or antibodies.

A preferred kit for determining the concentration of TGF Beta 1 in a sample would typically comprise a labeled compound, e.g., binding composition or antibody, having known binding affinity for the TGF Beta 1 protein, a source of TGF Beta 1 protein (naturally occurring or recombinant), and a means for separating the bound from free labeled compound, for example, a solid phase for immobilizing the TGF Beta 1 protein. Compartments containing reagents, and instructions, will normally be provided.

Antibodies, including antigen binding fragments, specific for a TGF Beta 1 protein or fragments thereof are useful in diagnostic applications to detect the presence of elevated levels of TGF Beta 1 protein and/or its fragments. Such diagnostic assays can employ lysates, live cells, fixed cells, immunofluorescence, cell cultures, body fluids, and further can involve the detection of antigens related to the protein in serum, or the like. Diagnostic assays may be homogeneous (without a separation step between free reagent and antigen-TGF Beta 1 or -WDS protein complex) or heterogeneous (with a separation step). Various commercial assays exist, such as radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA), enzyme immunoassay (EIA), enzyme-multiplied immunoassay technique (EMIT), substrate-labeled fluorescent immunoassay (SLFIA), and the like. For example, unlabeled antibodies are employed by using a second antibody which is labeled and which recognizes an antibody to a TGF Beta 1 protein or to a particular fragment thereof. Similar assays are also extensively discussed in the literature (see, e.g., Harlow and Lane (1988) Antibodies: A Laboratory Manual, CSH Press, NY; Chan (ed.) (1987) Immunoassay: A Practical Guide Academic Press, Orlando, FL; Price and Newman (eds.) (1991) Principles and Practice of Immunoassay Stockton Press, NY; and Ngo (ed.) (1988) Nonisotopic Immunoassay Plenum Press, NY).

Anti-idiotypic antibodies may have similar use to diagnose the presence of antibodies against an TGF Beta 1 protein or polypeptide, as such may be diagnostic of various abnormal states, conditions, disorders, or syndromes. For example,

overproduction of TGF Beta 1 protein may result in production of various immunological or other physiological reactions which may be diagnostic of abnormal physiological states, e.g., in cell growth, activation, or differentiation.

Frequently, the reagents for diagnostic assays are supplied in kits, to optimize the sensitivity of the assay. For the instant invention, depending upon the nature of the assay, the protocol, and the label, either labeled or unlabeled antibody or binding composition, or labeled TGF Beta 1 protein is provided. This is usually in conjunction with other additives, such as buffers, stabilizers, materials necessary for signal production such as substrates for enzymes, and the like. Preferably, the kit contains instructions for proper use and disposal of the contents after use. Typically, the kit has compartments for each useful reagent. Desirably, the reagents are provided as a dry lyophilized powder, where the reagents may be reconstituted in an aqueous medium providing appropriate concentrations of reagents for performing the assay.

Many of the aforementioned constituents of the drug screening and the diagnostic assays may be used without modification, or may be modified in a variety of ways. For example, labeling may be achieved by covalently or non-covalently joining a moiety that directly or indirectly provides a detectable signal. In any of these assays, the protein, test compound, TGF Beta 1 protein or polypeptide (or antibodies thereto) are labeled either directly or indirectly. Possibilities for direct labeling include label groups such as, e.g., without limitation, radiolabels (e.g., ^{125}I); enzymes (U.S. Pat. No. 3,645,090) such as peroxidase and alkaline phosphatase; and fluorescent labels (U.S. Pat. No. 3,940,475) that are capable of monitoring the change in fluorescence intensity, wavelength shift, or fluorescence polarization. Possibilities for indirect labeling include biotinylation of one constituent followed by binding to avidin coupled to an art known label such as one of the above.

There are also numerous methods of separating the bound from the free protein, or alternatively bound from free test compound. A TGF Beta 1 protein is immobilized on various matrices followed by washing. Suitable matrices include plastic such as an ELISA plate, filters, and beads. Methods of immobilizing the TGF Beta 1 protein to a matrix include, without limitation, direct adhesion to plastic, use of a capture antibody, chemical coupling, and biotin-avidin. The last step in this approach involves the precipitation of protein/binding composition or antigen/antibody complex by any of

several methods including those utilizing, e.g., an organic solvent such as polyethylene glycol or a salt such as ammonium sulfate. Other suitable separation techniques include, without limitation, a fluorescein antibody magnetizable particle method described in Rattle, et al. (1984) Clin. Chem. 30:1457-1461, and the double antibody magnetic particle separation as described in U.S. Pat. No. 4,659,678.

Methods for linking proteins or their fragments to the various labels have been extensively reported in the literature and do not require detailed discussion here. Many of the techniques involve the use of activated carboxyl groups either through the use of carbodiimide or active esters to form peptide bonds, the formation of thioethers by reaction of a mercapto group with an activated halogen such as chloroacetyl, or an activated olefin such as maleimide, for linkage, or the like. Fusion proteins will also find use in these applications.

Diagnostic kits, which also test for the qualitative or quantitative presence of other markers, are also contemplated. Diagnosis or prognosis may depend on the combination of multiple indications used as markers. Thus, kits may test for combinations of markers. See, e.g., Viallet, et al. (1989) Progress in Growth Factor Res. 1:89-97.

In specific embodiments, a kit may include, e.g., a recombinantly produced or chemically synthesized polypeptide antigen. The polypeptide antigen of the kit may also be attached to a solid support. In a more specific embodiment the detecting means of the above-described kit includes, e.g., a solid support to which said polypeptide antigen is attached. Such a kit may also include, e.g., a non-attached reporter-labeled anti-human antibody. In this embodiment, binding of the antibody to the polypeptide antigen is detected by binding of the reporter-labeled antibody.

In an additional embodiment, the invention includes, e.g., a diagnostic kit for use in screening a biological sample, e.g., such as serum, containing an antigen of a polypeptide (or fragment thereof) of the invention. The diagnostic kit can include, e.g., a substantially isolated antibody specifically and/or selectively immunoreactive with a polypeptide or polynucleotide antigen, and, a means for detecting the binding of the polynucleotide or polypeptide antigen to the antibody.

In one embodiment, the antibody is attached to a solid support. In a specific embodiment, the antibody may be a monoclonal antibody. The detecting means of the kit

may include, e.g., a second, labeled monoclonal antibody. Alternatively, or in addition, the detecting means may include, e.g., a labeled, competing antigen.

In one diagnostic configuration, test serum is reacted with a solid phase reagent having a surface-bound antigen obtained by an art known method or as described herein. After binding with specific antigen antibody to the reagent and removing unbound serum components, e.g., by washing; the reagent is reacted with reporter-labeled anti-human antibody to bind reporter to the reagent in proportion to the amount of bound anti-antigen antibody on the solid support. The reagent is again washed to remove unbound, labeled antibody, and the amount of reporter associated with the reagent is determined. Typically, the reporter is an enzyme that is detected by incubating the solid phase in the presence of a suitable fluorometric, luminescent or calorimetric substrate (Sigma, St. Louis, MO).

The solid surface reagent in the above assay is prepared by art known techniques for attaching proteinaceous material to a solid support, such as, e.g., polymeric beads, dip sticks, 96-well plate, or filter material. Methods for attachment generally include, e.g., non-specific adsorption of a protein or polypeptide (or fragment thereof) to a solid support or covalent attachment of a polypeptide, protein (or fragment thereof), typically, e.g., through a free amine group, to a chemically reactive group, such as, e.g., an activated carboxyl, hydroxyl, or aldehyde group on the solid support. Alternatively, streptavidin coated plates are used in conjunction with biotinylated antigen(s).

The broad scope of this invention is best understood with reference to the following examples, which are not intended to limit the invention to specific embodiments.

EXAMPLES

General Methods

Many of the standard methods described herein are described or referenced, e.g., in Maniatis, et al. (Cur. ed..) Molecular Cloning, A Laboratory Manual Cold Spring Harbor Laboratory, Cold Spring Harbor Press, NY; Sambrook, et al.; Ausubel, et al., Biology Greene Publishing Associates, Brooklyn, NY; or Ausubel, et al. (1987 and Supplements) Current Protocols in Molecular Biology Wiley/Greene, NY; Innis, et al. (eds.) (1990) PCR Protocols: A Guide to Methods and Applications Academic Press, NY. Methods for protein purification include such methods as ammonium sulfate

precipitation, column chromatography, electrophoresis, centrifugation, crystallization, and others. See, e.g., Ausubel, et al. (1987 and periodic supplements); Deutscher (1990) "Guide to Protein Purification," Methods in Enzymology vol. 182, and other volumes in this series; Coligan, et al. (1995 and supplements) Current Protocols in Protein Science John Wiley and Sons, New York, NY; P. Matsudaira (ed.) (1993) A Practical Guide to Protein and Peptide Purification for Microsequencing, Academic Press, San Diego, CA; and manufacturer's literature on use of protein purification products, e.g., Pharmacia, Piscataway, NJ, or Bio-Rad, Richmond, CA. Combination with recombinant techniques allows fusion to appropriate segments (epitope tags), e.g., to a FLAG sequence or an equivalent which can be fused, e.g., via a protease-removable sequence. See, e.g., Hochuli (1989) Chemische Industrie 12:69-70; Hochuli (1990) "Purification of Recombinant Proteins with Metal Chelate Absorbent" in Setlow (ed.) Genetic Engineering, Principle and Methods 12:87-98, Plenum Press, NY; and Crowe, et al. (1992) QIAexpress: The High Level Expression and Protein Purification System QUIAGEN, Inc., Chatsworth, CA.

Standard immunological techniques are described, e.g., in Hertzenberg, et al. (eds. 1996) Weir's Hanbook of Experimental Immunology vols. 1-4, Blackwell Science; Coligan (1991) Current Protocols in Immunology Wiley/Greene, NY; and Methods in Enzymology volumes. 70, 73, 74, 84, 92, 93, 108, 116, 121, 132, 150, 162, and 163. Assays for neural cell biological activities are described, e.g., in Wouterlood (ed. 1995) Neuroscience Protocols modules 10, Elsevier; Methods in Neurosciences Academic Press; and Neuromethods Humana Press, Totowa, NJ. Methodology of developmental systems is described, e.g., in Meisami (ed.) Handbook of Human Growth and Developmental Biology CRC Press; and Chrispeels (ed.) Molecular Techniques and Approaches in Developmental Biology Interscience.

FACS analyses are described in Melamed, et al. (1990) Flow Cytometry and Sorting Wiley-Liss, Inc., New York, NY; Shapiro (1988) Practical Flow Cytometry Liss, New York, NY; and Robinson, et al. (1993) Handbook of Flow Cytometry Methods Wiley-Liss, New York, NY.

Example 1: TGF Beta 1 Binding Composition Synthesis

The CDR and framework sequences of antibody binding compositions disclosed herein were identified from clones of Fab fragments, which were isolated from antibody libraries generated from an array of antibody RNA created by immunized C57/Black wild-type mice using OmniclonalTM antibody technology (Biosite®, San Diego, CA). The mice were immunized with human TGF Beta 1 using conjugated keyhole limpet hemoncyanin according to standard methods to improve immunogenicity.

Example 2: Fabs of the mAb Anti-TGF Beta 1 Binding Compositions

Table 1: Murine, e.g., mouse, mAb 1021 polynucleotide sequence (SEQ ID NO: 1) and corresponding polypeptide (SEQ ID NO: 2) of the light chain Fab of the mAb 1021 binding composition of the invention.

mAb 1021 Light Chain DNA Sequence (699 bp):

SEQ ID NO: 1: The first (5') codon (**ATG** coding for methionine) and last (3') codon (**TGT** coding for cysteine) are indicated in bold typeface and underlined.

ATGGAGACAGACACACTCCTGCTATGGGTACTGCTGCTCTGGGTTCCAGGATCTACCGGTCAAATTGTTCTC
ACCCAGTCTCCAGCAATCATGTCTGCATCTCCAGGGGAGAAGGTCACAATGACTTGCAGGGCCAGCTCAAGT
GTAAGTTACATGCACTGGTACCAGCAGAAGCCAGGATCCTCCCCCAAACCCTGGATTTATGCCACATCCAAC
CTGGCTTCTGGAGTCCCTGCTCGCTTCAGTGGCAGTGGGTCTGGGACCTCTTACTCTCTCACAATCAGCAGA
GTGGAGGCTGAAGATGCTGCCACTTATTACTGCCAGCAGTGGAATGGTAACCCACCCGCGTTCGGTGGAGGC
ACCAAGCTGGAAATCAAACGGGCTGATGCGGCGCCAACTGTATCCATCTTCCCACCATCCAGTGAGCAGTTA
ACATCTGGAGGTGCCTCAGTCGTGTGCTTCTTGAACAACTTCTACCCCAAAGACATCAATGTCAAGTGGAAG
ATTGATGGCAGTGAACGACAAAATGGCGTCCTGAACAGTTGGACTGATCAGGACAGCAAAGACAGCACCTAC
AGCATGAGCAGCACCCTCACGTTGACCAAGGACGAGTATGAACGACATAACAGCTATACCTGTGAGGCCACT
CACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAG**TGT**

mAb 1021 Light Chain Amino Acid Sequence (233 aa):

SEQ ID NO: 2: The predicted polypeptide sequence of the light chain Fab of the mAb 1021 binding composition encoded by the polynucleotide sequence of SEQ ID NO: 1. The three putative CDR regions are indicated in bold typeface and underlined starting with CDR 1 (RASSSVSYMH) through CDR2 (ATSNLAS) until CDR 3 (QQWNGNPPA).

METDTLLLWVLLLWVPGSTGQIVLTQSPAIMSASPGEKVTMTC**RASSSVSYMH**WYQQKPGSSPKPWIY**ATSN**
LASGVPARFSGSGSGTSYSLTISRVEAEDAATYYC**QQWNGNPPA**FGGGTKLEIKRADAAPTVSIFPPSSEQL
TSGGASVVCFLNNFYPKDINVKWKIDGSERQNGVLNSWTDQDSKDSTYSMSSTLTLTKDEYERHNSYTCEAT
HKTSTSPIVKSFNRNEC

Table 2: Murine, e.g., mouse, mAb 1021 polynucleotide sequence (SEQ ID NO: 3) and corresponding polypeptide (SEQ ID NO: 4) of the heavy chain Fab of the mAb 1021 binding composition of the invention.

mAb 1021 Heavy Chain DNA Sequence (1380 bp):

SEQ ID NO: 3: The first (5') codon (ATG coding for methionine) and last (3') codon (AAA coding for lysine) are indicated in bold typeface and underlined).

ATGGAGACAGACACACTCCTGCTATGGGTACTGCTGCTCTGGGTTCCAGGATCTACCGGTGAGGTCCAGCTT
CAGCAGTCAGGTCCTGAGCTGGTGAAACCTGGGGCCTCAGTGAAGATATCCTGCAAGGCTTCTGGATACACA
TTCACTGACTACAACATGCACTGGGTGAAGCAGAGCCATGGAAAGAGCCTTGAGTGGCTTGGATATATTTAT
CCTTACAATGGTGATACTGGCTACAACCAGAAGTTCAAGAGCAAGGCCACATTGACTGTAGACAATTCCTCC
AGCACAGCCTATATGGAGCTCCGCAGCCTGACATCTGAGGACTCTGCAGTCTATTATTGTGTAAGAGGATAC
TACTGGTTTGCTTACTGGGGCCGAGGGACTCTGGTCACTGTCTCTACAGCCAAAACGACACCCCCATCTGTC
TATCCACTGGCCCCTGGATCTGCTGCCCAAACTAACTCCATGGTGACCCTGGGATGCCTGGTCAAGGGCTAT
TTCCCTGAGCCAGTGACAGTGACCTGGAACTCTGGATCCCTGTCCAGCGGTGTGCACACCTTCCCAGCTGTC
CTGCAGTCTGACCTCTACACTCTGAGCAGCTCAGTGACTGTCCCCTCCAGCACCTGGCCCAGCGAGACCGTC
ACCTGCAACGTTGCCCACCCGGCCAGCAGCACCAAGGTGGACAAGAAAATTGTGCCCAGGGATTGTGGTTGT
AAGCCTTGCATATGTACAGTCCCAGAAGTATCATCTGTCTTCATCTTCCCCCCAAAGCCCAAGGATGTGCTC
ACCATTACTCTGACTCCTAAGGTCACGTGTGTTGTGGTAGACATCAGCAAGGATGATCCCGAGGTCCAGTTC
AGCTGGTTTGTAGATGATGTGGAGGTGCACACAGCTCAGACGCAACCCCGGGAGGAGCAGTTCAACAGCACT
TTCCGCTCAGTCAGTGAACTTCCCATCATGCACCAGGACTGGCTCAATGGCAAGGAGTTCAAATGCAGGGTC
AACAGTGCAGCTTTCCCTGCCCCCATCGAGAAAACCATCTCCAAAACCAAAGGCAGACCGAAGGCTCCACAG
GTGTACACCATTCCACCTCCCAAGGAGCAGATGGCCAAGGATAAAGTCAGTCTGACCTGCATGATAACAGAC
TTCTTCCCTGAAGACATTACTGTGGAGTGGCAGTGGAATGGGCAGCCAGCGGAGAACTACAAGAACACTCAG
CCCATCATGGACACAGATGGCTCTTACTTCGTCTACAGCAAGCTCAATGTGCAGAAGAGCAACTGGGAGGCA
GGAAATACTTTCACCTGCTCTGTGTTACATGAGGGCCTGCACAACCACCATACTGAGAAGAGCCTCTCCCAC
TCTCCTGGT**AAA**

mAb 1021 Heavy Chain Amino Acid Sequence (460 aa):

SEQ ID NO: 4: The predicted polypeptide sequence of the heavy chain Fab of the mAb 1021 binding composition encoded by the polynucleotide of SEQ ID NO: 3. The three putative CDR regions are indicated in bold typeface and underlined starting with CDR 1 (GYTFTDYNMH) through CDR2 (YIYPYNGDTGYNQKFKS) until CDR 3 (GYYWFAY). CDRs of the invention may include up to 1, 2, 3, 4, or 5 additional amino acids (at either the 5' or 3' end of an identified CDR region) as identified in a Table herein.

METDTLLLWVLLLWVPGSTGEVQLQQSGPELVKPGASVKISCKAS**GYTFTDYNMH**WVKQSHGKSLEWLG**YIY**
PYNGDTGYNQKFKSKATLTVDNSSSTAYMELRSLTSEDSAVYYCVR**GYYWFAY**WGRGTLVTVSTAKTTPPSV
YPLAPGSAAQTNSMVTLGCLVKGYFPEPVTVTWNSGSLSSGVHTFPAVLQSDLYTLSSSVTVPSSTWPSETV
TCNVAHPASSTKVDKKIVPRDCGCKPCICTVPEVSSVFIFPPKPKDVLTITLTPKVTCVVVDISKDDPEVQF
SWFVDDVEVHTAQTQPREEQFNSTFRSVSELPIMHQDWLNGKEFKCRVNSAAFPAPIEKTISKTKGRPKAPQ
VYTIPPPKEQMAKDKVSLTCMITDFFPEDITVEWQWNGQPAENYKNTQPIMDTDGSYFVYSKLNVQKSNWEA
GNTFTCSVLHEGLHNHHTEKSLSHSPGK

Table 3: Murine, e.g., mouse, mAb 2471 polynucleotide sequence (SEQ ID NO: 5) and corresponding polypeptide (SEQ ID NO: 6) for the light chain Fab of the mAb 1021 binding composition of the invention.

mAb 2471 Light Chain DNA Sequence (699 bp):

SEQ ID NO: 5: The first (5') codon (**ATG** coding for methionine) and last (3') codon (**TGT** coding for cysteine) are indicated in bold typeface and underlined.

ATGGAGACAGACACACTCCTGCTATGGGTACTGCTGCTCTGGGTTCCAGGATCTACCGGTCAAATTGTTCTC
ACCCAGTCTCCAGCAATCATGTCTGCATCTCCAGGGGAGAAGGTCACAATGACTTGCAGGGCCAGCTCAAGT
GTAAGTTACATGCACTGGTACCAGCAGAAGCCAGGATCCTCCCCCAAACCCTGGATTTATGCCACATCCAAC
CTGGCTTCTGGAGTCCCTGCTCGCTTCAGTGGCAGTGGGTCTGGGACCTCTTACTCTCTCACAATCAGCAGA
GTGGAGGCTGAAGATGCTGCCACTTATTACTGCCAGCAGTGGGATAGTAACCCACCCGCGTTCGGTGGAGGC
ACCAAGCTGGAAATCAAACGGGCTGATGCTGCACCAACTGTATCCATCTTCCCACCATCCAGTGAGCAGTTA
ACATCTGGAGGTGCCTCAGTCGTGTGCTTCTTGAACAACTTCTACCCCAAAGACATCAATGTCAAGTGGAAG
ATTGATGGCAGTGAACGACAAAATGGCGTCCTGAACAGTTGGACTGATCAGGACAGCAAAGACAGCACCTAC
AGCATGAGCAGCACCCTCACGTTGACCAAGGACGAGTATGAACGACATAACAGCTATACCTGTGAGGCCACT
CACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAG**TGT**

mAb 2471 Light Chain Amino Acid Sequence (233 aa):

SEQ ID NO: 6: The predicted polypeptide sequence of the light chain Fab of the mAb 2471 binding composition encoded by the polynucleotide sequence of SEQ ID NO: 5. The three putative CDR regions are indicated in bold typeface and underlined starting with CDR 1 (RASSSVSYMH) through CDR2 (ATSNLAS) until CDR 3 (QQWDSNP).

METDTLLLWVLLLWVPGSTGQIVLTQSPAIMSASPGEKVTMTC**RASSSVSYMH**WYQQKPGSSPKPWIY**ATSN**
LASGVPARFSGSGSGTSYSLTISRVEAEDAATYYC**QQWDSNP**PAFGGGTKLEIKRADAAPTVSIFPPSSEQL
TSGGASVVCFLNNFYPKDINVKWKIDGSERQNGVLNSWTDQDSKDSTYSMSSTLTLTKDEYERHNSYTCEAT
HKTSTSPIVKSFNRNEC

Table 4: Murine, e.g., mouse, mAb 2471 polynucleotide sequence (SEQ ID NO: 7) and corresponding polypeptide (SEQ ID NO: 8) of the heavy chain Fab of the mAb 2471 binding composition of the invention.

mAb 2471 Heavy Chain DNA Sequence (1380 bp):

SEQ ID NO: 7: The first (5') codon (**ATG** coding for methionine) and last (3') codon (**AAA** coding for lysine) are indicated in bold typeface and underlined.

ATGGAGACAGACACACTCCTGCTATGGGTACTGCTGCTCTGGGTTCCAGGATCTACCGGTGAGGTCCAGCTT
CAGCAGTCAGGTCCTGAGCTGGTGAAACCTGGGGCCTCAGTGAAGATATCCTGCAAGGCTTCTGGATACACA
TTCACTGACTACAACATGCACTGGGTGAAGCAGAGCCATGGAAAGAGCCTTGAGTGGCTTGGATATATTTAT
CCTTACAATGGTGATACTGGCTACAACCAGAAGTTCAAGAGCAAGGCCACATTGACTGTAGACAATTCCTCC
AGCACAGCCTATATGGAGCTCCGCAGCCTGACATCTGAGGACTCTGCAGTCTATTATTGTGTAAGAGGATAC
TACTGGTTTGCTTACTGGGGCCAAGGGACTCTGGTCACTGTCTCTACAGCCAAAACGACACCCCCATCTGTC
TATCCACTGGCCCCTGGATCTGCTGCCCAAACTAACTCCATGGTGACCCTGGGATGCCTGGTCAAGGGCTAT
TTCCCTGAGCCAGTGACAGTGACCTGGAACTCTGGATCCCTGTCCAGCGGTGTGCACACCTTCCCAGCTGTC
CTGCAGTCTGACCTCTACACTCTGAGCAGCTCAGTGACTGTCCCCTCCAGCACCTGGCCCAGCGAGACCGTC
ACCTGCAACGTTGCCCACCCGGCCAGCAGCACCAAGGTGGACAAGAAAATTGTGCCCAGGGATTGTGGTTGT
AAGCCTTGCATATGTACAGTCCCAGAAGTATCATCTGTCTTCATCTTCCCCCCAAAGCCCAAGGATGTGCTC
ACCATTACTCTGACTCCTAAGGTCACGTGTGTTGTGGTAGACATCAGCAAGGATGATCCCGAGGTCCAGTTC
AGCTGGTTTGTAGATGATGTGGAGGTGCACACAGCTCAGACGCAACCCCGGGAGGAGCAGTTCAACAGCACT
TTCCGCTCAGTCAGTGAACTTCCCATCATGCACCAGGACTGGCTCAATGGCAAGGAGTTCAAATGCAGGGTC
AACAGTGCAGCTTTCCCTGCCCCCATCGAGAAAACCATCTCCAAAACCAAAGGCAGACCGAAGGCTCCACAG
GTGTACACCATTCCACCTCCCAAGGAGCAGATGGCCAAGGATAAAGTCAGTCTGACCTGCATGATAACAGAC
TTCTTCCCTGAAGACATTACTGTGGAGTGGCAGTGGAATGGGCAGCCAGCGGAGAACTACAAGAACACTCAG
CCCATCATGGACACAGATGGCTCTTACTTCGTCTACAGCAAGCTCAATGTGCAGAAGAGCAACTGGGAGGCA
GGAAATACTTTCACCTGCTCTGTGTTACATGAGGGCCTGCACAACCACCATACTGAGAAGAGCCTCTCCCAC
TCTCCTGGT**AAA**

mAb 2471 Heavy Chain Amino Acid Sequence (460 aa):

SEQ ID NO: 8: The predicted polypeptide sequence of the heavy chain Fab of the mAb 2471 binding composition encoded by the polynucleotide of SEQ ID NO: 3. The three putative CDR regions are indicated in bold typeface and underlined starting with CDR 1 (GYTFTDYNMH) through CDR2 (YIYPYNGDTGYNQKFKS) until CDR 3 (GYYWFAY).

METDTLLLWVLLLWVPGSTGEVQLQQSGPELVKPGASVKISCKAS**GYTFTDYNMH**WVKQSHGKSLEWLG**YIY**
PYNGDTGYNQKFKSKATLTVDNSSSTAYMELRSLTSEDSAVYYCVR**GYYWFAY**WGQGTLVTVSTAKTTPPSV
YPLAPGSAAQTNSMVTLGCLVKGYFPEPVTVTWNSGSLSSGVHTFPAVLQSDLYTLSSSVTVPSSTWPSETV
TCNVAHPASSTKVDKKIVPRDCGCKPCICTVPEVSSVFIFPPKPKDVLTITLTPKVTCVVVDISKDDPEVQF
SWFVDDVEVHTAQTQPREEQFNSTFRSVSELPIMHQDWLNGKEFKCRVNSAAFPAPIEKTISKTKGRPKAPQ
VYTIPPPKEQMAKDKVSLTCMITDFFPEDITVEWQWNGQPAENYKNTQPIMDTDGSYFVYSKLNVQKSNWEA
GNTFTCSVLHEGLHNHHTEKSLSHSPGK

Table 5: Murine, e.g., mouse, mAb 3821 polynucleotide sequence (SEQ ID NO: 9) and corresponding polypeptide (SEQ ID NO: 10) of the light chain Fab of the mAb 3821 binding composition of the invention.

mAb 3821 Light Chain DNA Sequence (702bp):

SEQ ID NO: 9: The first (5') codon (**ATG** coding for methionine) and last (3') codon (**TGT** coding for cysteine) are indicated in bold typeface and underlined.

ATGGAGACAGACACACTCCTGCTATGGGTACTGCTGCTCTGGGTTCCAGGATCTACCGGTGACGTCCAGATA
ACCCAGTCTCCATCCTCCTTATCTGCCTCTCTGGGAGAAAGAGTCAGTCTCACTTGTCGGGCAAGTCAGGAA
ATTAGTGGTTACTTAAGCTGGCTTCAGCAGAAACCAGATGGAACTATTAAACGCCTGATCTACGCCACATCC
AGTTTAGATTCTGGTGTCCCCAAAAGGTTCAGTGGCAGTAGGTCTGGGTCAGATTATTCTCTCACCATCAGC
AGCCCTGAGTCTGAAGATTTTGTAGACTATTACTGTCTACAATATGCTAGTTCTCCGTACACGTTTGGAGGG
GGGACCAAGCTGGAAATAAAACGGGCTGATGCTGCACCAACTGTATCCATCTTCCCACCATCCAGTGAGCAG
TTAACATCTGGAGGTGCCTCAGTCGTGTGCTTCTTGAACAACTTCTACCCCAAAGACATCAATGTCAAGTGG
AAGATTGATGGCAGTGAACGACAAAATGGCGTCCTGAACAGTTGGACTGATCGGGACAGCAAAGACAGCACC
TACAGCATGAGCAGCACCCTCACGTTGACCAAGGACGAGTATGAACGACATAACAGTTATACCTGTGAGGCC
ACTCACAAGACATCAACTTCACCCATTGTCAAGAGCTTCAACAGGAATGAG**TGT**

mAb 3821 Light Chain Amino Acid Sequence (234 aa):

SEQ ID NO: 10: The predicted polypeptide sequence of the light chain Fab of the mAb 3821 binding composition encoded by the polynucleotide sequence of SEQ ID NO: 9. The three putative CDR regions are indicated in bold typeface and underlined starting with CDR 1 (RASQEISGYLS) through CDR2 (ATSSLDS) until CDR 3 (LQYASSPYT).

METDTLLLWVLLLWVPGSTGDVQITQSPSSLSASLGERVSLTC**RASQEISGYLS**WLQQKPDGTIKRLIY**ATS**
SLDSGVPKRFSGSRSGSDYSLTISSPESEDFVDYYC**LQYASSPYT**FGGGTKLEIKRADAAPTVSIFPPSSEQ
LTSGGASVVCFLNNFYPKDINVKWKIDGSERQNGVLNSWTDRDSKDSTYSMSSTLTLTKDEYERHNSYTCEA
THKTSTSPIVKSFNRNEC

Table 6: Murine, e.g., mouse, mAb 3821 polynucleotide sequence (SEQ ID NO: 11) and corresponding polypeptide (SEQ ID NO: 12) for the heavy chain Fab of the mAb 3821 binding composition of the invention.

mAb 3821 Heavy Chain DNA Sequence (1392 bp):

SEQ ID NO: 11: The first (5') codon (**ATG** coding for methionine) and last (3') codon (**AAA** coding for lysine) are indicated in bold typeface and underlined).

ATGGAGACAGACACACTCCTGCTATGGGTACTGCTGCTCTGGGTTCCAGGATCTACCGGTGAGGTTCAGCTG
CAGCAGTCTGGGGCTGCGCTGATGAGGCCTGGGGTCTCAGTGAAGATTTCCTGCAAGGGTTCTGGCTACACA
TTCACTGATTATACTATGCACTGGGTGAAGCAGAGTCATGCAAAGAGTCTAGAGTGGATTGGACTTATTACT
CCTTTCTATGGTGATGCTATATACAACCAGAAGTTCAAGGGCAAGGCCACAATGACTGTAGACAAATCCTCC
AGCACAGCCTATATGGAACTTGCCAGACTGACATCTGATGATTCTGCCATCTATTACTGTACAAGGGGGGGA
TTACGACGCGGACCCCCTTTTGCTTACTGGGGCCAAGGGACTCTGGTCACTGTCTCTGCAGCCAAAACGACA
CCCCCATCTGTCTATCCACTGGCCCCTGGATCTGCTGCCCAAACTAACTCCATGGTGACCCTGGGATGCCTG
GTCAAGGGCTATTTCCCTGAGCCAGTGACAGTGACCTGGAACTCTGGATCCCTGTCCAGCGGTGTGCACACC
TTCCCAGCTGTCCTGCAGTCTGACCTCTACACTCTGAGCAGCTCAGTGACTGTCCCCTCCAGCACCTGGCCC
AGCGAGACCGTCACCTGCAACGTTGCCCACCCGGCCAGCAGCACCAAGGTGGACAAGAAAATTGTGCCCAGG
GATTGTGGTTGTAAGCCTTGCATATGTACAGTCCCAGAAGTATCATCTGTCTTCATCTTCCCCCCAAAGCCC
AAGGATGTGCTCACCATTACTCTGACTCCTAAGGTCACGTGTGTTGTGGTAGACATCAGCAAGGATGATCCC
GAGGTCCAGTTCAGCTGGTTTGTAGATGATGTGGAGGTGCACACAGCTCAGACGCAACCCCGGGAGGAGCAG
TTCAACAGCACTTTCCGCTCAGTCAGTGAACTTCCCATCATGCACCAGGACTGGCTCAATGGCAAGGAGTTC
AAATGCAGGGTCAACAGTGCAGCTTTCCCTGCCCCCATCGAGAAAACCATCTCCAAAACCAAAGGCAGACCG
AAGGCTCCACAGGTGTACACCATTCCACCTCCCAAGGAGCAGATGGCCAAGGATAAAGTCAGTCTGACCTGC
ATGATAACAGACTTCTTCCCTGAAGACATTACTGTGGAGTGGCAGTGGAATGGGCAGCCAGCGGAGAACTAC
AAGAACACTCAGCCCATCATGGACACAGATGGCTCTTACTTCGTCTACAGCAAGCTCAATGTGCAGAAGAGC
AACTGGGAGGCAGGAAATACTTTCACCTGCTCTGTGTTACATGAGGGCCTGCACAACCACCATACTGAGAAG
AGCCTCTCCCACTCTCCTGGT**AAA**

mAb 3821 Heavy Chain Amino Acid Sequence (464 aa):

SEQ ID NO: 12: The predicted polypeptide sequence of the heavy chain Fab of the mAb 3821 binding composition encoded by the polynucleotide of SEQ ID NO: 11. The three putative CDR regions are indicated in bold typeface and underlined starting with CDR 1 (GYTFTDYTMH) through CDR2 (LITPFYGDAIYNQKFKG) until CDR 3 (GGLRRGPPFAY).

METDTLLLWVLLLWVPGSTGEVQLQQSGAALMRPGVSVKISCKGS**GYTFTDYTMH**WVKQSHAKSLEWIG**LIT**
PFYGDAIYNQKFKGKATMTVDKSSSTAYMELARLTSDDSAIYYCTR**GGLRRGPPFAY**WGQGTLVTVSAAKTT
PPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPVTVTWNSGSLSSGVHTFPAVLQSDLYTLSSSVTVPSSTWP
SETVTCNVAHPASSTKVDKKIVPRDCGCKPCICTVPEVSSVFIFPPKPKDVLTITLTPKVTCVVVDISKDDP
EVQFSWFVDDVEVHTAQTQPREEQFNSTFRSVSELPIMHQDWLNGKEFKCRVNSAAFPAPIEKTISKTKGRP
KAPQVYTIPPPKEQMAKDKVSLTCMITDFFPEDITVEWQWNGQPAENYKNTQPIMDTDGSYFVYSKLNVQKS
NWEAGNTFTCSVLHEGLHNHHTEKSLSHSPGK

Table 7: A BLOSUM62 amino acid substitution matrix was used to conduct a PILEUP sequence alignment (Henikoff and Henikoff 1992 Proc. Natl. Acad. Sci. USA 89: 10915-10919) of the light chain domains of the mAb binding compositions of the invention (mAb 2471, mAb 1021 and mAb 3821). Putative CDRs are indicated by underlining and italic typeface.

```
                      1                                                CDR1 50
    1021LC     (1)  METDTLLLWVLLLWVPGSTGQIVLTQSPAIMSASPGEKVTMTCRASSSVS
    2471LC     (1)  METDTLLLWVLLLWVPGSTGQIVLTQSPAIMSASPGEKVTMTCRASSSVS
    3821LC     (1)  METDTLLLWVLLLWVPGSTGDVQITQSPSSLSASLGERVSLTCRASQEIS
  Consensus    (1)  METDTLLLWVLLLWVPGSTGQIVLTQSPAIMSASPGEKVTMTCRASSSVS

                     51                  CDR2                        100
    1021LC    (51)  -YMHWYQQKPGSSPKPWIYATSNLASGVPARFSGSGSGTSYSLTISRVEA
    2471LC    (51)  -YMHWYQQKPGSSPKPWIYATSNLASGVPARFSGSGSGTSYSLTISRVEA
    3821LC    (51)  GYLSWLQQKPDGTIKRLIYATSSLDSGVPKRFSGSRSGSDYSLTISSPES
  Consensus   (51)   YMHWYQQKPGSSPKPWIYATSNLASGVPARFSGSGSGTSYSLTISRVEA

                    101        CDR3                         150
    1021LC   (100)  EDAATYYCQQWNGNPPAFGGGTKLEIKRADAAPTVSIFPPSSEQLTSGGA
    2471LC   (100)  EDAATYYCQQWDSNPPAFGGGTKLEIKRADAAPTVSIFPPSSEQLTSGGA
    3821LC   (101)  EDFVDYYCLQYASSPYTFGGGTKLEIKRADAAPTVSIFPPSSEQLTSGGA
  Consensus  (101)  EDAATYYCQQW SNPPAFGGGTKLEIKRADAAPTVSIFPPSSEQLTSGGA

                    151                                          200
    1021LC   (150)  SVVCFLNNFYPKDINVKWKIDGSERQNGVLNSWTDQDSKDSTYSMSSTLT
    2471LC   (150)  SVVCFLNNFYPKDINVKWKIDGSERQNGVLNSWTDQDSKDSTYSMSSTLT
    3821LC   (151)  SVVCFLNNFYPKDINVKWKIDGSERQNGVLNSWTDRDSKDSTYSMSSTLT
  Consensus  (151)  SVVCFLNNFYPKDINVKWKIDGSERQNGVLNSWTDQDSKDSTYSMSSTLT

                    201                                  234
    1021LC   (200)  LTKDEYERHNSYTCEATHKTSTSPIVKSFNRNEC
    2471LC   (200)  LTKDEYERHNSYTCEATHKTSTSPIVKSFNRNEC
    3821LC   (201)  LTKDEYERHNSYTCEATHKTSTSPIVKSFNRNEC
  Consensus  (201)  LTKDEYERHNSYTCEATHKTSTSPIVKSFNRNEC
```

Table 8: A BLOSUM62 amino acid substitution matrix was used to conduct a PILEUP sequence alignment (Henikoff and Henikoff 1992 Proc. Natl. Acad. Sci. USA 89: 10915-10919) of the heavy chain domains of the mAb binding compositions of the invention (mAb 2471, mAb 1021 and mAb 3821). Putative CDRs are indicated by underlining and italic typeface.

1021 and 2471 HC's differ by 1 aa after CDR3

```
                    1                                                CDR1 50
   1021HC     (1)   METDTLLLWVLLLWVPGSTGEVQLQQSGPELVKPGASVKISCKASGYTFT
   2471HC     (1)   METDTLLLWVLLLWVPGSTGEVQLQQSGPELVKPGASVKISCKASGYTFT
   3821HC     (1)   METDTLLLWVLLLWVPGSTGEVQLQQSGAALMRPGVSVKISCKGSGYTFT
Consensus     (1)   METDTLLLWVLLLWVPGSTGEVQLQQSGPELVKPGASVKISCKASGYTFT

                   51                        CDR2                   100
   1021HC    (51)   DYNMHWVKQSHGKSLEWLGYIYPYNGDTGYNQKFKSKATLTVDNSSSTAY
   2471HC    (51)   DYNMHWVKQSHGKSLEWLGYIYPYNGDTGYNQKFKSKATLTVDNSSSTAY
   3821HC    (51)   DYTMHWVKQSHAKSLEWIGLITPFYGDAIYNQKFKGKATMTVDKSSSTAY
Consensus    (51)   DYNMHWVKQSHGKSLEWLGYIYPYNGDTGYNQKFKSKATLTVDNSSSTAY

                  101                       CDR3                    150
   1021HC   (101)   MELRSLTSEDSAVYYCVRGYY----WFAYWGRGTLVTVSTAKTTPPSVYP
   2471HC   (101)   MELRSLTSEDSAVYYCVRGYY----WFAYWGQGTLVTVSTAKTTPPSVYP
   3821HC   (101)   MELARLTSDDSAIYYCTRGGLRRGPPFAYWGQGTLVTVSAAKTTPPSVYP
Consensus   (101)   MELRSLTSEDSAVYYCVRGYY    WFAYWGQGTLVTVSTAKTTPPSVYP

                  151                                                200
   1021HC   (147)   LAPGSAAQTNSMVTLGCLVKGYFPEPVTVTWNSGSLSSGVHTFPAVLQSD
   2471HC   (147)   LAPGSAAQTNSMVTLGCLVKGYFPEPVTVTWNSGSLSSGVHTFPAVLQSD
   3821HC   (151)   LAPGSAAQTNSMVTLGCLVKGYFPEPVTVTWNSGSLSSGVHTFPAVLQSD
Consensus   (151)   LAPGSAAQTNSMVTLGCLVKGYFPEPVTVTWNSGSLSSGVHTFPAVLQSD

                  201                                                250
   1021HC   (197)   LYTLSSSVTVPSSTWPSETVTCNVAHPASSTKVDKKIVPRDCGCKPCICT
   2471HC   (197)   LYTLSSSVTVPSSTWPSETVTCNVAHPASSTKVDKKIVPRDCGCKPCICT
   3821HC   (201)   LYTLSSSVTVPSSTWPSETVTCNVAHPASSTKVDKKIVPRDCGCKPCICT
Consensus   (201)   LYTLSSSVTVPSSTWPSETVTCNVAHPASSTKVDKKIVPRDCGCKPCICT

                  251                                                300
   1021HC   (247)   VPEVSSVFIFPPKPKDVLTITLTPKVTCVVVDISKDDPEVQFSWFVDDVE
   2471HC   (247)   VPEVSSVFIFPPKPKDVLTITLTPKVTCVVVDISKDDPEVQFSWFVDDVE
   3821HC   (251)   VPEVSSVFIFPPKPKDVLTITLTPKVTCVVVDISKDDPEVQFSWFVDDVE
Consensus   (251)   VPEVSSVFIFPPKPKDVLTITLTPKVTCVVVDISKDDPEVQFSWFVDDVE

                  301                                                350
   1021HC   (297)   VHTAQTQPREEQFNSTFRSVSELPIMHQDWLNGKEFKCRVNSAAFPAPIE
   2471HC   (297)   VHTAQTQPREEQFNSTFRSVSELPIMHQDWLNGKEFKCRVNSAAFPAPIE
   3821HC   (301)   VHTAQTQPREEQFNSTFRSVSELPIMHQDWLNGKEFKCRVNSAAFPAPIE
Consensus   (301)   VHTAQTQPREEQFNSTFRSVSELPIMHQDWLNGKEFKCRVNSAAFPAPIE

                  351                                                400
   1021HC   (347)   KTISKTKGRPKAPQVYTIPPPKEQMAKDKVSLTCMITDFFPEDITVEWQW
   2471HC   (347)   KTISKTKGRPKAPQVYTIPPPKEQMAKDKVSLTCMITDFFPEDITVEWQW
   3821HC   (351)   KTISKTKGRPKAPQVYTIPPPKEQMAKDKVSLTCMITDFFPEDITVEWQW
Consensus   (351)   KTISKTKGRPKAPQVYTIPPPKEQMAKDKVSLTCMITDFFPEDITVEWQW
```

```
                    401                                                 450
     1021HC  (397)  NGQPAENYKNTQPIMDTDGSYFVYSKLNVQKSNWEAGNTFTCSVLHEGLH
     2471HC  (397)  NGQPAENYKNTQPIMDTDGSYFVYSKLNVQKSNWEAGNTFTCSVLHEGLH
     3821HC  (401)  NGQPAENYKNTQPIMDTDGSYFVYSKLNVQKSNWEAGNTFTCSVLHEGLH
  Consensus  (401)  NGQPAENYKNTQPIMDTDGSYFVYSKLNVQKSNWEAGNTFTCSVLHEGLH

                    451       464
     1021HC  (447)  NHHTEKSLSHSPGK
     2471HC  (447)  NHHTEKSLSHSPGK
     3821HC  (451)  NHHTEKSLSHSPGK
  Consensus  (451)  NHHTEKSLSHSPGK
```

Table 9 CDR Sequences of mAb Binding Compositions #1021, #2471, and #3821

	CDR1	CDR2	CDR3
1021 VH	GYTFTDYNMH (SEQ ID NO:13)	YIYPYNGDTGYNQKFKS (SEQ ID NO: 14)	GYYWFAY (SEQ ID NO: 15)
2471 VH	GYTFTDYNMH (SEQ ID NO: 16)	YIYPYNGDTGYNQKFKS (SEQ ID NO: 17)	GYYWFAY (SEQ ID NO: 18)
3821 VH	GYTFTDYTMH (SEQ ID NO: 19)	LITPFYGDAIYNQKFKG (SEQ ID NO: 20)	GGLRRGPPFAY (SEQ ID NO: 21)
1021 VL	RASSSVSYMH (SEQ ID NO: 22)	ATSNLAS (SEQ ID NO: 23)	QQWNGNPPA (SEQ ID NO: 24)
2471 VL	RASSSVSYMH (SEQ ID NO: 25)	ATSNLAS (SEQ ID NO: 26)	QQWDSNPPA (SEQ ID NO: 27)
3821 VL	RASQEISGYLS (SEQ ID NO: 28)	ATSSLDS (SEQ ID NO: 29)	LQYASSPYT (SEQ ID NO: 30)

Example 2: TGF Beta 1 ELISA

Binding compositions of the invention (e.g., anti-TGF Beta Fabs designated 1021, 2471, and 3821) were tested in a competitive ELISA assay as described herein. Typically, a solution phase assay is performed in which a compound that might compete with an antigen for binding to a binding composition, such as an antibody, is combined first with the antibody in solution phase, then the degree of binding of the antibody with the antigen is subsequently measured.

Materials:

Carbonate coating buffer consists of 50 mM sodium carbonate pH 9.6. Antigens used were TGF-β 1 (R&D Systems, Cat # 240-B/CF, 239 ug/ml), TGF-β 2 (RDI, Cat #

RDI-1035, 50 ug/ml) and TGF-β 3 (RDI, Cat # RDI-1036/CF, 50 ug/ml) diluted to 0.4 ug/mL in coating buffer. Wash buffer consisted of 0.02 M Tris pH 7.4, 0.15 M NaCl, 0.1% Tween 20 and blocking solution of 10 mg/ml BSA (Sigma A-4503) dissolved in wash buffer. Proteins used as positive control were mouse-anti-human TGF-β 1, 2, or 3 (R&D Systems, cat# 1D11), mouse-anti-human TGF-β2 – B (R&D Systems, cat# BAF302) and mouse-anti-human TGF-β3 (R&D Systems, cat# BAF243), which were diluted to 1ug/ml in block buffer. The detection antibody conjugate used was anti-mouse kappa – peroxidase conjugate (Southern Biotech, cat# 1050-05), at a working concentration of 1:2000 in blocking solution. The substrate used for the color reaction was O-phenylenediamine (OPD) tablets (Sigma cat# P-6912), which were dissolved in substrate buffer: 0.1 M Na_2HPO_4, pH to 5.0 with 0.05 M citric Acid. The OPD substrate working solution (i.e., the volume for one 196-well-plate) was freshly made prior to each plate development by dissolving 1 x 5mg OPD tablet in 12.5 mL of substrate buffer followed by the addition of 5 ul of 30% H_2O_2.

Protocol:

A single 96 well plate was coated with antigen (TGF-β1, 2, or 3 at 0.4 ug/ml and dispense 50 uL per well) and then sealed with adhesive tape before storage (16-20 hours at 4°C). The plate was subsequently washed (2X, twice) in wash buffer (described above) before adding 100 uL of blocking solution (10 mg/ml BSA in wash buffer) into each well. After incubation (approximately 1 hour at 22 °C), the plate was washed (2X, twice) with wash buffer. Then, 100 uL of either sample (diluted in buffer) or control (diluted in PBS) was added to each well and incubated (1.5 hours at 22 °C). After incubation, the plate was washed (6X, six times) with wash buffer (described above) before adding 100 uL per well of either anti-mouse kappa – peroxidase conjugate (diluted to 1:2000 in Blocking solution) or SA-HRP (diluted 1:10,000 in blocking solution). The test samples were left to incubate (1 hour at 22 °C) before adding 100 uL of OPD substrate to each well. After color development (approximately 10 minutes), the 96-well plate was measured at an absorbance of 490nm.

Results:

A TGF-β ELISA was used to screen Fab binding compositions of the invention to determine their specificity and/or selectivity for a particular TGF-β isoform. Results

from these experiments (shown below in Table 10) show that the Fabs designated 1021 and 2471 produced an absorbance greater than 1.6 units at 490 nm with TGF-β1 but gave significantly lower values with TGF- β 2 and TGF- β 3 thus demonstrating that the 1021 and 2471 binding compositions comprise binding sites that are specific and/or selective for TGF-β1. Support for this conclusion was provided by the similarity of the absorbance readings obtained with the known TGF-β1 specific control mAb (designated mAb240), which was obtained from R&D Systems.

When the control mAb240 is used in combination with the biotinylated anti-TGF-β1 detection antibody (R&D # BAF240) in sandwich ELISAs, less than 2% cross-reactivity is observed with TGF-β3 and TGF-β5 and no cross-reactivity is observed with the following cytokines: rhAng, rhCNTF, rhβ-ECGF, rmEGF, rhEGF, rmEGF, rhEpo, bFGF acidic, rhFGF acidic, bFGF basic, rhFGF basic, rhFGF-5, rhFGF-6, rhG-CSF, rmGM-CSF, rhGROα, rhHB-EGF, rhHGF, rhIFN-γ, rhIGF-I, rhIGF-II, rhLIF, rmLIF, rhM-CSF, rhMCP-1, rhMIP-1α, rmMIP-1α, rhMIP-1α, rmMIP-1α, rhβ-NGF, rhOSM, rhPDGF, rhPDGF-AA, rhPDGF-AB, rhPDGF-BB, rhPTN, rhRANTES, rhSCF, rmSCF, rhSLPI, pTGF-β2, rhTGF-β2, rhTNF-α, rmTNF-α, rhTNF-β, rhsTNF RI, rhsTNF RII, and, rhVEGF.

However, in contrast, the control readings (from a known non-selective mAb, designated 1D11) produced high absorbance readings for all three TGF-β isoforms (i.e., TGF- β 1, 2, and 3) supporting the conclusion that the Fab 1021 and 2471 are specific and/or selective for TGF-Beta 1 (see Table 10 below).

TABLE 10

Test or Control	TGF-β1	TGF-β2	TGF-β3
Fab 1021	1.661	0.102	0.041
Fab 2471	1.999	0.072	0.042
mAb 1D11	2.095	1.942	1.197
mAb240	1.604	0.086	0.041
anti TGF-b2	0.61	3.94	0.292
anti TGF-b3	2.348	3.924	4.173
Anti mouse-HRP	0.046	0.078	0.037
SA-HRP	0.046	0.052	0.04
SA-HRP	0.041	0.041	0.041

Values of Table 10 represent the absorbance at 490 nm from duplicate determinations with the Fabs 1021 and 2471 used at a concentration of 10 ug/ml. Assay controls included Anti mouse-HRP, SA-HRP and SA-HRP.

Example 3: Neutralization of TGF-β Activity

To test the ability of a binding composition of the invention to neutralize TGF beta bioactivity and to neutralize a particular TGF Beta isoform, applicants adapted the HT-2 cell proliferation assay of Tsang, et al., (1995 Cytokine 7:389-97). Briefly, the human cell line HT-2 proliferates in response to IL-4 but that proliferation is inhibited by TGF-β1, TGF-β2, or TGF-β3. Consequently, a binding composition that is specific and/or selective for TGF-β1 can be determined to be neutralizing if it can be shown to prevent the normal inhibitory effect that TGF-β1 has on IL-4 induced HT-2 cells. Accordingly, IL-4-induced cell proliferation should proceed unconstrained if sufficient TGF Beta 1-specific binding composition is added to a mixture of HT-2 cells and TGF Beta 1. Consequently, the dose response neutralizing capability of binding compositions of the invention was assessed in the HT-2 assay in the presence of particular TGF isoforms and the IL-4 proliferation signal. Degree of cell proliferation was assessed using a commercial colorimetric cell proliferation measure (CellTiter 96® AQueous One Solution Cell Proliferation Assay from Promega).

HT-2 assay:

HT-2 cells were maintained in RPMI 1640 supplemented with 10% FBS, 50 μM β-Mercaptoethanol and 10 ng/ml human IL-2 (R&D Systems). Cells were centrifuged (1000 RPM in a Jouan CR422 centrifuge) and re-suspended in PBS. After washing (2X), the cells were finally re-suspended (1.5×10^6 cells/ml) in assay media (phenol red free RPMI 1640 supplemented with 2% FBS, 50 μM β-Mercaptoethanol). The cells were subsequently added (50 μl) to each well of a 96 well plate. Varying concentrations (0.0, 3.0, 1.0, 0.3, 0.1, 0.03, 0.01 ug/ml) of the binding compositions (such as the Fabs or mAbs designated 1D11, 2471, 1021, and 3821) were pre-incubated with TGF-β1 (300 pg/ml) before they were added to separate wells of the 96 well plate. After incubation (30 min at RT), 50 μl of pre-incubation mixture was added to the cells in a test well, followed immediately thereafter by 50 μl of assay media containing IL-4 (45 ng/ml murine; R&D

Systems). Assays are performed by adding a small amount of the CellTiter 96® AQueous One Solution Reagent directly to culture wells, incubating for 1–4 hours and then recording absorbance at 490nm with a 96-well plate reader. Specifically, after an incubation of 20 to 48 hr, 35 µl of CellTiter 96 Aqueous solution (Promega Corp) was added to each test well. After a further 2 to 3 hr incubation (37°C in a humidified, 5% CO2 atmosphere), the assay was quantitated by analysis on an ELISA plate reader at 490 nM (the quantity of formazan product generated using the CellTiter 96® colorimetric assay and as measured by the amount of 490nm absorbance is directly proportional to the number of living cells in culture).

Results:

The neutralization results of the TGF Beta 1 specific binding compositions of the invention (mAb 2471, mAb 1021 and mAb 3821) compared to the non-specific mAb 1D11 are shown below in Table 11. The values of the table show the extent of the neutralization as a measure of cell proliferation that has been rescued from inhibition by TGF Beta 1. Each value represents the mean of at least three data points with standard deviation (SD) indicated.

TABLE 11

	mAb 1D11		**mAb 2471**	
[mAb] (µg/ml)	**Mean**	**SD**	**Mean**	**SD**
3			106.29	2.96
1	130.20	7.43	78.78	6.36
0.3	14.79	4.33	32.53	10.3
0.1	92.42	9.19	14.16	7.00
0.03	47.30	2.90	4.12	5.09
0.01	17.16	3.60		
0			0	2.59

	mAb 1D11		mAb 1021	
[mAb] (µg/ml)	Mean	SD	Mean	SD
10			72.00	8.30
3			42.86	16.83
1	122.41	3.66	14.89	5.41
0.3	109.69	3.96	5.76	3.13
0.1	91.42	7.32	4.14	2.56
0.03	44.55	1.77		
0.01	11.17	1.54		
0			0	2.59

	mAb 1D11		mAb 3821	
[mAb] (µg/ml)	**Mean**	**Sdev**	**Mean**	**Sdev**
10			98.12	10.81
3			68.91	11.34
1	100.43	10.09	48.44	9.27
0.3	93.34	8.90	17.67	4.3
0.1	87.83	5.4	11.27	8.37
0.03	61.98	4.04		
0.01	22.81	4.90		
0			0	2.41

Example 4: Specificity of Binding Compositions for the TGF-β 1, 2 and 3 Isoforms

As stated herein, TGF-β1, TGF-β2, and TGF-β3 can inhibit IL-4 induced cell proliferation of HT-2 cells. Accordingly, the HT-2 assay described above was used to assess the specificity of the neutralizing ability of a binding composition of the invention (mAb 3821). The HT-2 assay was performed as described above except that TGF-β2 and TGF- β3 were additionally tested. Consequently, blocking inhibition of the IL-4 induced cell proliferation response by mAb 3821 with TGF-β2 and TGF- β3 would indicate no specificity of its neutralizing capability.

Results:

The data below in Table 12 show that the binding composition (mAb 3821) specifically and/or selectively neutralizes only TGF-β1 inhibition of IL-4 induced cell proliferation, whereas the control mAb 1D11 non-specifically blocks all three TGF-β1 isoforms (TGF-β1, TGF-β2, and TGF-β3) from inhibiting IL-4 induced cell proliferation.

The values of the table show the extent of the neutralization as a measure of cell proliferation that has been rescued from inhibition by TGF Beta 1, 2, or 3.

TABLE 12

	1		2		3		4	
	0 ug/ml mAb + 0 pg/ml TGF-β		1 ug/ml mAb 1D11 + 100 pg/ml TGFβ		10 ug/ml mAb 3821 + 100 pg/ml TGF-β		0 ug/ml mAb + 100 pg/ml TGF-β	
	Mean	Sdev	Mean	Sdev	Mean	Sdev	Mean	SDev
TGF-β1	100.00	9.00	100.95	3.19	93.09	5.91	0.00	8.46
TGF-β2	109.98	4.84	100.42	1.95	20.65	0.91	12.43	4.69
TGF-β3	106.26	2.59	112.75	3.99	7.75	3.30	1.16	5.71

Column 1 is a control showing the effect of no inhibitory TGF Beta isoform added to IL-4 induced HT-2 cell proliferation (note, the average colorimetric value indicating normal cell proliferation is approximately 100). Column 4, however, by contrast shows the unmitigated inhibitory effect of 100pg/ml of each inhibitory TGF Beta isoform (indicated by the row on the far left) on IL-4 induced cell proliferation (note the average degree of cell proliferation is below 5). Column 2 shows the lack of any neutralizing effect of mAb1D11 on 100pg/ml of each inhibitory TGF Beta isoform. Column 3 indicates the specific neutralizing effect of the binding composition mAb 3821 on 100pg/ml of each inhibitory TGF Beta isoform (note, only TGF Beta 1 is neutralized almost to the same extent as if no TGF Beta had been added as shown in Col. 1). Each value represents the mean of at least three data points with standard deviation (SD) indicated.

Example 3: Affinity Measurement

The affinity of mAb 2471, mAb 1021 and mAb 3821 for human TGF-β 1 (R&D Systems, Cat # 240-B/CF), TGF-β 2 (RDI, Cat # RDI-1035) and TGF-β 3 (RDI, Cat # RDI-1036/CF) is measured using a BIAcore® 2000 instrument. The BIAcore® utilizes the optical properties of surface plasmon resonance to detect alteration in protein concentration of interacting molecules within a dextran biosensor matrix. Except where noted, all reagents and materials are purchased from BIAcore® AB (Upsala, Sweden). All measurements are performed at room temperature. Samples are dissolved in HBS-EP buffer (150mM sodium chloride, 3mM EDTA, 0.005% (w/v) surfactant P-20, and 10mM HEPES, pH7.4). Goat anti-human Fc antibody is immobilized on flow cells 1 and 2 of a B1 sensor chip at a level of 500 response units (RUs) using an amine coupling kit.

Binding is evaluated using multiple analytical cycles. Each cycle is performed at a flow rate of 50μL/minute and consists of the following steps: injection of 10μL of an mAb 2471, mAb 1021 and mAb 3821 antibody at 1μg/mL, injection of 240μL of TGF-β 1, TGF-β 2, and TGF-β 3 (starting at 100nM and using two-fold serial dilutions for each cycle) followed by 20 minutes for dissociation, and regeneration using 50μL of 10mM glycine hydrochloride, pH1.5. Association and dissociation rates for each cycle are evaluated using a "Langmuir 1:1 with mass transport" binding model in the BIAevaluation software.

WHAT IS CLAIMED IS:

1. (1021 & 2471) A binding composition which specifically and/or selectively binds TGF Beta 1 isoform over TGF Beta 2 and/or TGF Beta 3 and which neutralizes TGF Beta 1 comprising at least one binding site comprising:
   a) at least four contiguous amino acids from QQWNGNPPA (SEQ ID NO: 24)(VL CDR3);
   b) at least six contiguous amino acids from QQWDSNPPA (SEQ ID NO: 27)(VL CDR3);
   c) at least five contiguous amino acids from YIYPYNGDTGYNQKFKS (SEQ ID NO: 14), wherein one of said at least five contiguous amino acids is D (VH CDR2); or
   d) at least five contiguous amino acids from GYYWFAY (SEQ ID NO: 15) (VH CDR3);or

(3821) a binding composition which specifically and/or selectively binds human TGF Beta 1 isoform over TGF Beta 2 and/or TGF Beta 3 and which neutralizes TGF Beta 1 comprising at least one antibody binding site comprising:
   a) at least six contiguous amino acids from LQYASSPYT (SEQ ID NO: 30)(VL CDR3);
   b) at least five contiguous amino acids from GYTFTDYTMH (SEQ ID NO: 19)(VH CDR1);
   c) at least eight contiguous amino acids from LITPFYGDAIYNQKFKG (SEQ ID NO: 20)(VH CDR2); or
   d) at least seven contiguous amino acids from GGLRRGPPFAY (SEQ ID NO: 21)(VH CDR3).

2. The binding composition of Claim 1 further comprises an additional binding site selected from the following:
   a) RASSSVSYMH (SEQ ID NO: 22)(1021 VL CDR1);
   b) ATSNLAS (SEQ ID NO: 23)(1021 VL CDR2);

c) GYTFTDYNMH (SEQ ID NO: 13)(1021 VH CDR1);

d) RASQEISGYLS (SEQ ID NO: 28)(3821 VL CDR1);

e) ATSSLDS (SEQ ID NO: 29)(3821 VL CDR2); and

f) GYTFTDYTMH (SEQ ID NO: 19)(3821 VH CDR1).

3. The binding composition of Claim 2 comprising:

a) at least three said binding sites;

b) at least four said binding sites;

c) at least five said binding sites;

d) at least six said binding sites; or

e) at least seven said binding sites.

4. The binding composition of Claim 3, wherein:

a) said binding site:

i) is specifically immunoreactive with mature human TGF Beta-1;

ii) is specifically immunoreactive with mature murine TGF Beta-1;

iii) is raised against a purified or recombinantly produced human TGF Beta-1 protein or fragment thereof;

iv) is in a monoclonal antibody, Fab, Fv, scFv, F(ab)2, or a variable domain of an antibody;

v) has at least one, two, or three conservative substitutions; or

vi) is in a human or a humanized antibody framework; or

b) said binding composition:

i) is an antibody molecule;

ii) is a monoclonal antibody molecule;

iii) is a diabody molecule;

iv) is a triabody molecule;

v) is a tetrabody molecule;

vi) is a minibody molecule;

vii) is a monoclonal antiserum;

viii) is detectably labeled;

ix) is lyophilized;

x) is sterile; or

xi) is in a buffered composition.

5. The binding composition of Claim 4 that is a monoclonal antibody, wherein said monoclonal antibody comprises at least one sequence at a CDR1, CDR2, or CDR3 of the light chain variable region (LCVR); or at a CDR1, CDR2, or CDR3 of the heavy chain variable region (HCVR) selected from:

a) GYTFTDYNMH (SEQ ID NO: 13)(1021 VH CDR1);
b) RASSSVSYMH (SEQ ID NO: 22)(1021 VL CDR1);
c) YIYPYNGDTGYNQKFKS (SEQ ID NO: 14)(1021 VH CDR2);
d) ATSNLAS (SEQ ID NO: 23) (1021 VL CDR2);
e) GYYWFAY (SEQ ID NO: 15) (1021 VH CDR3);
f) QQWNGNPPA (SEQ ID NO: 24)(1021 VL CDR3); or
g) QQWDSNPPA (SEQ ID NO: 27)(2471 VL CDR3).

6. The monoclonal antibody of Claim 5 wherein said CDR2 of the light chain variable region (LCVR) is ATSNPASYK or F (SEQ ID NO: 23) (2471 VL CDR2).

7. The monoclonal antibody of Claim 6 wherein said CDR2 of the heavy chain variable region (HCVR) is YIYPYDGETGYNQKFKS (SEQ ID NO: 14)(2471 VH CDR2).

8. The monoclonal antibody of Claim 7, wherein said CDR1 of the light chain variable region (LCVR) is RASSSVSYMH (SEQ ID NO: 22)(1021 VL CDR1).

9. The monoclonal antibody of Claim 8, wherein said CDR1 of the heavy chain variable region (HCVR) is GYTFTDYNMH (SEQ ID NO: 13)(1021 VH CDR1).

10. The monoclonal antibody of Claim 9 wherein said CDR3 of the light chain variable region (LCVR) is QQWNGNPPA (SEQ ID NO: 24)(1021 VL CDR3).

11. The monoclonal antibody of Claim 10 wherein said CDR3 of the heavy chain variable region (HCVR) is GYYWFAY (SEQ ID NO: 15) (1021 VH CDR3).

12. The monoclonal antibody of Claim 11, wherein said light chain variable region comprises at least 10 contiguous residues of SEQ ID NO: 2 (QIVLTQSPAIMSASPGEKVTMTCRASSSVSYMHWYQQKPGSSPKPWI YATSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQQWNGNPP AFGGGTKLEIKRA).

13. The monoclonal antibody of Claim 12, wherein said heavy chain variable region comprises at least 10 contiguous residues of SEQ ID NO: 4 (PELVKPGASVKISCKASGYTFTDYNMHWVKQSHGKSLEWLGYIYPY NGDTGYNQKFKSKATLTVDNSSSTAYMELRSLTSEDSAVYYCVRGYY WFAYWGRGTLVTVST).

14. The monoclonal antibody of Claim 13 that:

a) is humanized;
b) comprises of human or humanized constant regions;
c) is a Fab fragment;
d) is an Fv fragment;
e) is a scFv fragment;
f) is a F(ab)2 fragment;
g) is detectably labeled;
h) is lyophilized;
i) is encoded in an isolated nucleic acid molecule;
j) is encoded in an isolated nucleic acid molecule that is operably linked in an expression vector;
k) is encoded in an isolated nucleic acid molecule that is operably linked in an expression vector that is incorporated into a host cell;
l) is a chimeric antibody;
m) is conjugated to another chemical moiety;
n) is sterile;

o) is a pharmaceutical composition;

15. The monoclonal antibody of Claim 11, wherein said CDR1, CDR2, and CDR3 of said heavy variable region and said CDR1, CDR2, and CDR3 said light chain variable region are embedded within a human or humanized framework.

16. The monoclonal antibody of Claim 15, wherein said framework is human or humanized.

17. The monoclonal antibody of Claim 15 that:

a) is a Fab fragment;
b) is an Fv fragment;
c) is an scFv fragment;
d) is a F(ab)2 fragment;
e) is fused to a human constant region;
f) is conjugated to another chemical moiety;
g) comprises a heavy chain constant region selected from: IgG1, IgG2, IgG3, IgG4, IgA, IgE, IgM, and IgD;
h) comprises a human light chain constant region;
i) is detectably labeled;
j) is lyophilized;
k) is a fusion protein;
l) is sterile; or
m) comprises a pharmaceutical composition.

18. The binding composition of Claim 4 that is a monoclonal antibody, wherein said monoclonal antibody comprises at least one sequence at a CDR1, CDR2, or CDR3 of the light chain variable region (LCVR); or at a CDR1, CDR2, or CDR3 of the heavy chain variable region (HCVR) selected from:

a) RASQEISGYLS (SEQ ID NO: 28)(3821 VL CDR1);
b) ATSSLDS (SEQ ID NO: 29)(3821 VL CDR2);
c) LQYASSPYT (SEQ ID NO: 30)(3821 VL CDR3);

d) GYTFTDYTMH (SEQ ID NO: 19)(3821 VH CDR1);

e) LITPFYGDAIYNQKFKG (SEQ ID NO: 20)(3821 VH CDR2); and

f) GGLRRGPPFAY (SEQ ID NO: 21)(3821 VH CDR3).

19. The monoclonal antibody of Claim 18 wherein said CDR2 of the light chain variable region (LCVR) is ATSSLDS (SEQ ID NO: 29) (3821 VL CDR2).

20. The monoclonal antibody of Claim 19 wherein said CDR2 of the heavy chain variable region (HCVR) is LITPFYGDAIYNQKFKG (SEQ ID NO: 20)(3821 VH CDR2).

21. The monoclonal antibody of Claim 20, wherein said CDR1 of the light chain variable region (LCVR) is RASQEISGYLS (SEQ ID NO: 28) (3821 VL CDR1).

22. The monoclonal antibody of Claim 21 wherein said CDR1 of the heavy chain variable region (HCVR) is GYTFTDYTMH (SEQ ID NO: 19)(3821 VH CDR1).

23. The monoclonal antibody of Claim 22 wherein said CDR3 of the light chain variable region (LCVR) is LQYASSPYT (SEQ ID NO: 30)(3821 VL CDR3).

24. The monoclonal antibody of Claim 23 wherein said CDR3 of the heavy chain variable region (HCVR) is GGLRRGPPFAY (SEQ ID NO: 21)(3821 VH CDR3).

25. The monoclonal antibody of Claim 24, wherein said light chain variable region comprises residues 21-129 of SEQ ID NO: 10 (DVQITQSPSSLSASLGERVSLTCRASQEISGYLSWLQQKPDGTIKRLIY ATSSLDSGVPKRFSGSRSGSDYSLTISSPESEDFVDYYCLQYASSPYTFG GGTKLEIKRA).

26. The monoclonal antibody of Claim 25, wherein said heavy chain variable region comprises residues 29-140 of SEQ ID NO: 12 (AALMRPGVSVKISCKGSGYTFTDYTMHWVKQSHAKSLEWIGLITPFY GDAIYNQKFKGKATMTVDKSSSTAYMELARLTSDDSAIYYCTRGGLR RGPPFAYWGQGTLVTVSA).

27. The monoclonal antibody of Claim 26 that is:
a) humanized;
b) fused to a human constant region;
c) a Fab fragment;
d) an Fv fragment;
e) a scFv fragment;
f) a F(ab)2 fragment;
g) detectably labeled;
h) lyophilized;
i) a chimeric antibody;
j) used in the manufacture of a medicament for administration to a mammal for the treatment of a fibrotic condition selected from:

k) conjugated to another chemical moiety;
l) sterile; or
m) a pharmaceutical composition.

28. The monoclonal antibody of Claim 24, wherein said CDR1, CDR2, and CDR3 of said heavy variable region and said CDR1, CDR2, and CDR3 said light chain variable region are linked within a human or humanized framework.

29. The monoclonal antibody of Claim 28 that:
a) is a Fab fragment;
b) is an Fv fragment;
c) is an scFv fragment;
d) is a F(ab)2 fragment;

e) is fused to a human constant region;

f) is conjugated to another chemical moiety;

g) comprises a heavy chain constant region selected from: IgG1, IgG2, IgG3, IgG4, IgA, IgE, IgM, and IgD;

h) comprises a human light chain constant region;

i) is detectably labeled;

j) is a fusion protein;

k) is lyophilized;

l) is sterile; or

m) comprises a pharmaceutical composition.

30. A method of using the binding composition of Claim 3, comprising contacting said binding composition with a biological sample comprising an antigen, thereby forming a TGF Beta 1 binding composition:antigen complex.

31. The method of Claim 30, wherein said biological sample is from a human, and wherein said binding composition is an antibody.

32. A detection kit comprising said binding composition of Claim 3, and:

a) instructional material for the use of said binding composition for said detection; or

b) a compartment providing segregation of said binding composition.

SEQUENCE LISTING

SEQ ID NO: 1 is murine mAb 1021 Light Chain nucleic acid sequence.
SEQ ID NO: 2 is murine mAb 1021 Light Chain amino acid sequence.
SEQ ID NO: 3 is murine mAb 1021 Heavy Chain nucleic acid sequence.
SEQ ID NO: 4 is murine mAb 1021 Heavy Chain amino acid sequence.
SEQ ID NO: 5 is murine mAb 2471 Light Chain nucleic acid sequence.
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SEQ ID NO: 7 is murine mAb 2471 Heavy Chain nucleic acid sequence.
SEQ ID NO: 8 is murine mAb 2471 Heavy Chain amino acid sequence.
SEQ ID NO: 9 is murine mAb 3821 Light Chain nucleic acid sequence.
SEQ ID NO: 10 is murine mAb 3821 Light Chain amino acid sequence.
SEQ ID NO: 11 is murine mAb 3821 Heavy Chain nucleic acid sequence.
SEQ ID NO: 12 is murine mAb 3821 Heavy Chain amino acid sequence.
SEQ ID NO: 13 is murine mAb 1021 Heavy Chain CDR1 amino acid sequence.
SEQ ID NO: 14 is murine mAb 1021 Heavy Chain CDR2 amino acid sequence.
SEQ ID NO: 15 is murine mAb 1021 Heavy Chain CDR3 amino acid sequence.
SEQ ID NO: 16 is murine mAb 2471 Heavy Chain CDR1 amino acid sequence.
SEQ ID NO: 17 is murine mAb 2471 Heavy Chain CDR2 amino acid sequence.
SEQ ID NO: 18 is murine mAb 2471 Heavy Chain CDR3 amino acid sequence.
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SEQ ID NO: 20 is murine mAb 3821 Heavy Chain CDR2 amino acid sequence.
SEQ ID NO: 21 is murine mAb 3821 Heavy Chain CDR3 amino acid sequence.
SEQ ID NO: 22 is murine mAb 1021 Light Chain CDR1 amino acid sequence.
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SEQ ID NO: 24 is murine mAb 1021 Light Chain CDR3 amino acid sequence.
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SEQ ID NO: 26 is murine mAb 2471 Light Chain CDR2 amino acid sequence.
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acttcaccca ttgtcaagag cttcaacagg aatgagtgt 699

<210> 2
<211> 233
<212> PRT
<213> mus sp.

<400> 2

Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
1 5 10 15

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

Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Arg Ala Ser Ser Ser
35 40 45

Val Ser Tyr Met His Trp Tyr Gln Gln Lys Pro Gly Ser Ser Pro Lys
50 55 60

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

Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Arg
85 90 95

Val Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Asn Gly
100 105 110

Asn Pro Pro Ala Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Ala
115 120 125

Asp Ala Ala Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu Gln Leu
130 135 140

Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe Tyr Pro
145 150 155 160

Lys Asp Ile Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg Gln Asn
165 170 175

Gly Val Leu Asn Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser Thr Tyr
180 185 190

Ser Met Ser Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu Arg His
195 200 205

Asn Ser Tyr Thr Cys Glu Ala Thr His Lys Thr Ser Thr Ser Pro Ile
210 215 220

Val Lys Ser Phe Asn Arg Asn Glu Cys
225 230

<210> 3
<211> 1380
<212> DNA
<213> mus sp.

<400> 3
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tcctgcaagg cttctggata cacattcact gactacaaca tgcactgggt gaagcagagc 180

catggaaaga gccttgagtg gcttggatat atttatcctt acaatggtga tactggctac 240

aaccagaagt tcaagagcaa ggccacattg actgtagaca attcctccag cacagcctat 300

atggagctcc gcagcctgac atctgaggac tctgcagtct attattgtgt aagaggatac 360

tactggtttg cttactgggg ccgagggact ctggtcactg tctctacagc caaaacgaca 420

cccccatctg tctatccact ggcccctgga tctgctgccc aaactaactc catggtgacc 480

ctgggatgcc tggtcaaggg ctatttccct gagccagtga cagtgacctg gaactctgga 540

tccctgtcca gcggtgtgca caccttccca gctgtcctgc agtctgacct ctacactctg 600

agcagctcag tgactgtccc ctccagcacc tggcccagcg agaccgtcac ctgcaacgtt 660

gcccacccgg ccagcagcac caaggtggac aagaaaattg tgcccaggga ttgtggttgt 720

aagccttgca tatgtacagt cccagaagta tcatctgtct tcatcttccc cccaaagccc 780

aaggatgtgc tcaccattac tctgactcct aaggtcacgt gtgttgtggt agacatcagc 840

aaggatgatc ccgaggtcca gttcagctgg tttgtagatg atgtggaggt gcacacagct 900

cagacgcaac cccgggagga gcagttcaac agcactttcc gctcagtcag tgaacttccc 960

atcatgcacc aggactggct caatggcaag gagttcaaat gcagggtcaa cagtgcagct 1020

ttccctgccc ccatcgagaa aaccatctcc aaaaccaaag gcagaccgaa ggctccacag 1080

gtgtacacca ttccacctcc caaggagcag atggccaagg ataaagtcag tctgacctgc 1140

atgataacag acttcttccc tgaagacatt actgtggagt ggcagtggaa tgggcagcca 1200

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ttacatgagg gcctgcacaa ccaccatact gagaagagcc tctcccactc tcctggtaaa 1380

<210> 4
<211> 459
<212> PRT
<213> mus sp.

<400> 4

Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro Gly
1 5 10 15

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

Pro Gly Ala Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe
35 40 45

Thr Asp Tyr Asn Met His Trp Val Lys Gln Ser His Gly Lys Ser Leu
50 55 60

Glu Trp Leu Gly Tyr Ile Tyr Pro Tyr Asn Gly Asp Thr Gly Tyr Asn
65 70 75 80

Gln Lys Phe Lys Ser Lys Ala Thr Leu Thr Val Asp Asn Ser Ser Ser
85 90 95

Thr Ala Tyr Met Glu Leu Arg Ser Leu Thr Ser Glu Asp Ser Ala Val
100 105 110

Tyr Tyr Cys Val Arg Gly Tyr Tyr Trp Phe Ala Tyr Trp Gly Arg Gly
115 120 125

Thr Leu Val Thr Val Ser Thr Ala Lys Thr Thr Pro Pro Ser Val Tyr
130 135 140

Pro Leu Ala Pro Gly Ser Ala Ala Gln Thr Asn Ser Met Val Thr Leu
145 150 155 160

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

Asn Ser Gly Ser Leu Ser Ser Gly Val His Thr Phe Pro Ala Val Leu
180 185 190

Gln Ser Asp Leu Tyr Thr Leu Ser Ser Ser Val Thr Val Pro Ser Ser
195 200 205

Thr Trp Pro Ser Glu Thr Val Thr Cys Asn Val Ala His Pro Ala Ser
210 215 220

Ser Thr Lys Val Asp Lys Lys Ile Val Pro Arg Asp Cys Gly Cys Lys
225 230 235 240

Pro Cys Ile Cys Thr Val Pro Glu Val Ser Ser Val Phe Ile Phe Pro
245 250 255

Pro Lys Pro Lys Asp Val Leu Thr Ile Thr Leu Thr Pro Lys Val Thr
260 265 270

Cys Val Val Val Asp Ile Ser Lys Asp Asp Pro Glu Val Gln Phe Ser
275 280 285

Trp Phe Val Asp Asp Val Glu Val His Thr Ala Gln Thr Gln Pro Arg
290 295 300

Glu Glu Gln Phe Asn Ser Thr Phe Arg Ser Val Ser Glu Leu Pro Ile
305 310 315 320

Met His Gln Asp Trp Leu Asn Gly Lys Glu Phe Lys Cys Arg Val Asn
325 330 335

Ser Ala Ala Phe Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys
340 345 350

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

Gln Met Ala Lys Asp Lys Val Ser Leu Thr Cys Met Ile Thr Asp Phe
370 375 380

Phe Pro Glu Asp Ile Thr Val Glu Trp Gln Trp Asn Gly Gln Pro Ala
385 390 395 400

Glu Asn Tyr Lys Asn Thr Gln Pro Ile Met Asp Thr Asp Gly Ser Tyr
405 410 415

Phe Val Tyr Ser Lys Leu Asn Val Gln Lys Ser Asn Trp Glu Ala Gly
420 425 430

Asn Thr Phe Thr Cys Ser Val Leu His Glu Gly Leu His Asn His His
435 440 445

Thr Glu Lys Ser Leu Ser His Ser Pro Gly Lys
450 455

<210> 5
<211> 699
<212> DNA
<213> mus sp.

<400> 5
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caaattgttc tcacccagtc tccagcaatc atgtctgcat ctccagggga gaaggtcaca 120

atgacttgca gggccagctc aagtgtaagt tacatgcact ggtaccagca gaagccagga 180

tcctccccca aaccctggat ttatgccaca tccaacctgg cttctggagt ccctgctcgc 240

ttcagtggca gtgggtctgg gacctcttac tctctcacaa tcagcagagt ggaggctgaa 300

gatgctgcca cttattactg ccagcagtgg gatagtaacc cacccgcgtt cggtggaggc 360

accaagctgg aaatcaaacg ggctgatgct gcaccaactg tatccatctt cccaccatcc 420

agtgagcagt taacatctgg aggtgcctca gtcgtgtgct tcttgaacaa cttctacccc 480

aaagacatca atgtcaagtg gaagattgat ggcagtgaac gacaaaatgg cgtcctgaac 540

agttggactg atcaggacag caaagacagc acctacagca tgagcagcac cctcacgttg 600

accaaggacg agtatgaacg acataacagc tatacctgtg aggccactca caagacatca 660

acttcaccca ttgtcaagag cttcaacagg aatgagtgt 699

<210> 6
<211> 233
<212> PRT
<213> mus sp.

<400> 6

Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
1 5 10 15

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

Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Arg Ala Ser Ser Ser
35 40 45

Val Ser Tyr Met His Trp Tyr Gln Gln Lys Pro Gly Ser Ser Pro Lys
50 55 60

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

Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Arg
85 90 95

Val Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Asp Ser
100 105 110

Asn Pro Pro Ala Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Ala
115 120 125

Asp Ala Ala Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu Gln Leu
130 135 140

Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe Tyr Pro
145 150 155 160

Lys Asp Ile Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg Gln Asn
165 170 175

Gly Val Leu Asn Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser Thr Tyr
180 185 190

Ser Met Ser Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu Arg His
195 200 205

Asn Ser Tyr Thr Cys Glu Ala Thr His Lys Thr Ser Thr Ser Pro Ile
210 215 220

Val Lys Ser Phe Asn Arg Asn Glu Cys
225 230

<210> 7
<211> 1380
<212> DNA
<213> mus sp.

<400> 7
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gaggtccagc ttcagcagtc aggtcctgag ctggtgaaac ctggggcctc agtgaagata 120

tcctgcaagg cttctggata cacattcact gactacaaca tgcactgggt gaagcagagc 180

catggaaaga gccttgagtg gcttggatat atttatcctt acaatggtga tactggctac 240

aaccagaagt tcaagagcaa ggccacattg actgtagaca attcctccag cacagcctat 300

atggagctcc gcagcctgac atctgaggac tctgcagtct attattgtgt aagaggatac 360

tactggtttg cttactgggg ccaagggact ctggtcactg tctctacagc caaaacgaca 420

cccccatctg tctatccact ggcccctgga tctgctgccc aaactaactc catggtgacc 480

ctgggatgcc tggtcaaggg ctatttccct gagccagtga cagtgacctg gaactctgga 540

tccctgtcca gcggtgtgca caccttccca gctgtcctgc agtctgacct ctacactctg 600

agcagctcag tgactgtccc ctccagcacc tggcccagcg agaccgtcac ctgcaacgtt 660

gcccacccgg ccagcagcac caaggtggac aagaaaattg tgcccaggga ttgtggttgt 720

aagccttgca tatgtacagt cccagaagta tcatctgtct tcatcttccc cccaaagccc 780

aaggatgtgc tcaccattac tctgactcct aaggtcacgt gtgttgtggt agacatcagc 840

aaggatgatc ccgaggtcca gttcagctgg tttgtagatg atgtggaggt gcacacagct 900

cagacgcaac cccgggagga gcagttcaac agcactttcc gctcagtcag tgaacttccc 960

atcatgcacc aggactggct caatggcaag gagttcaaat gcagggtcaa cagtgcagct 1020

ttccctgccc ccatcgagaa aaccatctcc aaaaccaaag gcagaccgaa ggctccacag 1080

gtgtacacca ttccacctcc caaggagcag atggccaagg ataaagtcag tctgacctgc 1140

atgataacag acttcttccc tgaagacatt actgtggagt ggcagtggaa tgggcagcca 1200

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ttacatgagg gcctgcacaa ccaccatact gagaagagcc tctcccactc tcctggtaaa 1380

<210> 8
<211> 460
<212> PRT
<213> mus sp.

<400> 8

Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
1 5 10 15

Gly Ser Thr Gly Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val
20 25 30

Lys Pro Gly Ala Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr
35 40 45

Phe Thr Asp Tyr Asn Met His Trp Val Lys Gln Ser His Gly Lys Ser
50 55 60

Leu Glu Trp Leu Gly Tyr Ile Tyr Pro Tyr Asn Gly Asp Thr Gly Tyr
65 70 75 80

Asn Gln Lys Phe Lys Ser Lys Ala Thr Leu Thr Val Asp Asn Ser Ser
85 90 95

Ser Thr Ala Tyr Met Glu Leu Arg Ser Leu Thr Ser Glu Asp Ser Ala
100 105 110

Val Tyr Tyr Cys Val Arg Gly Tyr Tyr Trp Phe Ala Tyr Trp Gly Gln
115 120 125

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

Tyr Pro Leu Ala Pro Gly Ser Ala Ala Gln Thr Asn Ser Met Val Thr
145 150 155 160

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

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

Leu Gln Ser Asp Leu Tyr Thr Leu Ser Ser Ser Val Thr Val Pro Ser
195 200 205

Ser Thr Trp Pro Ser Glu Thr Val Thr Cys Asn Val Ala His Pro Ala
210 215 220

Ser Ser Thr Lys Val Asp Lys Lys Ile Val Pro Arg Asp Cys Gly Cys
225 230 235 240

Lys Pro Cys Ile Cys Thr Val Pro Glu Val Ser Ser Val Phe Ile Phe
245 250 255

Pro Pro Lys Pro Lys Asp Val Leu Thr Ile Thr Leu Thr Pro Lys Val
260 265 270

Thr Cys Val Val Val Asp Ile Ser Lys Asp Asp Pro Glu Val Gln Phe
275 280 285

Ser Trp Phe Val Asp Asp Val Glu Val His Thr Ala Gln Thr Gln Pro
290 295 300

Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Ser Val Ser Glu Leu Pro
305 310 315 320

Ile Met His Gln Asp Trp Leu Asn Gly Lys Glu Phe Lys Cys Arg Val
325 330 335

Asn Ser Ala Ala Phe Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr
340 345 350

Lys Gly Arg Pro Lys Ala Pro Gln Val Tyr Thr Ile Pro Pro Pro Lys

355 360 365

Glu Gln Met Ala Lys Asp Lys Val Ser Leu Thr Cys Met Ile Thr Asp
370 375 380

Phe Phe Pro Glu Asp Ile Thr Val Glu Trp Gln Trp Asn Gly Gln Pro
385 390 395 400

Ala Glu Asn Tyr Lys Asn Thr Gln Pro Ile Met Asp Thr Asp Gly Ser
405 410 415

Tyr Phe Val Tyr Ser Lys Leu Asn Val Gln Lys Ser Asn Trp Glu Ala
420 425 430

Gly Asn Thr Phe Thr Cys Ser Val Leu His Glu Gly Leu His Asn His
435 440 445

His Thr Glu Lys Ser Leu Ser His Ser Pro Gly Lys
450 455 460

<210> 9
<211> 702
<212> DNA
<213> mus sp.

<400> 9
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ctcacttgtc gggcaagtca ggaaattagt ggttacttaa gctggcttca gcagaaacca 180

gatggaacta ttaaacgcct gatctacgcc acatccagtt tagattctgg tgtccccaaa 240

aggttcagtg gcagtaggtc tgggtcagat tattctctca ccatcagcag ccctgagtct 300

gaagattttg tagactatta ctgtctacaa tatgctagtt ctccgtacac gtttggaggg 360

gggaccaagc tggaaataaa acgggctgat gctgcaccaa ctgtatccat cttcccacca 420

tccagtgagc agttaacatc tggaggtgcc tcagtcgtgt gcttcttgaa caacttctac 480

cccaaagaca tcaatgtcaa gtggaagatt gatggcagtg aacgacaaaa tggcgtcctg 540

aacagttgga ctgatcggga cagcaaagac agcacctaca gcatgagcag caccctcacg 600

ttgaccaagg acgagtatga acgacataac agttatacct gtgaggccac tcacaagaca 660

tcaacttcac ccattgtcaa gagcttcaac aggaatgagt gt 702

<210> 10
<211> 234
<212> PRT
<213> mus sp.

<400> 10

Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
1 5 10 15

Gly Ser Thr Gly Asp Val Gln Ile Thr Gln Ser Pro Ser Ser Leu Ser
20 25 30

Ala Ser Leu Gly Glu Arg Val Ser Leu Thr Cys Arg Ala Ser Gln Glu
35 40 45

Ile Ser Gly Tyr Leu Ser Trp Leu Gln Gln Lys Pro Asp Gly Thr Ile
50 55 60

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

Arg Phe Ser Gly Ser Arg Ser Gly Ser Asp Tyr Ser Leu Thr Ile Ser
85 90 95

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

Ser Ser Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg
115 120 125

Ala Asp Ala Ala Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu Gln
130 135 140

Leu Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe Tyr
145 150 155 160

Pro Lys Asp Ile Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg Gln
165 170 175

Asn Gly Val Leu Asn Ser Trp Thr Asp Arg Asp Ser Lys Asp Ser Thr
180 185 190

Tyr Ser Met Ser Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu Arg
195 200 205

His Asn Ser Tyr Thr Cys Glu Ala Thr His Lys Thr Ser Thr Ser Pro
210 215 220

Ile Val Lys Ser Phe Asn Arg Asn Glu Cys
225 230

<210> 11
<211> 1392
<212> DNA
<213> mus sp.

<400> 11
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gaggttcagc tgcagcagtc tggggctgcg ctgatgaggc ctggggtctc agtgaagatt 120

tcctgcaagg gttctggcta cacattcact gattatacta tgcactgggt gaagcagagt 180
catgcaaaga gtctagagtg gattggactt attactcctt tctatggtga tgctatatac 240
aaccagaagt tcaagggcaa ggccacaatg actgtagaca aatcctccag cacagcctat 300
atggaacttg ccagactgac atctgatgat tctgccatct attactgtac aaggggggga 360
ttacgacgcg gacccccttt tgcttactgg ggccaaggga ctctggtcac tgtctctgca 420
gccaaaacga cacccccatc tgtctatcca ctggcccctg gatctgctgc ccaaactaac 480
tccatggtga ccctgggatg cctggtcaag ggctatttcc ctgagccagt gacagtgacc 540
tggaactctg gatccctgtc cagcggtgtg cacaccttcc cagctgtcct gcagtctgac 600
ctctacactc tgagcagctc agtgactgtc ccctccagca cctggcccag cgagaccgtc 660
acctgcaacg ttgcccaccc ggccagcagc accaaggtgg acaagaaaat tgtgcccagg 720
gattgtggtt gtaagccttg catatgtaca gtcccagaag tatcatctgt cttcatcttc 780
cccccaaagc ccaaggatgt gctcaccatt actctgactc ctaaggtcac gtgtgttgtg 840
gtagacatca gcaaggatga tcccgaggtc cagttcagct ggtttgtaga tgatgtggag 900
gtgcacacag ctcagacgca accccgggag gagcagttca acagcacttt ccgctcagtc 960
agtgaacttc ccatcatgca ccaggactgg ctcaatggca aggagttcaa atgcagggtc 1020
aacagtgcag ctttccctgc ccccatcgag aaaaccatct ccaaaaccaa aggcagaccg 1080
aaggctccac aggtgtacac cattccacct cccaaggagc agatggccaa ggataaagtc 1140
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aatgggcagc cagcggagaa ctacaagaac actcagccca tcatggacac agatggctct 1260
tacttcgtct acagcaagct caatgtgcag aagagcaact gggaggcagg aaatactttc 1320
acctgctctg tgttacatga gggcctgcac aaccaccata ctgagaagag cctctcccac 1380
tctcctggta aa 1392

<210> 12
<211> 464
<212> PRT
<213> mus sp.

<400> 12

Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
1 5 10 15

Gly Ser Thr Gly Glu Val Gln Leu Gln Gln Ser Gly Ala Ala Leu Met
20 25 30

Arg Pro Gly Val Ser Val Lys Ile Ser Cys Lys Gly Ser Gly Tyr Thr
35 40 45

Phe Thr Asp Tyr Thr Met His Trp Val Lys Gln Ser His Ala Lys Ser
50 55 60

Leu Glu Trp Ile Gly Leu Ile Thr Pro Phe Tyr Gly Asp Ala Ile Tyr
65 70 75 80

Asn Gln Lys Phe Lys Gly Lys Ala Thr Met Thr Val Asp Lys Ser Ser
85 90 95

Ser Thr Ala Tyr Met Glu Leu Ala Arg Leu Thr Ser Asp Asp Ser Ala
100 105 110

Ile Tyr Tyr Cys Thr Arg Gly Gly Leu Arg Arg Gly Pro Pro Phe Ala
115 120 125

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

Pro Pro Ser Val Tyr Pro Leu Ala Pro Gly Ser Ala Ala Gln Thr Asn
145 150 155 160

Ser Met Val Thr Leu Gly Cys Leu Val Lys Gly Tyr Phe Pro Glu Pro
165 170 175

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

Phe Pro Ala Val Leu Gln Ser Asp Leu Tyr Thr Leu Ser Ser Ser Val
195 200 205

Thr Val Pro Ser Ser Thr Trp Pro Ser Glu Thr Val Thr Cys Asn Val
210 215 220

Ala His Pro Ala Ser Ser Thr Lys Val Asp Lys Lys Ile Val Pro Arg
225 230 235 240

Asp Cys Gly Cys Lys Pro Cys Ile Cys Thr Val Pro Glu Val Ser Ser
245 250 255

Val Phe Ile Phe Pro Pro Lys Pro Lys Asp Val Leu Thr Ile Thr Leu
260 265 270

Thr Pro Lys Val Thr Cys Val Val Val Asp Ile Ser Lys Asp Asp Pro
275 280 285

Glu Val Gln Phe Ser Trp Phe Val Asp Asp Val Glu Val His Thr Ala
290 295 300

Gln Thr Gln Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Ser Val
305 310 315 320

Ser Glu Leu Pro Ile Met His Gln Asp Trp Leu Asn Gly Lys Glu Phe

325 330 335

Lys Cys Arg Val Asn Ser Ala Ala Phe Pro Ala Pro Ile Glu Lys Thr
340 345 350

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

Pro Pro Pro Lys Glu Gln Met Ala Lys Asp Lys Val Ser Leu Thr Cys
370 375 380

Met Ile Thr Asp Phe Phe Pro Glu Asp Ile Thr Val Glu Trp Gln Trp
385 390 395 400

Asn Gly Gln Pro Ala Glu Asn Tyr Lys Asn Thr Gln Pro Ile Met Asp
405 410 415

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

Asn Trp Glu Ala Gly Asn Thr Phe Thr Cys Ser Val Leu His Glu Gly
435 440 445

Leu His Asn His His Thr Glu Lys Ser Leu Ser His Ser Pro Gly Lys
450 455 460

<210> 13
<211> 10
<212> PRT
<213> mus sp.

<400> 13

Gly Tyr Thr Phe Thr Asp Tyr Asn Met His
1 5 10

<210> 14
<211> 17
<212> PRT
<213> mus sp.

<400> 14

Tyr Ile Tyr Pro Tyr Asn Gly Asp Thr Gly Tyr Asn Gln Lys Phe Lys
1 5 10 15

Ser

<210> 15
<211> 7
<212> PRT
<213> mus sp.

<400> 15

Gly Tyr Tyr Trp Phe Ala Tyr
1 5

<210> 16
<211> 10
<212> PRT
<213> mus sp.

<400> 16

Gly Tyr Thr Phe Thr Asp Tyr Asn Met His
1 5 10

<210> 17
<211> 17
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<213> mus sp.

<400> 17

Tyr Ile Tyr Pro Tyr Asn Gly Asp Thr Gly Tyr Asn Gln Lys Phe Lys
1 5 10 15

Ser

<210> 18
<211> 7
<212> PRT
<213> mus sp.

<400> 18

Gly Tyr Tyr Trp Phe Ala Tyr
1 5

<210> 19
<211> 10
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<213> mus sp.

<400> 19

Gly Tyr Thr Phe Thr Asp Tyr Thr Met His
1 5 10

<210> 20
<211> 17
<212> PRT
<213> mus sp.

<400> 20

Leu Ile Thr Pro Phe Tyr Gly Asp Ala Ile Tyr Asn Gln Lys Phe Lys
1 5 10 15

Gly

<210> 21
<211> 11
<212> PRT
<213> mus sp.

<400> 21

Gly Gly Leu Arg Arg Gly Pro Pro Phe Ala Tyr
1 5 10

<210> 22
<211> 10
<212> PRT
<213> mus sp.

<400> 22

Arg Ala Ser Ser Ser Val Ser Tyr Met His
1 5 10

<210> 23
<211> 7
<212> PRT
<213> mus sp.

<400> 23

Ala Thr Ser Asn Leu Ala Ser
1 5

<210> 24
<211> 9
<212> PRT
<213> mus sp.

<400> 24

Gln Gln Trp Asn Gly Asn Pro Pro Ala
1 5

<210> 25
<211> 10
<212> PRT
<213> mus sp.

<400> 25

Arg Ala Ser Ser Ser Val Ser Tyr Met His
1 5 10

<210> 26
<211> 7
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<213> mus sp.

<400> 26

Ala Thr Ser Asn Leu Ala Ser
1 5

<210> 27
<211> 9
<212> PRT
<213> mus sp.

<400> 27

Gln Gln Trp Asp Ser Asn Pro Pro Ala
1 5

<210> 28
<211> 11
<212> PRT
<213> mus sp.

<400> 28

Arg Ala Ser Gln Glu Ile Ser Gly Tyr Leu Ser
1 5 10

<210> 29
<211> 7
<212> PRT
<213> mus sp.

<400> 29

Ala Thr Ser Ser Leu Asp Ser
1 5

<210> 30
<211> 9
<212> PRT
<213> mus sp.

<400> 30

Leu Gln Tyr Ala Ser Ser Pro Tyr Thr
1 5