

Description

Title of Invention: ANTI-LATENT TGF-BETA 1 ANTIBODIES AND METHODS OF USE

Technical Field

[0001] The present invention relates to anti-latent TGF-beta 1 antibodies and methods of using the same.

Background Art

[0002] Transforming growth factor-beta (transforming growth factor beta; TGF-beta) is a member of the TGF-beta superfamily of cytokines, which consists of TGF-beta isoforms, activins, inhibins, Nodal, bone morphogenetic proteins (BMPs), anti-Mullerian hormone (AMH), as well as growth and differentiation factors (GDFs). Members of this superfamily are dimeric proteins with conserved structures and have pleiotropic functions in vitro and in vivo (NPL 1, 2). The TGF-beta isoforms are involved in many cellular processes, including growth inhibition, cell migration, invasion, epithelial-mesenchymal transition (EMT), extracellular matrix (ECM) remodeling, and immune-suppression (NPL 3). However, although normally dynamically regulated and involved in maintenance of tissue homeostasis, TGF-beta isoforms are often chronically overexpressed in disease states, including cancer, fibrosis, and inflammation, and this excessive production of TGF-beta drives disease progression by modulating cell growth, migration, or phenotype.

[0003] Three separate TGF-beta isoforms (TGF-beta 1, TGF-beta 2, and TGF-beta 3) have been identified in mammals, and share 70-82% homology at the amino acid level (NPL 4). All three TGF-beta isoforms bind to TGF-beta receptor type 2 (TGFR2) as homodimers (their active form); TGFR2 then recruits and activates TGF-beta receptor type 1 (TGFR1) to activate receptor signaling (NPL 5). However, expression levels of the three isoforms vary depending on the tissue (NPL 6), and their functions are distinct, as demonstrated by the phenotypes of knockout mice (NPL 7-11).

[0004] Like other members of the TGF-beta superfamily, TGF-beta is synthesized as a precursor protein, which forms a homodimer that interacts with its latency-associated peptide (LAP) and a latent TGF-beta-binding protein (LTBP) to form a larger complex called the large latent complex (LLC). The TGF-beta gene encodes a preproprotein sequence consisting of a signal peptide, a propeptide that ends with a proprotein convertase (PPC) cleavage site, and the mature TGF-beta sequence. Furin hydrolyzes the PPC cleavage site, creating separate TGF-beta- and propeptide-derived homodimers. The two homodimers remain noncovalently associated and are secreted. This latent complex keeps TGF-beta in an inactive form that is incapable of binding to

its receptors (NPL 12, 13). The TGF-beta activation process involves the release of the LLC from the ECM, followed by further proteolysis of LAP to release active TGF-beta to its receptors (NPL 3). Latent TGF-beta is cleaved to release active TGF-beta by a wide range of proteases, including plasmin (PLN), plasma kallikrein (PLK), matrix metalloproteinase (MMP) 2, and MMP9 (NPL 14), and by thrombospondin 1 (TSP-1) (NPL 15). Without wishing to be bound by any theory, MMP2, as well as MMP9, proteolytically cleaves latent TGF-beta 1 and release mature TGF-beta 1 from latent form. Both MMP2 and MMP9 are synthesized as inactive pro-MMP. Pro-MMP2 is activated by a complex of membrane type 1 MMP (MT1-MMP/MMP14) and tissue inhibitor of metalloproteinase 2 (TIMP-2). Pro-MMP9 is activated through an interacting protease cascade involving plasmin and stromelysin 1 (MMP-3). Plasmin generates active MMP-3 from its zymogen. Active MMP-3 cleaves the propeptide from the 92-kDa pro-MMP-9, yielding an 82-kDa enzymatically active enzyme. The cleavage sites of MMPs are not specifically determined; however, it is reported that MMP3 specifically cleaves the site between 79 Ala and 80 Leu of latent TGF-beta, so as to activate TGF-beta (WO2005/023870). Alternatively, upon mechanical stretch, integrins can activate TGF-beta by binding to the RGD motif present in LAP to induce the release of mature TGF-beta from its latent complex (NPL 16, 17).

[0005] After activation, the dimeric TGF-beta ligand binds to the extracellular domains of type I and type II receptors and induces close proximity, placing the intracellular serine/threonine kinase domains of the receptors in a conformation that facilitates the phosphorylation and subsequent activation of the type I receptor. This activation of the type I receptor leads to the propagation of signaling by at least two seemingly independent routes: the SMAD-dependent canonical pathway and the SMAD-independent or non-canonical pathway. In the SMAD-dependent pathway, activation of TGFR1 (also known as ALK5) leads to phosphorylation of SMAD proteins. SMAD2 and SMAD3 are substrates of TGFR1. Upon phosphorylation by the receptor, SMADs together with the common mediator SMAD4 translocate to the nucleus, where they interact with other transcription factors to regulate transcriptional responses (NPL 18). In the non-canonical pathway, the activated TGF-beta receptor complex transmits a signal through other factors, such as tumor necrosis factor (TNF) receptor-associated factor 4 (TRAF4), TRAF6, TGF-beta-activated kinase 1 (TAK1, also known as MAP3K7), p38 mitogen-activated protein kinase (p38 MAPK), RHO, phosphoinositide 3-kinase (PI3K), AKT (also known as protein kinase B), extracellular signal-regulated kinase (ERK), JUN N-terminal kinase (JNK), or nuclear factor-kappa B (NF-kappa B). Thus, cellular responses to TGF-beta signaling result from the dynamic combination of canonical and non-canonical signaling cascades.

[0006] Fibrosis, or the accumulation of ECM molecules that make up scar tissue, is a

common feature of chronic tissue injury. Renal fibrosis, liver fibrosis, and lung/pulmonary fibrosis are among the more common fibrotic diseases, which in aggregate represent a huge unmet clinical need. TGF-beta strongly promotes generation of the extracellular matrices of mesenchymal cells, while at the same time it suppresses the growth of epithelial cells, which contributes to the pathogenesis of sclerotic diseases. Overexpression of the active form of TGF-beta 1 in the liver of transgenic mice is sufficient to induce fibrotic disease in multiple organs (NPL 19). On the other hand, TGF-beta also plays an important role in maintaining our health. For example, TGF-beta suppresses excessive generation of proteases in the lung and prevents the destruction of lung tissue that leads to emphysema. Also, mice with deleted TGF-beta 1 show prenatal lethality (around 50% at 10.5 days post coitus) or their offspring die shortly after birth, with massive inflammatory lesions seen in many organs, including the lungs (vasculitis, perivascular cuffing, and interstitial pneumonia) and heart (endocarditis and myocarditis), which suggests that TGF-beta 1 plays a crucial role in maintaining immune homeostasis (NPL 7).

- [0007] Results of studies using a neutralizing antibody to TGF-beta and animal models revealed that sclerotic diseases can be prevented or cured by suppressing the action of TGF-beta. As TGF-beta is produced as a precursor protein, there are several reported approaches to prevent activation from the latent form. Another method of preventing activation from the latent form is to use an inhibitor or antibody that binds to latent TGF-beta to block cleavage by proteases, such as PLK and PLN. Several antibodies that use this method of suppressing TGF-beta activation were reported as preventing or treating hepatic cirrhosis (PTL 1). In addition, there have been some documents mentioning anti-LAP antibodies for treating cancer (PTL 2), and TGF beta 1-binding immunoglobulins for treating TGF beta 1-related disorders (PTL 3).

Some anti-latent TGF-beta 1 antibodies have been described (PTL 4, PTL 5, and PTL 6).

Citation List

Patent Literature

- [0008] [PTL 1] WO 2011102483
 [PTL 2] WO 2016115345
 [PTL 3] WO 2017156500
 [PTL 4] WO 2018/043734
 [PTL 5] WO 2019/163927
 [PTL 6] WO 2021/039945

Non Patent Literature

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- [NPL 2] Massague, J. Annu. Rev. Biochem. 67, 753-791 (1998)
- [NPL 3] Derynck, R. & Miyazono, K. Cold Spring Harbor Press (2008)
- [NPL 4] Yu, L. et al. Kidney Int. 64, 844-856 (2003).
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- [NPL 6] Millan, F. A. et al. Development 111, 131-143 (1991).
- [NPL 7] Kulkarni, A. B. et al. Proc. Natl Acad. Sci. USA 90, 770-774 (1993).
- [NPL 8] Shull, M. M. et al. Nature 359, 693-699 (1992).
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- [NPL 14] Annes, J. et al. J. Cell Sci. 116, 217-224 (2003).
- [NPL 15] Schultz-Cherry, S. et al. J. Biol. Chem. 269, 26775-26782 (1994).
- [NPL 16] Munger, J. S. et al. Cell 96, 319-328 (1999).
- [NPL 17] Shi, M. et al. Nature 474, 343-349 (2011).
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- [NPL 19] Sanderson, N. et al. Proc. Natl Acad. Sci. USA 92, 2572-2576 (1995).

Summary of Invention

Technical Problem

- [0010] An object of the invention is to provide cross-species, humanized and optimized anti-latent TGF-beta 1 antibodies which inhibit a protease mediated activation of latent TGF-beta 1 without inhibiting integrin mediated activation of latent TGF-beta 1 or with partially inhibiting integrin mediated activation of latent TGF-beta 1, or which inhibit a protease mediated activation of latent TGF-beta 1 but have less impact on inhibiting integrin mediated activation of latent TGF-beta 1. The invention also provides anti-TGF-beta 1 antibodies which have pH-dependent binding affinity towards latent TGF-beta 1.

Solution to Problem

- [0011] The present inventors have conducted diligent studies under the situations as described above and consequently created cross-species, humanized and optimized anti-latent TGF-beta 1 antibodies which inhibit a protease mediated activation of TGF-beta 1 without inhibiting integrin mediated activation of latent TGF-beta 1 or with partially inhibiting integrin mediated activation of latent TGF-beta 1, or which inhibit a protease mediated activation of latent TGF-beta 1 but have less impact on inhibiting integrin mediated activation of latent TGF-beta 1. Further, the present inventors created anti-TGF-beta 1 antibodies which have pH-dependent binding affinity towards

latent TGF-beta 1.

[0012] More specifically, the present invention provides:

A1. An anti-latent TGF-beta 1 antibody comprising:

(a) HVR-H1, HVR-H2 and HVR-H3 comprising the amino acid sequences of SEQ ID NO: 29, 30 and 36, respectively;

(b) HVR-H1, HVR-H2 and HVR-H3 comprising the amino acid sequences of SEQ ID NO: 28, 32 and 37, respectively;

(c) HVR-H1, HVR-H2 and HVR-H3 comprising the amino acid sequences of SEQ ID NO: 27, 30 and 39, respectively; or

(d) HVR-H1, HVR-H2 and HVR-H3 comprising the amino acid sequences of SEQ ID NO: 27, 33 and 40, respectively.

A1-1. An anti-latent TGF-beta 1 antibody comprising:

(a) HVR-H1, HVR-H2 and HVR-H3 consisting of the amino acid sequences of SEQ ID NO: 29, 30 and 36, respectively;

(b) HVR-H1, HVR-H2 and HVR-H3 consisting of the amino acid sequences of SEQ ID NO: 28, 32 and 37, respectively;

(c) HVR-H1, HVR-H2 and HVR-H3 consisting of the amino acid sequences of SEQ ID NO: 27, 30 and 39, respectively; or

(d) HVR-H1, HVR-H2 and HVR-H3 consisting of the amino acid sequences of SEQ ID NO: 27, 33 and 40, respectively.

A2. The anti-latent TGF-beta 1 antibody of A1 which further comprises:

(a) HVR-L1, HVR-L2 and HVR-L3 comprising the amino acid sequences of SEQ ID NO: 49, 52 and 56, respectively;

(b) HVR-L1, HVR-L2 and HVR-L3 comprising the amino acid sequences of SEQ ID NO: 50, 52 and 56, respectively;

(c) HVR-L1, HVR-L2 and HVR-L3 comprising the amino acid sequences of SEQ ID NO: 48, 53 and 56, respectively; and

(d) HVR-L1, HVR-L2 and HVR-L3 comprising the amino acid sequences of SEQ ID NO: 51, 52 and 56, respectively.

A2-1. The anti-latent TGF-beta 1 antibody of A1-1 which further comprises:

(a) HVR-L1, HVR-L2 and HVR-L3 consisting of the amino acid sequences of SEQ ID NO: 49, 52 and 56, respectively;

(b) HVR-L1, HVR-L2 and HVR-L3 consisting of the amino acid sequences of SEQ ID NO: 50, 52 and 56, respectively;

(c) HVR-L1, HVR-L2 and HVR-L3 consisting of the amino acid sequences of SEQ ID NO: 48, 53 and 56, respectively; and

(d) HVR-L1, HVR-L2 and HVR-L3 consisting of the amino acid sequences of SEQ ID NO: 51, 52 and 56, respectively.

A3. An anti-latent TGF-beta 1 antibody comprising:

- (a) HVR-H1, HVR-H2 and HVR-H3 comprising the amino acid sequences of SEQ ID NO: 29, 30 and 36, respectively, and HVR-L1, HVR-L2 and HVR-L3 comprising the amino acid sequences of SEQ ID NO: 49, 52 and 56, respectively;
- (b) HVR-H1, HVR-H2 and HVR-H3 comprising the amino acid sequences of SEQ ID NO: 28, 32 and 37, respectively, and HVR-L1, HVR-L2 and HVR-L3 comprising the amino acid sequences of SEQ ID NO: 50, 52 and 56, respectively;
- (c) HVR-H1, HVR-H2 and HVR-H3 comprising the amino acid sequences of SEQ ID NO: 27, 30 and 39, respectively, and HVR-L1, HVR-L2 and HVR-L3 comprising the amino acid sequences of SEQ ID NO: 48, 53 and 56, respectively; or
- (d) HVR-H1, HVR-H2 and HVR-H3 comprising the amino acid sequences of SEQ ID NO: 27, 33 and 40, respectively, and HVR-L1, HVR-L2 and HVR-L3 comprising the amino acid sequences of SEQ ID NO: 51, 52 and 56, respectively.

A3-1. An anti-latent TGF-beta 1 antibody comprising:

- (a) HVR-H1, HVR-H2 and HVR-H3 consisting of the amino acid sequences of SEQ ID NO: 29, 30 and 36, respectively, and HVR-L1, HVR-L2 and HVR-L3 consisting of the amino acid sequences of SEQ ID NO: 49, 52 and 56, respectively;
- (b) HVR-H1, HVR-H2 and HVR-H3 consisting of the amino acid sequences of SEQ ID NO: 28, 32 and 37, respectively, and HVR-L1, HVR-L2 and HVR-L3 consisting of the amino acid sequences of SEQ ID NO: 50, 52 and 56, respectively;
- (c) HVR-H1, HVR-H2 and HVR-H3 consisting of the amino acid sequences of SEQ ID NO: 27, 30 and 39, respectively, and HVR-L1, HVR-L2 and HVR-L3 consisting of the amino acid sequences of SEQ ID NO: 48, 53 and 56, respectively; or
- (d) HVR-H1, HVR-H2 and HVR-H3 consisting of the amino acid sequences of SEQ ID NO: 27, 33 and 40, respectively, and HVR-L1, HVR-L2 and HVR-L3 consisting of the amino acid sequences of SEQ ID NO: 51, 52 and 56, respectively.

A4. The anti-latent TGF-beta 1 antibody of any one of A1 to A3-1, comprising:

- (a) (i) a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 4, (ii) a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 16, or (iii) a VH sequence as in (i) and a VL sequence as in (ii);
- (b) (i) a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 9, (ii) a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 21, or (iii) a VH sequence as in (i) and a VL sequence as in (ii);
- (c) (i) a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 11, (ii) a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 17, or (iii) a VH sequence as in (i) and a

VL sequence as in (ii); or

(d) (i) a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 10, (ii) a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 20, or (iii) a VH sequence as in (i) and a VL sequence as in (ii).

A4-1. The anti-latent TGF-beta 1 antibody of any one of A1 to A4, which retains a binding activity of the antibody to human latent TGF-beta 1.

A5. The anti-latent TGF-beta 1 antibody of A4 or A4-1, comprising a VH sequence of SEQ ID NO: 4, 9, 11 or 10.

A6. The anti-latent TGF-beta 1 antibody of any one of A4 to A5, comprising a VL sequence of SEQ ID NO: 16, 21, 17 or 20.

A7. The anti-latent TGF-beta 1 antibody of any one of A4 to A6, comprising:

(a) a VH sequence of SEQ ID NO: 4 and a VL sequence of SEQ ID NO: 16;

(b) a VH sequence of SEQ ID NO: 9 and a VL sequence of SEQ ID NO: 21;

(c) a VH sequence of SEQ ID NO: 11 and a VL sequence of SEQ ID NO: 17; or

(d) a VH sequence of SEQ ID NO: 10 and a VL sequence of SEQ ID NO: 20.

A8. An anti-latent TGF-beta 1 antibody comprising:

(a) a VH sequence of SEQ ID NO: 4 and a VL sequence of SEQ ID NO: 16;

(b) a VH sequence of SEQ ID NO: 9 and a VL sequence of SEQ ID NO: 21;

(c) a VH sequence of SEQ ID NO: 11 and a VL sequence of SEQ ID NO: 17; or

(d) a VH sequence of SEQ ID NO: 10 and a VL sequence of SEQ ID NO: 20.

A9. The anti-latent TGF-beta 1 antibody of any one of A1 to A8, which is a human, humanized, or chimeric antibody.

A9-1. The anti-latent TGF-beta 1 antibody of A9, which is a human antibody.

A9-2. The anti-latent TGF-beta 1 antibody of A9, which is a humanized antibody.

A9-3. The anti-latent TGF-beta 1 antibody of A9, which is a chimeric antibody.

A10. The anti-latent TGF-beta 1 antibody of any one of A1 to A9-3, which is a IgG antibody.

A10-1. The anti-latent TGF-beta 1 antibody of any one of A1 to A10, which is a IgG1 antibody.

A11. The anti-latent TGF-beta 1 antibody of any one of A1 to A9-3, which is a bispecific antibody.

A12. The anti-latent TGF-beta 1 antibody of any one of A1 to A11, wherein the anti-latent TGF-beta 1 antibody comprises a modified IgG1 Fc region having reduced effector function compared with a wild type IgG1 Fc region.

A13. The anti-latent TGF-beta 1 antibody of A12, wherein the modified IgG1 Fc region comprises amino acid substitutions at positions at 235 and/or 236, according to the EU index.

A14. The anti-latent TGF-beta 1 antibody of A12 or A13, wherein, in the modified IgG1 Fc region, amino acid residues at positions 235 and 236, according to the EU index, are altered to be arginine (R).

A14-1. The anti-latent TGF-beta 1 antibody of any one of A12 to A14, wherein the modified IgG1 Fc region comprises amino acid substitutions of L235R and G236R, according to the EU index.

A15. The anti-latent TGF-beta 1 antibody of any one of A12 to A14-1, wherein the modified IgG1 Fc region further has enhanced binding activity to FcRn compared with a wild type IgG1 Fc region.

A16. The anti-latent TGF-beta 1 antibody of A15, wherein the modified IgG1 Fc region comprises one or more amino acid substitutions at positions selected from the group consisting of: 428, 434, 438 and 440, according to the EU index.

A17. The anti-latent TGF-beta 1 antibody of A15 or A16, wherein, in the modified IgG1 Fc region, amino acid residues at positions at 428, 434, 438, and 440 are altered to be leucine (L), alanine (A), arginine (R), and glutamic acid (E), respectively.

A17-1. The anti-latent TGF-beta 1 antibody of any one of A15 to A17, wherein the modified IgG1 Fc region comprises amino acid substitutions of M428L, N434A, Q438R and S440E.

A18. The anti-latent TGF-beta 1 antibody of any one of A1 to A11, wherein the anti-latent TGF-beta 1 antibody comprises a modified IgG1 Fc region, wherein, in the modified IgG1 Fc region, amino acid residues at positions 214, 235, and 236 are each altered to be arginine (R).

A18-1. The anti-latent TGF-beta 1 antibody of any one of A1 to A11, wherein the anti-latent TGF-beta 1 antibody comprises a modified IgG1 Fc region, wherein the modified IgG1 Fc region comprises amino acid substitutions of K214R, L235R and G236R.

A19. The anti-latent TGF-beta 1 antibody of any one of A1 to A11, wherein the anti-latent TGF-beta 1 antibody comprises a modified IgG1 Fc region, wherein, in the modified IgG1 Fc region, amino acid residues at positions 214, 235 236, 428, 434, 438, and 440 are altered to be arginine (R), arginine (R), arginine (R), leucine (L), alanine (A), arginine (R), and glutamic acid (E), respectively.

A19-1. The anti-latent TGF-beta 1 antibody of any one of A1 to A11, wherein the anti-latent TGF-beta 1 antibody comprises a modified IgG1 Fc region, wherein the modified IgG1 Fc region comprises amino acid substitutions of K214R, L235R, G236R, M428L, N434A, Q438R and S440E.

A20-1. The anti-latent TGF-beta 1 antibody of any one of A1 to A19-1, wherein the anti-latent TGF beta 1 binds to latent TGF-beta 1 in a pH-dependent manner.

A20-2. The anti-latent TGF-beta 1 antibody of A20-1, wherein the anti-latent TGF-

beta 1 antibody binds to latent TGF-beta 1 with a higher antigen-binding affinity in a neutral pH condition than in an acidic pH condition.

A20-3. The anti-latent TGF-beta 1 antibody of A20-2, wherein the anti-latent TGF-beta 1 antibody binds to latent TGF-beta 1 with a higher antigen-binding affinity at pH 7.4 than at pH 5.8.

A20-4. The anti-latent TGF-beta 1 antibody of A20-3, wherein the dissociation constant ratio between pH 5.8 and pH 7.4 ($[K_D(\text{pH } 5.8)/K_D(\text{pH } 7.4)]$) for latent TGF-beta 1 of the anti-latent TGF-beta 1 antibody is 5 or more.

A20-5. The anti-latent TGF-beta 1 antibody of A20-4, wherein the dissociation constant ratio is 50 or more.

A20-6. The anti-latent TGF-beta 1 antibody of A20-4, wherein the dissociation constant ratio is 100 or more.

A20-7. The anti-latent TGF-beta 1 antibody of A20-4, wherein the dissociation constant ratio is 120 or more.

A20-8. The anti-latent TGF-beta 1 antibody of A20-4, wherein the dissociation constant ratio is 130 or more.

A20-9. The anti-latent TGF-beta 1 antibody of A20-4, wherein the dissociation constant ratio is 160 or more.

A20-10. The anti-latent TGF-beta 1 antibody of any one of A20-4 to A20-9, wherein the dissociation constant ratio is measured using a surface plasmon resonance method.

A21. An anti-latent TGF-beta 1 antibody comprising:

(a) a heavy chain sequence of SEQ ID NO: 82 and a light chain sequence of SEQ ID NO: 83;

(b) a heavy chain sequence of SEQ ID NO: 84 and a light chain sequence of SEQ ID NO: 85;

(c) a heavy chain sequence of SEQ ID NO: 86 and a light chain sequence of SEQ ID NO: 87;

(d) a heavy chain sequence of SEQ ID NO: 88 and a light chain sequence of SEQ ID NO: 89;

(e) a heavy chain sequence of SEQ ID NO: 90 and a light chain sequence of SEQ ID NO: 83;

(f) a heavy chain sequence of SEQ ID NO: 91 and a light chain sequence of SEQ ID NO: 85;

(g) a heavy chain sequence of SEQ ID NO: 92 and a light chain sequence of SEQ ID NO: 87; or

(h) a heavy chain sequence of SEQ ID NO: 93 and a light chain sequence of SEQ ID NO: 89.

A22. The anti-latent TGF-beta 1 antibody of any one of A1 to A21, wherein the latent TGF beta-1 is a human latent TGF beta-1, a mouse latent TGF beta-1, or a cynomolgus

monkey latent TGF beta-1.

A23. The anti-latent TGF-beta 1 antibody of any one of A1 to A22, wherein the anti-latent TGF-beta 1 antibody binds to a human latent TGF-beta 1, a mouse latent TGF-beta 1, and a cynomolgus monkey latent TGF-beta 1.

A24. The anti-latent TGF-beta 1 antibody of any one of A1 to A23, wherein the anti-latent TGF-beta 1 antibody binds to a latency-associated peptide (LAP) region of a latent TGF-beta 1.

A24-1. The anti-latent TGF-beta 1 antibody of any one of A1 to A24, wherein the anti-latent TGF-beta 1 antibody:

- i) inhibits activation of a latent TGF-beta 1;
- ii) inhibits the release of mature TGF-beta 1 from latent TGF-beta 1;
- iii) inhibits protease mediated and/or integrin mediated release of mature TGF-beta 1 from latent TGF-beta 1;
- iv) inhibits protease mediated release of mature TGF-beta 1 from latent TGF-beta 1 without inhibiting protease mediated cleavage of LAP portion of latent TGF-beta 1;
- v) inhibits protease mediated release of mature TGF-beta 1 from latent TGF-beta 1 and allows a protease to cleave the LAP region while the anti-latent TGF-beta 1 antibody binds to the LAP region of the latent TGF-beta 1;
- vi) does not block access of a protease to latent TGF-beta 1;
- vii) does not bind to protease cleavage sites of LAP portion of a latent TGF-beta 1;
- viii) inhibits a protease mediated activation of latent TGF-beta 1 without inhibiting integrin mediated activation of latent TGF-beta 1 or with partially inhibiting integrin mediated activation of latent TGF-beta 1; and/or
- ix) inhibits a protease mediated activation of latent TGF-beta 1 but has less impact on inhibiting integrin mediated activation of latent TGF-beta 1.

A24-1a. The anti-latent TGF-beta 1 antibody of A24-1, wherein the protease is selected from the group consisting of plasmin (PLN), plasma kallikrein (PLK), matrix metalloproteinase 2 (MMP2), and matrix metalloproteinase 9 (MMP9).

A24-2. An antibody fragment of the anti-latent TGF-beta 1 antibody of any one of A1 to A24-1a.

A25. An immunoconjugate comprising the anti-latent TGF beta-1 antibody of any one of A1 to A24-1a or the antibody fragment of A24-2 and a cytotoxic agent.

A26. An isolated nucleic acid encoding the anti-latent TGF beta-1 antibody of any one of A1 to A24-1a or the antibody fragment of A24-2.

A27. A vector comprising the nucleic acid of A26.

A28. A host cell comprising the nucleic acid of A26 or the vector of A27.

A29. A method of producing an anti-latent TGF beta-1 antibody comprising culturing the host cell of A28 so that the antibody is produced.

A29-2. A method of producing an anti-latent TGF beta-1 antibody comprising:

- (a) contacting a biological sample comprising latent TGF-beta 1 and a protease with a test antibody;
- (b) detecting (i) whether a test antibody inhibits the cleavage of the LAP region of latent TGF-beta 1 and (ii) whether a test antibody inhibits activation of latent TGF-beta 1;
- (c) selecting the test antibody that inhibits activation of latent TGF-beta 1 without inhibiting protease mediated cleavage of the LAP portion of latent TGF-beta 1.
- (d) obtaining amino acid sequence information of the anti-latent TGF-beta 1 antibody selected in step (c); and
- (e) introducing a gene encoding the anti-latent TGF-beta 1 antibody into a host cell; and
- (f) culturing the host cell so that the antibody is produced.

A30. The method of A29 or A29-2, further comprising recovering the antibody from the host cell.

B1. The anti-latent TGF beta-1 antibody of any one of A1 to A24-1a or the antibody fragment of A24-2 or the immunoconjugate of A25, for use as a medicament.

B1-1. The anti-latent TGF beta-1 antibody of B1, for use as a medicament for subcutaneous administration.

B2. The anti-latent TGF beta-1 antibody of any one of A1 to A24-1a or the antibody fragment of A24-2 or the immunoconjugate of A25, for use in treating fibrosis.

B2a. The anti-latent TGF beta-1 antibody of any one of A1 to A24-1a or the antibody fragment of A24-2 or the immunoconjugate of A25, for use in treating cancer.

B2-1. The anti-latent TGF beta-1 antibody of B2 or B2a, for use in subcutaneously administering the antibody.

B2-2. The anti-latent TGF beta-1 antibody of B2 or B2-1, wherein the fibrosis is selected from the group consisting of renal fibrosis, liver fibrosis, and lung fibrosis.

B2-3. The anti-latent TGF beta-1 antibody of B2-2, wherein the fibrosis is renal fibrosis.

B2-4. The anti-latent TGF beta-1 antibody of B2-2, wherein the fibrosis is liver fibrosis.

B2-5. The anti-latent TGF beta-1 antibody of B2-2, wherein the fibrosis is lung fibrosis.

B3. Use of the anti-latent TGF beta-1 antibody of any one of A1 to A24-1a or the antibody fragment of A24-2 or the immunoconjugate of A25, in the manufacture of a medicament for treatment of fibrosis.

B3a. Use of the anti-latent TGF beta-1 antibody of any one of A1 to A24-1a or the antibody fragment of A24-2 or the immunoconjugate of A25, in the manufacture of a

medicament for treatment of cancer.

B3-1. The use of B3 or B3a, the medicament is for subcutaneous administration.

B3-2. The use of B3 or B3-1, wherein the fibrosis is selected from the group consisting of renal fibrosis, liver fibrosis, and lung fibrosis.

B3-3. The anti-latent TGF beta-1 antibody of B3-2, wherein the fibrosis is renal fibrosis.

B3-4. The anti-latent TGF beta-1 antibody of B3-2, wherein the fibrosis is liver fibrosis.

B3-5. The anti-latent TGF beta-1 antibody of B3-2, wherein the fibrosis is lung fibrosis.

C1. A pharmaceutical formulation comprising the anti-latent TGF beta-1 antibody of any one of A1 to A24-1a or the antibody fragment of A24-2 or the immunoconjugate of A25, and a pharmaceutically acceptable carrier.

C2. The pharmaceutical formulation of C1, wherein the viscosity of the pharmaceutical formulation is less than 70 mPa-s.

C3. The pharmaceutical formulation of C1 or C2, wherein the concentration of the antibody in the pharmaceutical formulation is 50 to 350 mg/ml.

C4. The pharmaceutical formulation of any one of C1 to C3, which is administered subcutaneously.

C5. The pharmaceutical formulation of any one of C1 to C4, for use in treatment of fibrosis.

C5a. The pharmaceutical formulation of any one of C1 to C4, for use in treatment of cancer.

C6. The pharmaceutical formulation of C5, wherein the fibrosis is selected from the group consisting of renal fibrosis, liver fibrosis, and lung fibrosis.

C7. The anti-latent TGF beta-1 antibody of C6, wherein the fibrosis is renal fibrosis.

C8. The anti-latent TGF beta-1 antibody of C6, wherein the fibrosis is liver fibrosis.

C9. The anti-latent TGF beta-1 antibody of C6, wherein the fibrosis is lung fibrosis.

E1. A method of treating an individual having fibrosis comprising administering to the individual an effective amount of the anti-latent TGF beta-1 antibody of any one of A1 to A24-1a or the antibody fragment of A24-2 or the immunoconjugate of A25.

E1a. A method of treating an individual having cancer comprising administering to the individual an effective amount of the anti-latent TGF beta-1 antibody of any one of A1 to A24-1a or the antibody fragment of A24-2 or the immunoconjugate of A25.

E2. The method of E1 or E1a, which comprises subcutaneously administering the effective amount of the anti-latent TGF beta-1 antibody to the individual.

E3. The method of E1 or E2, wherein the fibrosis is selected from the group consisting of renal fibrosis, liver fibrosis, and lung fibrosis.

E4. The anti-latent TGF beta-1 antibody of E3, wherein the fibrosis is renal fibrosis.

E5. The anti-latent TGF beta-1 antibody of E3, wherein the fibrosis is liver fibrosis.

E6. The anti-latent TGF beta-1 antibody of E3, wherein the fibrosis is lung fibrosis.

Brief Description of Drawings

- [0013] [Fig.1]FIG. 1 depicts an evaluation of human latent TGF-beta 1 neutralization activity towards PLN.
- [Fig.2]FIG. 2 depicts an evaluation of antibody kinetics towards human latent TGF-beta 1.
- [Fig.3]FIG. 3 depicts an evaluation of KD at pH7.4 and koff at pH 5.8 towards human latent TGF-beta 1 for antibody variants generated in the antibody optimization process.
- [Fig.4]FIG. 4 depicts an evaluation of IL-2 secretion from human CD8 CD25^{low} PBMCs upon antibody incubation for immunogenicity risk assessment.
- [Fig.5]FIG. 5 shows ka, kd and KD values of anti-latent TGF-beta 1 antibodies binding towards recombinant human, cynomolgus ("cyno"), and mouse latent TGF-beta 1 at pH7.4.
- [Fig.6]FIG. 6 shows ka, kd and KD values of anti-latent TGF-beta 1 antibodies binding towards recombinant human, cynomolgus, and mouse latent TGF-beta 1 at pH 5.8.
- [Fig.7]FIG. 7 shows koff and KD ratios (pH 5.8/pH 7.4) of anti-latent TGF-beta 1 antibodies based on FIGS. 5 and 6.
- [Fig.8]FIG. 8 depicts the results of binding of anti-latent TGF-beta 1 antibodies to cell surface latent TGF-beta 1 on FreeStyle™ 293-F cells and BaF3 cells.
- [Fig.9]FIG. 9 depicts the results of the activities of anti-latent TGF-beta 1 antibodies against spontaneous latent TGF-beta 1 activation.
- [Fig.10]FIG. 10 depicts the results of the activities of anti-latent TGF-beta 1 antibodies against plasmin (PLN)-mediated latent TGF-beta 1 activation.
- [Fig.11]FIG. 11 depicts the results of the activities of anti-latent TGF-beta 1 antibodies against plasma kallikrein-, matrix metalloproteinase (MMP) 2, and MMP9-mediated human latent TGF-beta 1 activation.
- [Fig.12]FIG. 12 depicts the results of the activities of anti-latent TGF-beta 1 antibodies against integrin-mediated mouse TGF-beta 1 activation in mouse PBMC.
- [Fig.13]FIG. 13 depicts the results of the activities of anti-latent TGF-beta 1 antibodies against integrin avb6-mediated human TGF-beta 1 activation in Detroit562.
- [Fig.14-1]FIG. 14-1 depicts a pharmacokinetics (PK) study of anti-human latent TGF-beta 1 antibodies in cynomolgus monkey.
- [Fig.14-2]FIG. 14-2 is a continuation of FIG. 14-1.
- [Fig.14-3]FIG. 14-3 is a continuation of FIG. 14-2.
- [Fig.14-4]FIG. 14-4 is a continuation of FIG. 14-3.

[Fig.14-5]FIG. 14-5 is a continuation of FIG. 14-4.

[Fig.14-6]FIG. 14-6 is a continuation of FIG. 14-5.

[Fig.14-7]FIG. 14-7 depicts a PK study of anti-human latent TGF-beta 1 antibodies in mouse.

[Fig.14-8]FIG. 14-8 is a continuation of FIG. 14-7.

[Fig.14-9]FIG. 14-9 is a continuation of FIG. 14-8.

[Fig.14-10]FIG. 14-10 is a continuation of FIG. 14-9.

[Fig.15]FIG. 15 depicts the results of hydroxyproline content in kidney. Efficacy of antibodies was evaluated in Unilateral Ureteral Obstruction (UUO) induced mouse renal fibrosis model. Sham operated group represents as non-disease-induced control.

[Fig.16]FIG. 16 depicts the results of glomerular sclerosis in kidney. Efficacy of antibodies was evaluated in anti-GBM serum induced mouse nephritis model (aGBM model). Normal mice group represents as non-disease-induced control.

[Fig.17]FIG. 17 depicts the results of plasma creatinine. Efficacy of antibodies was evaluated in COL4A3 KO mice (Alport mice model). Normal mice group represents as non-disease-induced control.

[Fig.18]FIG. 18 shows the viscosity of anti-latent TGF-beta 1 antibodies at 150 mg/mL antibody concentration in a formulation buffer at pH6.0.

[Fig.19]FIG. 19 shows SEC analysis of anti-latent TGF-beta 1 antibodies treated at 40 degrees Celsius for 2 and 4 weeks.

[Fig.20]FIG. 20 depicts the results of collagen type 1 alpha 1 (Col1a1) mRNA in liver. Efficacy of antibodies was evaluated in choline-deficient, L-amino acid-defined, high-fat (CDAHFD) induced mouse NASH/liver fibrosis model. Normal diet group represents as non-disease control.

[Fig.21]FIG. 21 depicts the results of hydroxyproline content, collagen type 1 alpha 1 (Col1a1) mRNA, and Serpine 1 mRNA in lung. Efficacy of antibodies was evaluated in bleomycin induced lung fibrosis model. Saline group represents as non-disease control.

[Fig.22]FIG. 22 depicts wild-type Fc region sequences of IgG1, IgG2, IgG3, and IgG4.

Description of Embodiments

[0014] The techniques and procedures described or referenced herein are generally well understood and commonly employed using conventional methodology by those skilled in the art, such as, for example, the widely utilized methodologies described in Sambrook et al., *Molecular Cloning: A Laboratory Manual* 3d edition (2001) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.; *Current Protocols in Molecular Biology* (F.M. Ausubel, et al. eds., (2003)); the series *Methods in Enzymology* (Academic Press, Inc.); *PCR 2: A Practical Approach* (M.J. MacPherson, B.D. Hames and G.R.

Taylor eds. (1995)), Harlow and Lane, eds. (1988) Antibodies, A Laboratory Manual, and Animal Cell Culture (R.I. Freshney, ed. (1987)); Oligonucleotide Synthesis (M.J. Gait, ed., 1984); Methods in Molecular Biology, Humana Press; Cell Biology: A Laboratory Notebook (J.E. Cellis, ed., 1998) Academic Press; Animal Cell Culture (R.I. Freshney), ed., 1987); Introduction to Cell and Tissue Culture (J. P. Mather and P.E. Roberts, 1998) Plenum Press; Cell and Tissue Culture: Laboratory Procedures (A. Doyle, J.B. Griffiths, and D.G. Newell, eds., 1993-8) J. Wiley and Sons; Handbook of Experimental Immunology (D.M. Weir and C.C. Blackwell, eds.); Gene Transfer Vectors for Mammalian Cells (J.M. Miller and M.P. Calos, eds., 1987); PCR: The Polymerase Chain Reaction, (Mullis et al., eds., 1994); Current Protocols in Immunology (J.E. Coligan et al., eds., 1991); Short Protocols in Molecular Biology (Wiley and Sons, 1999); Immunobiology (C.A. Janeway and P. Travers, 1997); Antibodies (P. Finch, 1997); Antibodies: A Practical Approach (D. Catty., ed., IRL Press, 1988-1989); Monoclonal Antibodies: A Practical Approach (P. Shepherd and C. Dean, eds., Oxford University Press, 2000); Using Antibodies: A Laboratory Manual (E. Harlow and D. Lane (Cold Spring Harbor Laboratory Press, 1999); The Antibodies (M. Zanetti and J. D. Capra, eds., Harwood Academic Publishers, 1995); and Cancer: Principles and Practice of Oncology (V.T. DeVita et al., eds., J.B. Lippincott Company, 1993).

[0015] **I. DEFINITIONS**

An "acceptor human framework" for the purposes herein is a framework comprising the amino acid sequence of a light chain variable domain (VL) framework or a heavy chain variable domain (VH) framework derived from a human immunoglobulin framework or a human consensus framework, as defined below. An acceptor human framework "derived from" a human immunoglobulin framework or a human consensus framework may comprise the same amino acid sequence thereof, or it may contain amino acid sequence changes. In some embodiments, the number of amino acid changes are 10 or less, 9 or less, 8 or less, 7 or less, 6 or less, 5 or less, 4 or less, 3 or less, or 2 or less. In some embodiments, the VL acceptor human framework is identical in sequence to the VL human immunoglobulin framework sequence or human consensus framework sequence.

[0016] The term "binding activity" refers to the strength of the sum total of noncovalent interactions between one or more binding sites of a molecule (e.g., an antibody) and its binding partner (e.g., an antigen). Herein, "binding activity" is not strictly limited to a 1:1 interaction between members of a binding pair (e.g., antibody and antigen). For example, when the members of a binding pair reflect a monovalent 1:1 interaction, the binding activity is particularly called the intrinsic binding affinity (affinity). When a member of a binding pair is capable of both monovalent binding and multivalent

binding, the binding activity is the sum of each binding strength. The binding activity of a molecule X for its partner Y can generally be represented by the dissociation constant (KD) or "binding amount of analyte per unit amount of ligand" (hereinbelow, may be referred to as "binding amount"). Those skilled in the art would understand that, generally, lower value of dissociation constant (KD) means higher binding activity, and higher value of "binding amount of analyte per unit amount of ligand" or "binding amount" means higher binding activity. Binding activity can be measured by common methods known in the art, including those described herein. Specific illustrative and exemplary embodiments for measuring binding activity are described in the following.

- [0017] A "binding activity-matured", "affinity-matured" antigen-binding molecule or antibody, "binding activity-increased (enhanced) ", or "affinity-increased (enhanced) " antigen-binding molecule or antibody refers to an antibody with one or more alterations (e.g., substitutions) in one or more hypervariable regions (HVRs), compared to a parent antigen-binding molecule or a parent antibody which does not carry such alterations, such alterations resulting in an improvement in the binding activity of the antigen-binding molecule or antibody for antigen.
- [0018] The terms "anti-latent TGF-beta 1 antibody" and "an antibody that can bind to latent TGF-beta 1" refer to an antibody that is capable of binding latent TGF-beta 1 with sufficient binding activity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting latent TGF-beta 1. In one embodiment, "an antibody that can bind to latent TGF-beta 1" is an antibody that specifically binds to latent TGF-beta 1. In one embodiment, the extent of binding activity of an anti-latent TGF-beta 1 antibody to an unrelated, non-latent TGF-beta 1 protein is less than about 10% of the binding activity of the antibody to latent TGF-beta 1 as measured, e.g., by a radioimmunoassay (RIA). In certain embodiments, an antibody that can bind to TGF-beta 1 has a dissociation constant (KD) of 1 micromolar or less, 100 nM or less, 10 nM or less, 1 nM or less, 0.1 nM or less, 0.01 nM or less, or 0.001 nM or less (e.g. 10^{-8} M or less, e.g. from 10^{-8} M to 10^{-13} M, e.g., from 10^{-9} M to 10^{-13} M). In certain embodiments, an anti-latent TGF-beta 1 antibody binds to an epitope of latent TGF-beta 1 that is conserved among latent TGF-beta 1 from different species.
- [0019] The term "antibody" herein is used in the broadest sense and encompasses various antibody structures, including but not limited to monoclonal antibodies, polyclonal antibodies, and multispecific antibodies (e.g., bispecific antibodies) so long as they exhibit the desired antigen-binding affinity/activity. The "antibody" may be an antigen binding molecule which comprises variable heavy chain and/or variable light chain structure(s) of immunoglobulin.
- [0020] An "antibody fragment" refers to a molecule other than an intact antibody that

comprises a portion of an intact antibody that binds the antigen to which the intact antibody binds. Examples of antibody fragments include but are not limited to Fv, Fab, Fab', Fab'-SH, F(ab')₂; diabodies; linear antibodies; single-chain antibody molecules (e.g. scFv); and multispecific antibodies formed from antibody fragments. The "antibody fragment" may be an antigen binding molecule which comprises variable heavy chain and/or variable light chain structure(s) of immunoglobulin.

- [0021] An "antibody that binds to the same epitope" as a reference antibody refers to an antibody that blocks binding of the reference antibody to its antigen in a competition assay by 50% or more, and conversely, the reference antibody blocks binding of the antibody to its antigen in a competition assay by 50% or more. An exemplary competition assay is provided herein.
- [0022] The terms "cancer" and "cancerous" refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth/proliferation. Examples of cancer include, but are not limited to, carcinoma, lymphoma (e.g., Hodgkin's and non-Hodgkin's lymphoma), blastoma, sarcoma, and leukemia. More particular examples of such cancers include squamous cell cancer, small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastrointestinal cancer, pancreatic cancer, glioma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney cancer, liver cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, leukemia and other lymphoproliferative disorders, and various types of head and neck cancer. In one example, cancer is resistant to immune-checkpoint inhibitors and/or shows limited response to immune-checkpoint inhibitors.
- [0023] The term "chimeric" antibody refers to an antibody in which a portion of the heavy and/or light chain is derived from a particular source or species, while the remainder of the heavy and/or light chain is derived from a different source or species.
- [0024] The "class" of an antibody refers to the type of constant domain or constant region possessed by its heavy chain. There are five major classes of antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called alpha, delta, epsilon, gamma, and mu, respectively.
- [0025] The term "cytotoxic agent" as used herein refers to a substance that inhibits or prevents a cellular function and/or causes cell death or destruction. Cytotoxic agents include, but are not limited to, radioactive isotopes (e.g., ²¹¹At, ¹³¹I, ¹²⁵I, ⁹⁰Y, ¹⁸⁶Re, ¹⁸⁸Re, ¹⁵³Sm, ²¹²Bi, ³²P, ²¹²Pb and radioactive isotopes of Lu); chemotherapeutic agents or

drugs (e.g., methotrexate, adriamycin, vinca alkaloids (vincristine, vinblastine, etoposide), doxorubicin, melphalan, mitomycin C, chlorambucil, daunorubicin or other intercalating agents); growth inhibitory agents; enzymes and fragments thereof such as nucleolytic enzymes; antibiotics; toxins such as small molecule toxins or enzymatically active toxins of bacterial, fungal, plant or animal origin, including fragments and/or variants thereof; and the various antitumor or anticancer agents disclosed below.

- [0026] "Effector functions" refer to those biological activities attributable to the Fc region of an antibody, which vary with the antibody isotype. Examples of antibody effector functions include: C1q binding and complement dependent cytotoxicity (CDC); Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (e.g. B cell receptor); and B cell activation.
- [0027] An "effective amount" of an agent, e.g., a pharmaceutical formulation, refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic or prophylactic result.
- [0028] The term "Fc region" herein is used to define a C-terminal region of an immunoglobulin heavy chain that contains at least a portion of the constant region. The term includes native sequence Fc regions and variant Fc regions. In one embodiment, a human IgG heavy chain Fc region extends from Cys226, or from Pro230, to the carboxyl-terminus of the heavy chain. However, the C-terminal lysine (Lys447) or glycine-lysine (residues 446-447) of the Fc region may or may not be present. Unless otherwise specified herein, numbering of amino acid residues in the Fc region or constant region is according to the EU numbering system, also called the EU index, as described in Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD, 1991.
- [0029] The Fc region of a naturally-occurring IgG, also called "a wild type Fc region", means an Fc region comprising an amino acid sequence identical to that of the Fc region derived from an IgG found in nature. The Fc region of a naturally-occurring IgG, also called "a wild type IgG1 Fc region", is shown in FIG. 22 (SEQ ID NOs: 105-108). For example, this figure refers to an Fc region derived from naturally-occurring human IgG1 (also called "wild type human IgG1 Fc region"), Fc region derived from naturally-occurring human IgG2 (also called "wild type human IgG2 Fc region"), Fc region derived from naturally-occurring human IgG3 (also called "wild type human IgG3 Fc region"), and Fc region derived from naturally-occurring human IgG4 (also called "wild type human IgG4 Fc region"). The Fc regions of naturally-occurring IgGs may also include mutants spontaneously produced from them.
- [0030] "Framework" or "FR" refers to variable domain residues other than hypervariable region (HVR) residues. The FR of a variable domain generally consists of four FR

domains: FR1, FR2, FR3, and FR4. Accordingly, the HVR and FR sequences generally appear in the following sequence in VH (or VL):
FR1-H1(L1)-FR2-H2(L2)-FR3-H3(L3)-FR4.

- [0031] The terms "full length antibody," "intact antibody," and "whole antibody" are used herein interchangeably to refer to an antibody having a structure substantially similar to a native antibody structure or having heavy chains that contain an Fc region as defined herein.
- [0032] The terms "host cell," "host cell line," and "host cell culture" are used interchangeably and refer to cells into which exogenous nucleic acid has been introduced, including the progeny of such cells. Host cells include "transformants" and "transformed cells," which include the primary transformed cell and progeny derived therefrom without regard to the number of passages. Progeny may not be completely identical in nucleic acid content to a parent cell, but may contain mutations. Mutant progenies that have the same function or biological activity as screened or selected for in the originally transformed cell are included herein.
- [0033] A "human antibody" is one which possesses an amino acid sequence which corresponds to that of an antibody produced by a human or a human cell or derived from a non-human source that utilizes human antibody repertoires or other human antibody-encoding sequences. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues.
- [0034] A "human consensus framework" is a framework which represents the most commonly occurring amino acid residues in a selection of human immunoglobulin VL or VH framework sequences. Generally, the selection of human immunoglobulin VL or VH sequences is from a subgroup of variable domain sequences. Generally, the subgroup of sequences is a subgroup as in Kabat et al., *Sequences of Proteins of Immunological Interest*, Fifth Edition, NIH Publication 91-3242, Bethesda MD (1991), vols. 1-3. In one embodiment, for the VL, the subgroup is subgroup kappa I as in Kabat et al., *supra*. In one embodiment, for the VH, the subgroup is subgroup III as in Kabat et al., *supra*.
- [0035] A "humanized" antibody refers to a chimeric antibody comprising amino acid residues from non-human HVRs and amino acid residues from human FRs. In certain embodiments, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the HVRs (e.g., CDRs) correspond to those of a non-human antibody, and all or substantially all of the FRs correspond to those of a human antibody. A humanized antibody optionally may comprise at least a portion of an antibody constant region derived from a human antibody. A "humanized form" of an antibody, e.g., a non-human antibody, refers to an antibody that has undergone humanization.

- [0036] The term "hypervariable region" or "HVR" as used herein refers to each of the regions of an antibody variable domain which are hypervariable in sequence ("complementarity determining regions" or "CDRs") and/or form structurally defined loops ("hypervariable loops") and/or contain the antigen-contacting residues ("antigen contacts"). Generally, antibodies comprise six HVRs: three in the VH (H1, H2, H3), and three in the VL (L1, L2, L3). Exemplary HVRs herein include:
- (a) hypervariable loops occurring at amino acid residues 26-32 (L1), 50-52 (L2), 91-96 (L3), 26-32 (H1), 53-55 (H2), and 96-101 (H3) (Chothia and Lesk, *J. Mol. Biol.* 196:901-917 (1987));
 - (b) CDRs occurring at amino acid residues 24-34 (L1), 50-56 (L2), 89-97 (L3), 31-35b (H1), 50-65 (H2), and 95-102 (H3) (Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD (1991));
 - (c) antigen contacts occurring at amino acid residues 27c-36 (L1), 46-55 (L2), 89-96 (L3), 30-35b (H1), 47-58 (H2), and 93-101 (H3) (MacCallum et al. *J. Mol. Biol.* 262: 732-745 (1996)); and
 - (d) combinations of (a), (b), and/or (c), including HVR amino acid residues 46-56 (L2), 47-56 (L2), 48-56 (L2), 49-56 (L2), 26-35 (H1), 26-35b (H1), 49-65 (H2), 93-102 (H3), and 94-102 (H3).
- Unless otherwise indicated, HVR residues and other residues in the variable domain (e.g., FR residues) are numbered herein according to Kabat et al., *supra*.
- [0037] An "immunoconjugate" is an antibody conjugated to one or more heterologous molecule(s), including but not limited to a cytotoxic agent.
- [0038] An "individual" or "subject" is a mammal. Mammals include, but are not limited to, domesticated animals (e.g., cows, sheep, cats, dogs, and horses), primates (e.g., humans and non-human primates such as monkeys), rabbits, and rodents (e.g., mice and rats). In certain embodiments, the individual or subject is a human.
- [0039] An "isolated" antibody is one which has been separated from a component of its natural environment. In some embodiments, an antibody is purified to greater than 95% or 99% purity as determined by, for example, electrophoretic (e.g., SDS-PAGE, isoelectric focusing (IEF), capillary electrophoresis) or chromatographic (e.g., ion exchange or reverse phase HPLC). For review of methods for assessment of antibody purity, see, e.g., Flatman et al., *J. Chromatogr. B* 848:79-87 (2007).
- [0040] An "isolated" nucleic acid refers to a nucleic acid molecule that has been separated from a component of its natural environment. An isolated nucleic acid includes a nucleic acid molecule contained in cells that ordinarily contain the nucleic acid molecule, but the nucleic acid molecule is present extrachromosomally or at a chromosomal location that is different from its natural chromosomal location.

- [0041] "Isolated nucleic acid encoding an anti-latent TGF-beta 1 antibody" or "nucleic acid encoding an anti-latent TGF-beta 1 antibody" refers to one or more nucleic acid molecules encoding antibody heavy and light chains, including such nucleic acid molecule(s) in a single vector or separate vectors, and such nucleic acid molecule(s) present at one or more locations in a host cell.
- [0042] The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies composing the population are identical and/or bind the same epitope, except for possible variant antibodies, e.g., containing naturally occurring mutations or arising during production of a monoclonal antibody preparation, such variants generally being present in minor amounts. In contrast to polyclonal antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody of a monoclonal antibody preparation is directed against a single determinant on an antigen. Thus, the modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by a variety of techniques, including but not limited to the hybridoma method, recombinant DNA methods, phage-display methods, and methods utilizing transgenic animals containing all or part of the human immunoglobulin loci, such methods and other exemplary methods for making monoclonal antibodies being described herein.
- [0043] A "naked antibody" refers to an antibody that is not conjugated to a heterologous moiety (e.g., a cytotoxic moiety) or radiolabel. The naked antibody may be present in a pharmaceutical formulation.
- [0044] "Native antibodies" refer to naturally occurring immunoglobulin molecules with varying structures. For example, native IgG antibodies are heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light chains and two identical heavy chains that are disulfide-bonded. From N- to C-terminus, each heavy chain has a variable region (VH), also called a variable heavy domain or a heavy chain variable domain, followed by three constant domains (CH1, CH2, and CH3). Similarly, from N- to C-terminus, each light chain has a variable region (VL), also called a variable light domain or a light chain variable domain, followed by a constant light (CL) domain. The light chain of an antibody may be assigned to one of two types, called kappa (kappa) and lambda (lambda), based on the amino acid sequence of its constant domain.
- [0045] The term "package insert" is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the in-

dications, usage, dosage, administration, combination therapy, contraindications and/or warnings concerning the use of such therapeutic products.

[0046] "Percent (%) amino acid sequence identity" with respect to a reference polypeptide sequence is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the reference polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN, Megalign (DNASTAR) software, or GENETYX (registered trademark) (Genetyx Co., Ltd.). Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared.

[0047] The ALIGN-2 sequence comparison computer program was authored by Genentech, Inc., and the source code has been filed with user documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered under U.S. Copyright Registration No. TXU510087. The ALIGN-2 program is publicly available from Genentech, Inc., South San Francisco, California, or may be compiled from the source code. The ALIGN-2 program should be compiled for use on a UNIX operating system, including digital UNIX V4.0D. All sequence comparison parameters are set by the ALIGN-2 program and do not vary. In situations where ALIGN-2 is employed for amino acid sequence comparisons, the % amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows:

100 times the fraction X/Y

where X is the number of amino acid residues scored as identical matches by the sequence alignment program ALIGN-2 in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid sequence A is not equal to the length of amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B to A. Unless specifically stated otherwise, all % amino acid sequence identity values used herein are obtained as described in the immediately preceding paragraph using the ALIGN-2 computer program.

[0048] The term "pharmaceutical formulation" refers to a preparation which is in such form as to permit the biological activity of an active ingredient contained therein to be

effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered.

[0049] A "pharmaceutically acceptable carrier" refers to an ingredient in a pharmaceutical formulation, other than an active ingredient, which is nontoxic to a subject. A pharmaceutically acceptable carrier includes, but is not limited to, a buffer, excipient, stabilizer, or preservative.

[0050] The term "TGF-beta 1," as used herein, refers to any native TGF-beta 1 from any vertebrate source, including mammals such as primates (e.g. humans) and rodents (e.g., mice and rats), unless otherwise indicated. The term encompasses "full-length" unprocessed TGF-beta 1 as well as any form of TGF-beta 1 that results from processing in the cell. The term also encompasses naturally occurring variants of TGF-beta 1, e.g., splice variants or allelic variants. The amino acid sequence of an exemplary human TGF-beta 1 preproprotein is shown in SEQ ID NO: 94 (NCBI RefSeq: NP_000651.3) and the nucleic acid sequence encoding an exemplary human TGF-beta 1 is shown in SEQ ID NO: 95 (NCBI RefSeq: NM_000660.6). The amino acid sequence of an exemplary mouse TGF-beta 1 preproprotein is shown in SEQ ID NO: 96 (NCBI RefSeq: NP_035707.1) and the nucleic acid sequence encoding an exemplary mouse TGF-beta 1 is shown in SEQ ID NO: 97 (NCBI RefSeq: NM_011577.2). The amino acid sequence of an exemplary cynomolgus monkey TGF-beta 1 preproprotein is shown in SEQ ID NO: 98 (NCBI RefSeq: XP_005589396.1) and the nucleic acid sequence encoding an exemplary cynomolgus monkey TGF-beta 1 is shown in SEQ ID NO: 99 (NCBI RefSeq: XM_005589339.2). The term "TGF-beta 1" encompasses both latent TGF-beta 1 and mature TGF-beta 1.

[0051] The term "latent TGF-beta 1", as used herein, refers to any TGF-beta 1 which forms a latent TGF-beta 1 complex ("cell surface latent TGF-beta 1", LLC or SLC (see below)) and/or which is incapable of binding to its receptors. Transforming growth factor-beta 1 (TGF-beta 1) is a member of TGF-beta, which is a member of TGF-beta superfamily. Like other members of TGF-beta superfamily, TGF-beta is synthesized as a precursor protein, which forms a homodimer that interacts with its latency-associated peptide (LAP) and a latent TGF-beta-binding protein (LTBP), forming a larger complex called the large latent complex (LLC). The amino acid sequence of an exemplary latent human TGF-beta 1 (TGF-beta homodimer and its LAP) is amino acids 30-390 of SEQ ID NO: 94. The amino acid sequence of an exemplary mouse latent TGF-beta 1 (TGF-beta homodimer and its LAP) is amino acids 30-390 of SEQ ID NO: 96. The amino acid sequence of an exemplary latent cynomolgus monkey TGF-beta 1 (TGF-beta homodimer and its LAP) is amino acids 30-390 of SEQ ID NO: 98.

[0052] A complex formed from the TGF-beta homodimer and its LAP is called Small Latent

Complex (SLC). This latent complex keeps TGF-beta in an inactive form, which is incapable of binding to its receptors. SLC may be covalently linked to an additional protein, latent TGF-beta binding protein (LTBP), forming the large latent complex (LLC). There are four different LTBP isoforms known, LTBP-1, LTBP-2, LTBP-3 and LTBP-4. It has been reported that LTBP-1, LTBP-3 and LTBP-4 bind to SLC (See, e.g., Rifkin et al., J Biol Chem. 2005 Mar 4;280(9):7409-12). SLC may also be covalently linked to other additional proteins, such as glycoprotein A repetitions predominant (GARP) or leucine-rich repeat-containing protein 33 (LRRC33). GARP and LRRC have a transmembrane domain and associate with LAP on the cell surface (See, e.g., Wang et al., Mol Biol Cell. 2012 Mar;23(6):1129-39). As to LLCs, it is reported that LLCs associate covalently with the extracellular matrix (ECM) via the N-termini of the LTBPs (See, e.g., Saharinen et al., Cytokine Growth Factor Rev. 1999 Jun;10(2):99-117.). In some embodiments, latent TGF-beta 1 associated with the ECM on a cell surface is referred to as "cell surface latent TGF-beta 1".

[0053] The term "active TGF-beta 1", "mature TGF-beta 1", or "active mature TGF-beta 1", as used herein, refers to any TGF-beta 1 homodimer which does not form a latent TGF-beta 1 complex (LLC or SLC) and which is capable of binding to its receptors. The TGF-beta 1 activation process involves the release of the LLC from the ECM, followed by further proteolysis of LAP to release active TGF-beta to its receptors. Wide range of proteases including plasmin (PLN), plasma kallikrein (PLK), matrix metalloproteinase (MMP) 2, MMP9, MMP13, MMP14, Thrombin, Trypsin and Calpain are known to cleave latent TGF-beta and release active TGF-beta. These proteases may be collectively called "(latent) TGF-beta-cleaving proteases" or "(latent) TGF-beta 1-cleaving proteases" in the context of the present invention. In addition to proteases, thrombospondin 1 (TSP-1), Neuropilin-1 (Nrp1), ADAMSTS1 and F-spondin activate latent TGF-beta. Alternatively, upon mechanical stretch, integrins (preferably integrin alpha V beta 8 and/or integrin alpha V beta 6) can activate TGF-beta by binding to the RGD motif present in LAP and inducing the release of mature TGF-beta from its latent complex form.

[0054] As used herein, "treatment" (and grammatical variations thereof such as "treat" or "treating") refers to clinical intervention in an attempt to alter the natural course of the individual being treated, and can be performed either for prophylaxis or during the course of clinical pathology. Desirable effects of treatment include, but are not limited to, preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, preventing metastasis, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis. In some embodiments, antibodies of the invention are used to delay development of a disease or to

slow the progression of a disease.

- [0055] The term "variable region" or "variable domain" refers to the domain of an antibody heavy or light chain that is involved in binding the antibody to antigen. The variable domains of the heavy chain and light chain (VH and VL, respectively) of a native antibody generally have similar structures, with each domain comprising four conserved framework regions (FRs) and three hypervariable regions (HVRs). (See, e.g., Kindt et al. *Kuby Immunology*, 6th ed., W.H. Freeman and Co., page 91 (2007).) A single VH or VL domain may be sufficient to confer antigen-binding specificity. Furthermore, antibodies that bind a particular antigen may be isolated using a VH or VL domain from an antibody that binds the antigen to screen a library of complementary VL or VH domains, respectively. See, e.g., Portolano et al., *J. Immunol.* 150:880-887 (1993); Clarkson et al., *Nature* 352:624-628 (1991).
- [0056] The term "vector," as used herein, refers to a nucleic acid molecule capable of propagating another nucleic acid to which it is linked. The term includes the vector as a self-replicating nucleic acid structure as well as the vector incorporated into the genome of a host cell into which it has been introduced. Certain vectors are capable of directing the expression of nucleic acids to which they are operatively linked. Such vectors are referred to herein as "expression vectors."
- [0057] II. COMPOSITIONS AND METHODS
- In one aspect, the invention is based, in part, on anti-latent TGF-beta 1 antibodies and uses thereof. In certain embodiments, antibodies that bind to TGF-beta 1 are provided. Antibodies of the invention are useful, e.g., for the diagnosis or treatment of fibrosis, preferably myocardial fibrosis, renal fibrosis, ocular fibrosis myelofibrosis, liver fibrosis, and lung/pulmonary fibrosis. Antibodies of the invention are also useful, e.g., for the diagnosis or treatment of cancer. Examples of cancer include, but are not limited to, carcinoma, lymphoma (e.g., Hodgkin's and non-Hodgkin's lymphoma), blastoma, sarcoma, and leukemia. More particular examples of such cancers include squamous cell cancer, small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastrointestinal cancer, pancreatic cancer, glioma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney cancer, liver cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, leukemia and other lymphoproliferative disorders, and various types of head and neck cancer.
- [0058] In the context of diagnosis and treatment, renal fibrosis includes, but not limited to, the following: progressive renal diseases, IgA nephropathy, mesangial proliferative nephritis, mesangial proliferative glomerulonephritis, mesangiocapillary glomeru-

lonephritis, systemic lupus erythematosus, glomerular nephritis, renal interstitial fibrosis, renal failure, diabetic nephropathy, polycystic kidney disease, alport syndrome, focal segmental glomerular sclerosis, or membranous nephropathy.

[0059] A. Exemplary Anti-latent TGF-beta 1 Antibodies

In one aspect, the invention provides isolated antibodies that bind to latent TGF-beta 1. In further embodiments, the anti-latent TGF-beta 1 antibody binds to latent associated protein (LAP) region of the latent TGF-beta 1. An example of LAP region comprises amino acids 30-278 of a human TGF-beta 1 preproprotein (SEQ ID NO: 100). LAP is a component of latent TGF-beta 1, as described above. In some embodiments, an anti-latent TGF-beta 1 antibody binds to latent TGF-beta 1 with a dissociation constant (KD) of 10^{-8} nM or less, 10^{-9} nM or less, or 10^{-10} nM or less.

[0060] In one aspect, an anti-latent TGF-beta 1 antibody binds to a latent TGF-beta 1 forming LLC, and/or a latent TGF-beta 1 forming complex with GARP or LRRC33. In certain embodiments, an anti-latent TGF-beta 1 antibody binds to cell surface latent TGF-beta 1, which is a latent TGF-beta 1 associated with the extracellular matrix (ECM) on a cell surface. In another aspect, an anti-latent TGF-beta 1 antibody binds to a latent TGF-beta 1, wherein the LAP region of the latent TGF-beta 1 is not linked to LTBP, forming the small latent complex (SLC). In certain embodiments, SLCs exist in a soluble form. In some embodiments, an anti-latent TGF-beta 1 antibody binds to latent TGF-beta 1 (cell surface latent TGF-beta 1, LLC, or SLC) with a dissociation constant (KD) of 10^{-8} nM or less, 10^{-9} nM or less, or 10^{-10} nM or less.

[0061] In one aspect, an anti-latent TGF-beta 1 antibody inhibits activation of a latent TGF-beta 1. The term "activation" of latent TGF-beta 1, as used herein, refers to any process in which mature TGF-beta 1 is released from LAP, which is a component of the latent TGF-beta 1. The activation of latent TGF-beta 1 can be detected, for example, by measuring mature TGF-beta 1 and/or measuring mature TGF-beta 1 activity using various techniques known in the art or described herein. In some embodiments, an anti-latent TGF-beta 1 antibody inhibits the release of mature TGF-beta 1 from latent TGF-beta 1. As described above, it has been reported that mature TGF-beta 1 is released from latent TGF-beta 1 by activators such as proteases, integrins and other non-protease activators. Non-limited examples of proteases which activate latent TGF-beta 1 include plasmin (PLN), plasma kallikrein (PLK), matrix metalloproteinase (MMP) 2 and MMP9. In some embodiments, an anti-latent TGF-beta 1 antibody inhibits protease mediated and/or integrin mediated release of mature TGF-beta 1 from latent TGF-beta 1. As described above, proteases cleave LAP region of latent TGF-beta 1, which causes release of mature TGF-beta 1. In some embodiments, the cleavage sites by PLN and/or PLK locate within a fragment consisting of amino acids 56-59 of LAP polypeptide.

In preferred embodiments, the protease is selected from the group consisting of the following:

- (i) plasmin (PLN), e.g., Sigma-Aldrich Cat. #527624
(https://www.merckmillipore.com/JP/ja/product/Plasmin-EACA-and-Lysine-Free-Human-Plasma,EMD_BIO-527624);
- (ii) plasma kallikrein (PLK), e.g., Enzyme Research Laboratories Cat. #HPKa 1303
(<https://enzymeresearch.com/product/human-kallikrein/>);
- (iii) matrix metalloproteinase 2 (MMP2), e.g., R&D Systems Cat. #902-MP
(https://www.rndsystems.com/products/recombinant-human-mmp-2-protein-cf_902-mmp#product-datasheets); and
- (iv) matrix metalloproteinase 9 (MMP9), e.g., R&D Systems Cat. #909-MM
(https://www.rndsystems.com/products/recombinant-mouse-mmp-9-protein-cf_909-mm).

[0062] In some embodiments, an anti-latent TGF-beta 1 antibody inhibits a protease mediated activation of latent TGF-beta 1 without inhibiting integrin mediated activation of latent TGF-beta 1 or with partially inhibiting integrin mediated activation of latent TGF-beta 1. In some embodiments, an anti-latent TGF-beta 1 antibody inhibits a protease mediated activation of latent TGF-beta 1 but has less impact on inhibiting integrin mediated activation of latent TGF-beta 1.

[0063] In one aspect, an anti-latent TGF-beta 1 antibody inhibits protease mediated release of mature TGF-beta 1 from latent TGF-beta 1 without inhibiting protease mediated cleavage of LAP portion of latent TGF-beta 1 or with partially inhibiting protease mediated cleavage of LAP portion of latent TGF-beta 1. In some embodiments an anti-latent TGF-beta 1 antibody inhibits a protease mediated release of mature TGF-beta 1 from latent TGF-beta 1 but has less impact on inhibiting protease mediated cleavage of LAP portion of latent TGF-beta 1. In some embodiments, an anti-latent TGF-beta 1 antibody inhibits protease mediated release of mature TGF-beta 1 from latent TGF-beta 1 and allows a protease to cleave the LAP region while the anti-latent TGF-beta 1 antibody binds to the LAP region of the latent TGF-beta 1. In some embodiments, an anti-latent TGF-beta 1 antibody does not block access of a protease to latent TGF-beta 1, especially to the cleavage sites by PLN and/or PLK. In other embodiments, an anti-latent TGF-beta 1 antibody does not bind to protease cleavage sites of LAP portion of a latent TGF-beta 1, especially the cleavage sites by PLN and/or PLK.

[0064] In some embodiments, an anti-latent TGF-beta 1 antibody that inhibits protease mediated release of mature TGF-beta 1 from latent TGF-beta 1 is an antibody which (i) inhibits cleavage of LAP region mediated by one or more proteases, but (ii) does not inhibit cleavage of LAP region mediated by other proteases. For example, an anti-latent TGF-beta 1 antibody (1-i) inhibits MMP2 and/or MMP9 mediated release of

mature TGF-beta 1 by inhibiting MMP2 and/or MMP9 mediated cleavage of LAP portion of latent TGF-beta 1, and (1-ii) inhibits PLN and/or PLK mediated release of mature TGF-beta 1 without inhibiting PLN and/or PLK mediated cleavage of LAP portion of latent TGF-beta 1. Alternatively, an anti-latent TGF-beta 1 antibody (2-i) inhibits PLN and/or PLK mediated release of mature TGF-beta 1 by inhibiting PLN and/or PLK mediated cleavage of LAP portion of latent TGF-beta 1, and (2-ii) inhibits MMP2 and/or MMP9 mediated release of mature TGF-beta 1 without inhibiting MMP2 and/or MMP9 mediated cleavage of LAP portion of latent TGF-beta 1. Alternatively, an anti-latent TGF-beta 1 antibody (3-i) inhibits PLN and/or PLK mediated release of mature TGF-beta 1 without inhibiting PLN and/or PLK mediated cleavage of LAP portion of latent TGF-beta 1, and (3-ii) inhibits MMP2 and/or MMP9 mediated release of mature TGF-beta 1 without inhibiting MMP2 and/or MMP9 mediated cleavage of LAP portion of latent TGF-beta 1.

[0065] In some embodiments, antibodies "which inhibit activation of a latent TGF-beta 1" include antibodies that cause at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, or 40% or greater decrease in TGF-beta 1 activation. In other embodiments, antibodies "which inhibit protease mediated release of mature TGF-beta 1 from latent TGF-beta 1" include antibodies that cause at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, or 40% or greater decrease in protease mediated release of mature TGF-beta 1 from latent TGF-beta 1. In further embodiments, antibodies which inhibit protease mediated release of mature TGF-beta 1 from latent TGF-beta 1 "without inhibiting protease mediated cleavage of LAP region of latent TGF-beta 1" include antibodies that cause 50% or less, 45% or less, 40% or less, 35% or less, 30% or less, 25% or less, 20% or less, 15% or less, 10% or less, 5% or less decrease in protease mediated cleavage of LAP region of latent TGF-beta 1.

[0066] In some embodiments, an anti-latent TGF-beta 1 antibody stabilizes the structure of LAP region of the latent TGF-beta 1 without inhibiting the protease mediated cleavage of the LAP region of the latent TGF-beta 1. When an anti-latent TGF-beta 1 antibody "stabilize" the structure of LAP region, as used herein, the LAP region bounded by the anti-latent TGF-beta 1 antibody was kept in a certain structure from which mature TGF-beta 1 cannot be released. In further embodiments, latent TGF-beta 1 which is stabilized by an anti-latent TGF-beta 1 antibody can be activated by integrin (preferably integrin alpha V beta 8 and/or integrin alpha V beta 6). In certain embodiments, the LAP region which is stabilized by an anti-latent TGF-beta 1 antibody has been either cleaved or not cleaved by a protease. In some embodiments, an anti-latent TGF-beta 1 antibody stabilizes the structure of LAP region of the latent TGF-beta 1 and allows a protease to cleave the LAP region while the anti-latent TGF-beta 1 antibody binds to the LAP region of the latent TGF-beta 1. In some embodiments, an

anti-latent TGF-beta 1 antibody stabilizes the structure of LAP region of the latent TGF-beta 1 without blocking access of a protease to latent TGF-beta 1, especially to the cleavage sites by PLN and/or PLK. In other embodiments, an anti-latent TGF-beta 1 antibody stabilizes the structure of LAP region of the latent TGF-beta 1 without blocking access of a protease to latent TGF-beta 1, especially to the cleavage sites by MMP2 and/or MMP9.

- [0067] In one aspect, an anti-latent TGF-beta 1 antibody does not bind to mature TGF-beta 1. In some embodiments, an anti-latent TGF-beta 1 antibody binds to latent TGF-beta 1 with higher binding activity than mature TGF-beta 1. In certain embodiments, the antibodies of the present invention bind to latent TGF-beta 1 with at least 2, 3, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 200, 400, 1000, 10000, or more times higher binding activity than to mature TGF-beta 1.
- [0068] In one aspect, an anti-latent TGF-beta 1 antibody does not or does not significantly inhibit integrin mediated TGF-beta 1 activation, i.e., integrin mediated release of mature TGF-beta 1 from latent TGF-beta 1. In one aspect, an anti-latent TGF-beta 1 antibody has less impact on inhibiting integrin mediated TGF-beta 1 activation, i.e., integrin mediated release of mature TGF-beta 1 from latent TGF-beta 1. Preferably the integrin here is integrin alpha V beta 8 and/or integrin alpha V beta 6. In some embodiments, antibodies "which does not or does not significantly inhibit integrin mediated TGF-beta 1 activation" or "which have less impact on inhibiting integrin mediated TGF-beta 1 activation" include antibodies that cause 50% or less, 45% or less, 40% or less, 35% or less, 30% or less, 25% or less, 20% or less, 15% or less, 10% or less, 5% or less decrease in integrin mediated TGF-beta 1 activation, i.e., integrin mediated release of mature TGF-beta 1 from latent TGF-beta 1.
- [0069] In one aspect, an anti-latent TGF-beta 1 antibody causes reduced or fewer toxicities and/or adverse effects associated with anti-TGF-beta antagonists. In some embodiments, anti-latent TGF-beta 1 antibodies, such as those described herein, have a superior safety-efficacy profile, as compared to agents that elicit activities towards mature TGF-beta 1 or agent that elicit activities towards latent TGF-beta 1 but inhibits both protease and integrin mediated activation of latent TGF-beta 1. In some embodiments, an anti-latent TGF-beta 1 antibody of the present disclosure has reduced cardiotoxicity while has efficacy better than or comparable with anti-mature TGF-beta 1 antibody. Without being bound by any theory, an anti-latent TGF-beta 1 antibody of the present disclosure does not or does not significantly inhibit integrin mediated TGF-beta 1 activation or has less impact on inhibiting integrin mediated TGF-beta 1 activation, and therefore has reduced or fewer toxicities and/or adverse effects which are caused by: (i) integrin mediated TGF-beta 1 activation or (ii) TGF-beta 1 signaling inhibition at sites where TGF-beta 1 is activated by integrin. An anti-latent TGF-beta 1

antibody of the present disclosure can therefore be administered to subjects in need thereof at a therapeutically effective dose without causing adverse effects, especially cardiotoxicity. Such approach would therefore broaden the dosage range in which both efficacy and safety/tolerability can be achieved in patients. Thus, the invention provides methods for treating a disease associated with TGF-beta 1 signaling, by administering to a subject an effective amount of an anti-latent TGF-beta 1 antibody which does not or does not significantly inhibit integrin mediated TGF-beta 1 activation or has less impact on inhibiting integrin mediated TGF-beta 1 activation. Use of an anti-latent TGF-beta 1 antibody for reducing toxicities and/or adverse effects associated with TGF-beta 1 inhibition in a subject is encompassed by the present invention. In some embodiments, toxicity and/or adverse effects may include cardiovascular toxicities, gastrointestinal toxicities, immune toxicities, bone/cartilage toxicities, reproductive toxicities, and renal toxicities. In some embodiments, cardiovascular toxicities include, but are not limited to: heart valve lesions, e.g., hemorrhage, inflammation, degeneration and proliferation of valvular interstitial cells. In some embodiments, toxicities and/or adverse effects may include bleeding. In some embodiments, toxicities and/or adverse effects may include skin lesions or tumors. In some embodiments, toxicities and/or adverse effects may include tumor progression.

- [0070] In some embodiments, an anti-latent TGF-beta 1 antibody of the present invention:
- binds to latent TGF-beta 1;
 - binds to latent TGF-beta 1 forming SLC;
 - binds to latent TGF-beta 1 forming LLC;
 - binds to latent TGF-beta 1 forming complex with GARP or LRRC33;
 - binds to cell surface latent TGF-beta 1;
 - binds to LAP region of latent TGF-beta 1;
 - binds to LAP;
 - binds to latent TGF-beta 1 with a dissociation constant (KD) of 10^{-8} nM or less, 10^{-9} nM or less, or 10^{-10} nM or less;
 - inhibits protease mediated release of mature TGF-beta 1 from latent TGF-beta 1;
 - does not inhibit protease mediated cleavage of LAP region of latent TGF-beta 1;
 - partially inhibits integrin mediated TGF-beta 1 activation;
 - has less impact on inhibiting integrin mediated TGF-beta 1 activation;
 - does not or does not significantly inhibit integrin mediated release of mature TGF-beta 1 from latent TGF-beta 1; and/or
 - causes reduced or fewer toxicities and/or adverse effects associated with anti-TGF-beta 1 antagonists, for example, anti-mature TGF-beta antibody.

In further embodiments, the anti-latent TGF-beta 1 antibody of the present invention is:

a monoclonal antibody;
a human, humanized or chimeric antibody; and/or
an IgG antibody.

In some embodiments, the anti-latent TGF-beta 1 antibody is a monoclonal antibody. In some embodiments, the anti-latent TGF-beta 1 antibody is a human antibody. In some embodiments, the anti-latent TGF-beta 1 antibody is a humanized antibody. In some embodiments, the anti-latent TGF-beta 1 antibody is a chimeric antibody. In some embodiments, the anti-latent TGF-beta 1 antibody is an IgG antibody.

[0071] In one aspect, the invention provides an anti-latent TGF-beta 1 antibody comprising:

(a) a HVR-H1 comprising (or consisting of) the amino acid sequence selected from SEQ ID NO: 27, 28 and 29;

(b) a HVR-H2 comprising (or consisting of) the amino acid sequence selected from SEQ ID NO: 30-32 and 33;

(c) a HVR-H3 comprising (or consisting of) the amino acid sequence selected from SEQ ID NO: 34-39 and 40;

(d) a HVR-L1 comprising (or consisting of) the amino acid sequence selected from SEQ ID NO: 48-50 and 51;

(e) a HVR-L2 comprising (or consisting of) the amino acid sequence selected from SEQ ID NO: 52, 53 and 54; and

(f) a HVR-L3 comprising (or consisting of) the amino acid sequence selected from SEQ ID NO: 55-58 and 59,

wherein the antibody does not comprise a HVR-H1, HVR-H2, HVR-H3, HVR-L1, HVR-L2 and HVR-L3 comprising SEQ ID NO: 27, 30, 34, 48, 52 and 55, respectively.

[0072] In one aspect, the invention provides an anti-latent TGF-beta 1 antibody comprising at least one, two, three, four, five, or six HVRs selected from:

(a) HVR-H1 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 29;

(b) HVR-H2 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 30;

(c) HVR-H3 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 36;

(d) HVR-L1 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 49;

(e) HVR-L2 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 52; and

(f) HVR-L3 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 56.

- [0073] In one aspect, the invention provides an anti-latent TGF-beta 1 antibody comprising at least one, two, three, four, five, or six HVRs selected from:
- (a) HVR-H1 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 28;
 - (b) HVR-H2 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 32;
 - (c) HVR-H3 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 37;
 - (d) HVR-L1 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 50;
 - (e) HVR-L2 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 52; and
 - (f) HVR-L3 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 56.
- [0074] In one aspect, the invention provides an anti-latent TGF-beta 1 antibody comprising at least one, two, three, four, five, or six HVRs selected from:
- (a) HVR-H1 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 27;
 - (b) HVR-H2 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 30;
 - (c) HVR-H3 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 39;
 - (d) HVR-L1 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 48;
 - (e) HVR-L2 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 53; and
 - (f) HVR-L3 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 56.
- [0075] In one aspect, the invention provides an anti-latent TGF-beta 1 antibody comprising at least one, two, three, four, five, or six HVRs selected from:
- (a) HVR-H1 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 27;
 - (b) HVR-H2 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 33;
 - (c) HVR-H3 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 40;
 - (d) HVR-L1 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 51;

- (e) HVR-L2 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 52;
and
 - (f) HVR-L3 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 56.
- [0076] In one aspect, the invention provides an anti-latent TGF-beta 1 antibody comprising:
- (a) HVR-H1 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 29;
 - (b) HVR-H2 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 30;
 - (c) HVR-H3 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 36;
 - (d) HVR-L1 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 49;
 - (e) HVR-L2 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 52; and
 - (f) HVR-L3 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 56.
- [0077] In one aspect, the invention provides an anti-latent TGF-beta 1 antibody comprising:
- (a) HVR-H1 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 28;
 - (b) HVR-H2 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 32;
 - (c) HVR-H3 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 37;
 - (d) HVR-L1 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 50;
 - (e) HVR-L2 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 52; and
 - (f) HVR-L3 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 56.
- [0078] In one aspect, the invention provides an anti-latent TGF-beta 1 antibody comprising:
- (a) HVR-H1 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 27;
 - (b) HVR-H2 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 30;
 - (c) HVR-H3 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 39;
 - (d) HVR-L1 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 48;

- (e) HVR-L2 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 53; and
- (f) HVR-L3 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 56.
- [0079] In one aspect, the invention provides an anti-latent TGF-beta 1 antibody comprising:
- (a) HVR-H1 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 27;
- (b) HVR-H2 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 33;
- (c) HVR-H3 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 40;
- (d) HVR-L1 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 51;
- (e) HVR-L2 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 52; and
- (f) HVR-L3 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 56.
- [0080] In one aspect, the invention provides an anti-Latent TGF-beta 1 antibody comprising (or consisting of) HVR-H1, HVR-H2 and HVR-H3 of the VH sequence set forth in SEQ ID NO: 1-10 and 11, and HVR-L1, HVR-L2 and HVR-L3 of the VL sequence set forth in SEQ ID NO: 12-20 and 21, wherein the HVRs are defined by (a) Chothia; (b) Kabat; (c) MacCallum; or (d) combinations of (a), (b) and/or (c), wherein the HVR-H1, HVR-H2, HVR-H3, HVR-L1, HVR-L2 and HVR-L3 does not comprise SEQ ID NO: 27, 30, 34, 48, 52 and 55, respectively.
- [0081] In one aspect, the invention provides an anti-Latent TGF-beta 1 antibody comprising (or consisting of) HVR-H1, HVR-H2 and HVR-H3 of the VH sequence set forth in SEQ ID NO: 4, and HVR-L1, HVR-L2 and HVR-L3 of the VL sequence set forth in SEQ ID NO: 16, wherein the HVRs are defined by (a) Chothia; (b) Kabat; (c) MacCallum; or (d) combinations of (a), (b) and/or (c).
- [0082] In one aspect, the invention provides an anti-Latent TGF-beta 1 antibody comprising (or consisting of) HVR-H1, HVR-H2 and HVR-H3 of the VH sequence set forth in SEQ ID NO: 9, and HVR-L1, HVR-L2 and HVR-L3 of the VL sequence set forth in SEQ ID NO: 21, wherein the HVRs are defined by (a) Chothia; (b) Kabat; (c) MacCallum; or (d) combinations of (a), (b) and/or (c).
- [0083] In one aspect, the invention provides an anti-Latent TGF-beta 1 antibody comprising (or consisting of) HVR-H1, HVR-H2 and HVR-H3 of the VH sequence set forth in SEQ ID NO: 11, and HVR-L1, HVR-L2 and HVR-L3 of the VL sequence set forth in SEQ ID NO: 17, wherein the HVRs are defined by (a) Chothia; (b) Kabat; (c) MacCallum; or (d) combinations of (a), (b) and/or (c).

- [0084] In one aspect, the invention provides an anti-Latent TGF-beta 1 antibody comprising (or consisting of) HVR-H1, HVR-H2 and HVR-H3 of the VH sequence set forth in SEQ ID NO: 10, and HVR-L1, HVR-L2 and HVR-L3 of the VL sequence set forth in SEQ ID NO: 20, wherein the HVRs are defined by (a) Chothia; (b) Kabat; (c) MacCallum; or (d) combinations of (a), (b) and/or (c).
- [0085] In any of the above embodiments, an anti-latent TGF-beta 1 antibody is humanized. In one embodiment, an anti-latent TGF-beta 1 antibody comprises HVRs as in any of the above embodiments, and further comprises an acceptor human framework, e.g. a human immunoglobulin framework or a human consensus framework.
- [0086] In another aspect, an anti-latent TGF-beta 1 antibody comprises a heavy chain variable domain (VH) sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 4, 9, 11, or 10. In certain embodiments, an anti-latent TGF-beta 1 antibody comprises a heavy chain variable domain (VH) sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 2-10 or 11. In certain embodiments, a VH sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-latent TGF-beta 1 antibody comprising that sequence retains the ability to bind to latent TGF-beta 1. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO: 4, 9, 11, or 10. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO: 2-10 or 11. In certain embodiments, substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs). Optionally, the anti-latent TGF-beta 1 antibody comprises the VH sequence in SEQ ID NO: 4, 9, 11, or 10, including post-translational modifications of that sequence. Optionally, the anti-latent TGF-beta 1 antibody comprises the VH sequence in SEQ ID NO: 2-10 or 11, including post-translational modifications of that sequence. In a particular embodiment, the VH comprises one, two or three HVRs selected from: (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 29, 28, or 27, (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 30, 32, or 33, and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 36, 37, 39, or 40. In a particular embodiment, the VH comprises one, two or three HVRs selected from: (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 29, 28, or 27, (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 30-32 or 33, and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 34-39 or 40. In a particular embodiment, the VH comprises (a) a HVR-H1 comprising the amino acid sequence of SEQ ID NO: 29, 28, or 27, (b) a HVR-H2 comprising the amino acid sequence of SEQ

ID NO: 30-32 or 33, and (c) a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 34-39 or 40. Post-translational modifications include but are not limited to a modification of glutamine or glutamate in N-terminal of heavy chain or light chain to pyroglutamic acid by pyroglutamylation.

[0087] In another aspect, an anti-Latent TGF-beta 1 antibody is provided, wherein the antibody comprises a light chain variable domain (VL) having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 16, 21, 17, or 20. In certain embodiments, an anti-Latent TGF-beta 1 antibody is provided, wherein the antibody comprises a light chain variable domain (VL) having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 13-20 or 21. In certain embodiments, a VL sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-latent TGF-beta 1 antibody comprising that sequence retains the ability to bind to latent TGF-beta 1. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO: 16, 21, 17, or 20. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO: 13-20 or 21. In certain embodiments, the substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs). Optionally, the anti-latent TGF-beta 1 antibody comprises the VL sequence in SEQ ID NO: 16, 21, 17, or 20, including post-translational modifications of that sequence. In a particular embodiment, the VL comprises one, two or three HVRs selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 49, 50, 48, or 51; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 52 or 53; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 56. In a particular embodiment, the VL comprises one, two or three HVRs selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 49, 50, 48, or 51; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 52, 53 or 54; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 55-58 or 59. In a particular embodiment, the VL comprises (a) a HVR-L1 comprising the amino acid sequence of SEQ ID NO: 49, 50, 48, or 51; (b) a HVR-L2 comprising the amino acid sequence of SEQ ID NO: 52, 53 or 54; and (c) a HVR-L3 comprising the amino acid sequence of SEQ ID NO: 55-58 or 59. Post-translational modifications include but are not limited to a modification of glutamine or glutamate in N-terminal of heavy chain or light chain to pyroglutamic acid by pyroglutamylation.

[0088] In another aspect, an anti-latent TGF-beta 1 antibody is provided, wherein the antibody comprises a VH as in any of the embodiments provided above, and a VL as in

any of the embodiments provided above. In one embodiment, the antibody comprises the VH and VL sequences set forth in SEQ ID NO: 1-11 and SEQ ID NO: 12-21, respectively, including post-translational modifications of those sequences, wherein the antibody does not comprise a VH and VL of SEQ ID NO: 1 and 12, respectively. In one embodiment, the antibody comprises the VH and VL sequences set forth in SEQ ID NO: 2-11 and SEQ ID NO: 13-21, respectively. Post-translational modifications include but are not limited to a modification of glutamine or glutamate in N-terminal of heavy chain or light chain to pyroglutamic acid by pyroglutamylation. In one embodiment, the antibody comprises the VH and VL sequences set forth in SEQ ID NO: 4 and SEQ ID NO: 16, respectively, including post-translational modifications of those sequences. Post-translational modifications include but are not limited to a modification of glutamine or glutamate in N-terminal of heavy chain or light chain to pyroglutamic acid by pyroglutamylation.

- [0089] In one embodiment, the antibody comprises the VH and VL sequences set forth in SEQ ID NO: 9 and SEQ ID NO: 21, respectively, including post-translational modifications of those sequences. Post-translational modifications include but are not limited to a modification of glutamine or glutamate in N-terminal of heavy chain or light chain to pyroglutamic acid by pyroglutamylation.
- [0090] In one embodiment, the antibody comprises the VH and VL sequences set forth in SEQ ID NO: 11 and SEQ ID NO: 17, respectively, including post-translational modifications of those sequences. Post-translational modifications include but are not limited to a modification of glutamine or glutamate in N-terminal of heavy chain or light chain to pyroglutamic acid by pyroglutamylation.
- [0091] In one embodiment, the antibody comprises the VH and VL sequences set forth in SEQ ID NO: 10 and SEQ ID NO: 20, respectively, including post-translational modifications of those sequences. Post-translational modifications include but are not limited to a modification of glutamine or glutamate in N-terminal of heavy chain or light chain to pyroglutamic acid by pyroglutamylation.
- [0092] In some embodiments, the antibody comprises any of (a)-(h) below, including post-translational modifications of those sequences:
- (a) a heavy chain sequence of SEQ ID NO: 82 and a light chain sequence of SEQ ID NO: 83;
 - (b) a heavy chain sequence of SEQ ID NO: 84 and a light chain sequence of SEQ ID NO: 85;
 - (c) a heavy chain sequence of SEQ ID NO: 86 and a light chain sequence of SEQ ID NO: 87;
 - (d) a heavy chain sequence of SEQ ID NO: 88 and a light chain sequence of SEQ ID NO: 89;

- (e) a heavy chain sequence of SEQ ID NO: 90 and a light chain sequence of SEQ ID NO: 83;
- (f) a heavy chain sequence of SEQ ID NO: 91 and a light chain sequence of SEQ ID NO: 85;
- (g) a heavy chain sequence of SEQ ID NO: 92 and a light chain sequence of SEQ ID NO: 87; or
- (h) a heavy chain sequence of SEQ ID NO: 93 and a light chain sequence of SEQ ID NO: 89.

Post-translational modifications include but are not limited to a modification of glutamine or glutamate in N-terminal of heavy chain or light chain to pyroglutamic acid by pyroglutamylolation.

[0093] In some embodiments, the antibody of the invention comprises a heavy chain sequence and a light chain sequence which are disclosed herein.

The heavy chain sequence of each antibody consists of (i.e., is the combination of) the sequence (SEQ ID NO) of "VH" (N-terminal side) and the sequence (SEQ ID NO) of "CH" (C-terminal side) which are shown in Tables 1 and 3.

That is, the sequence (SEQ ID NO) of the heavy chain sequence can be deduced by linking the sequence (SEQ ID NO) of "VH" and the sequence (SEQ ID NO) of "CH".

The light chain sequence of each antibody consists of (i.e., is the combination of) the sequence (SEQ ID NO) of "VL" (N-terminal side) and the sequence (SEQ ID NO) of "CL" (C-terminal side) which are shown in Table 1 and 3.

That is, the sequence (SEQ ID NO) of the light chain sequence can be deduced by linking the sequence (SEQ ID NO) of "VL" and the sequence (SEQ ID NO) of "CL".

Examples of such combinations are shown in Table 1.

[0094]

[Table 1]

Antibody name	Heavy chain SEQ ID NO:	VH SEQ ID NO:	CH SEQ ID NO:	Light chain SEQ ID NO:	VL SEQ ID NO:	CL SEQ ID NO:
hT0947AE20-SG181	82	4	22 (SG181)	83	16	25 (SK1)
hT0947AE27-SG181	84	9	22	85	21	25
hT0947AE34-SG181	86	11	22	87	17	25
hT0947AE37-SG181	88	10	22	89	20	25
hT0947AE20-SG191	90	4	23 (SG191)	83	16	25
hT0947AE27-SG191	91	9	23	85	21	25
hT0947AE34-SG191	92	11	23	87	17	25
hT0947AE37-SG191	93	10	23	89	20	25

[0095] In a further aspect, the invention provides an antibody that binds to the same epitope as an anti-latent TGF-beta 1 antibody provided herein. For example, in certain embodiments, an antibody is provided that binds to the same epitope as:

(1) An anti-latent TGF-beta 1 antibody comprising:

(a) HVR-H1 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 29,

(b) HVR-H2 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 30,

(c) HVR-H3 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 36,

(d) HVR-L1 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 49,

(e) HVR-L2 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 52, and

(f) HVR-L3 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 56;

(2) An anti-latent TGF-beta 1 antibody comprising:

(a) HVR-H1 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 28,

(b) HVR-H2 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 32,

- (c) HVR-H3 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 37,
- (d) HVR-L1 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 50,
- (e) HVR-L2 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 52,
- and
- (f) HVR-L3 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 56;
- (3) An anti-latent TGF-beta 1 antibody comprising:
 - (a) HVR-H1 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 27,
 - (b) HVR-H2 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 30,
 - (c) HVR-H3 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 39,
 - (d) HVR-L1 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 48,
 - (e) HVR-L2 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 53,
 - and
 - (f) HVR-L3 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 56;
- or
- (4) An anti-latent TGF-beta 1 antibody comprising:
 - (a) HVR-H1 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 27,
 - (b) HVR-H2 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 33,
 - (c) HVR-H3 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 40,
 - (d) HVR-L1 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 51,
 - (e) HVR-L2 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 52,
 - and
 - (f) HVR-L3 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 56.

[0096] In certain embodiments, the invention provides an antibody that binds the same epitope as an antibody comprising any of (a)-(h) below:

- (a) a heavy chain sequence of SEQ ID NO: 82 and a light chain sequence of SEQ ID NO: 83;
- (b) a heavy chain sequence of SEQ ID NO: 84 and a light chain sequence of SEQ ID NO: 85;
- (c) a heavy chain sequence of SEQ ID NO: 86 and a light chain sequence of SEQ ID NO: 87;
- (d) a heavy chain sequence of SEQ ID NO: 88 and a light chain sequence of SEQ ID NO: 89;
- (e) a heavy chain sequence of SEQ ID NO: 90 and a light chain sequence of SEQ ID NO: 83;
- (f) a heavy chain sequence of SEQ ID NO: 91 and a light chain sequence of SEQ ID NO: 85;

(g) a heavy chain sequence of SEQ ID NO: 92 and a light chain sequence of SEQ ID NO: 87; or

(h) a heavy chain sequence of SEQ ID NO: 93 and a light chain sequence of SEQ ID NO: 89.

[0097] In a further aspect, the invention provides an antibody that binds to latent TGF-beta 1 of human, monkey, mouse, and/or rat. In certain embodiments, the invention provides an antibody that binds to latent TGF-beta 1 of human, monkey and mouse. In certain embodiments, the invention provides an antibody which binds to latent TGF-beta 1 forming SLC of human, monkey and mouse. In certain embodiments, the invention provides an antibody which binds to latent TGF-beta 1 forming LLC of human, monkey and mouse. In certain embodiments, the invention provides an antibody which binds to latent TGF-beta 1 forming LLC of human, monkey and mouse. In certain embodiments, the invention provides an antibody which binds to latent TGF-beta 1 forming complex with GARP or LRRC33 of human, monkey and mouse. In certain embodiments, the invention provides an antibody which binds to cell surface latent TGF-beta 1 of human, monkey and mouse.

[0098] In a further aspect, the invention provides an antibody that binds to the same epitope as any one of the anti-latent TGF-beta 1 antibodies provided herein. The epitope may exist on TGF-beta 1 of human, monkey, mouse and/or rat. For example, in certain embodiments, the invention provides an antibody that binds the same epitope as a reference antibody, wherein the reference antibody is:

(1) An anti-latent TGF-beta 1 antibody comprising:

(a) HVR-H1 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 29,

(b) HVR-H2 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 30,

(c) HVR-H3 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 36,

(d) HVR-L1 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 49,

(e) HVR-L2 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 52, and

(f) HVR-L3 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 56;

(2) An anti-latent TGF-beta 1 antibody comprising:

(a) HVR-H1 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 28,

(b) HVR-H2 comprising (or consisting of) the amino acid sequence of SEQ ID NO:

32,

(c) HVR-H3 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 37,
 (d) HVR-L1 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 50,
 (e) HVR-L2 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 52,
 and

(f) HVR-L3 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 56;
 (3) An anti-latent TGF-beta 1 antibody comprising:

(a) HVR-H1 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 27,
 (b) HVR-H2 comprising (or consisting of) the amino acid sequence of SEQ ID NO:

30,

(c) HVR-H3 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 39,
 (d) HVR-L1 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 48,
 (e) HVR-L2 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 53,
 and

(f) HVR-L3 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 56;
 or

(4) An anti-latent TGF-beta 1 antibody comprising:

(a) HVR-H1 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 27,
 (b) HVR-H2 comprising (or consisting of) the amino acid sequence of SEQ ID NO:

33,

(c) HVR-H3 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 40,
 (d) HVR-L1 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 51,
 (e) HVR-L2 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 52,
 and

(f) HVR-L3 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 56.

[0099] In certain embodiments, the invention provides an antibody that binds the same epitope as a reference antibody, wherein the reference antibody comprises any of (a)-(h) below:

(a) a heavy chain sequence of SEQ ID NO: 82 and a light chain sequence of SEQ ID NO: 83;

(b) a heavy chain sequence of SEQ ID NO: 84 and a light chain sequence of SEQ ID NO: 85;

(c) a heavy chain sequence of SEQ ID NO: 86 and a light chain sequence of SEQ ID NO: 87;

(d) a heavy chain sequence of SEQ ID NO: 88 and a light chain sequence of SEQ ID NO: 89;

(e) a heavy chain sequence of SEQ ID NO: 90 and a light chain sequence of SEQ ID NO: 83;

- (f) a heavy chain sequence of SEQ ID NO: 91 and a light chain sequence of SEQ ID NO: 85;
- (g) a heavy chain sequence of SEQ ID NO: 92 and a light chain sequence of SEQ ID NO: 87; or
- (h) a heavy chain sequence of SEQ ID NO: 93 and a light chain sequence of SEQ ID NO: 89.

[0100] In a further aspect, the invention provides an antibody that competes with an anti-latent TGF-beta 1 antibody provided herein for binding to TGF-beta 1 of human, monkey, mouse and/or rat. For example, in certain embodiments, an antibody is provided that competes for binding to TGF-beta 1 of human, monkey, mouse and/or rat with:

(1) An anti-latent TGF-beta 1 antibody comprising:

- (a) HVR-H1 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 29,
- (b) HVR-H2 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 30,
- (c) HVR-H3 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 36,
- (d) HVR-L1 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 49,
- (e) HVR-L2 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 52, and
- (f) HVR-L3 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 56;

(2) An anti-latent TGF-beta 1 antibody comprising:

- (a) HVR-H1 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 28,
- (b) HVR-H2 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 32,
- (c) HVR-H3 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 37,
- (d) HVR-L1 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 50,
- (e) HVR-L2 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 52, and
- (f) HVR-L3 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 56;

(3) An anti-latent TGF-beta 1 antibody comprising:

- (a) HVR-H1 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 27,
 - (b) HVR-H2 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 30,
 - (c) HVR-H3 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 39,
 - (d) HVR-L1 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 48,
 - (e) HVR-L2 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 53, and
 - (f) HVR-L3 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 56;
- or
- (4) An anti-latent TGF-beta 1 antibody comprising:
 - (a) HVR-H1 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 27,
 - (b) HVR-H2 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 33,
 - (c) HVR-H3 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 40,
 - (d) HVR-L1 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 51,
 - (e) HVR-L2 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 52, and
 - (f) HVR-L3 comprising (or consisting of) the amino acid sequence of SEQ ID NO: 56.

[0101] In certain embodiments, the invention provides an antibody that competes for binding to TGF-beta 1 of human, monkey, mouse and/or rat with an antibody comprising any of (a)-(h) below:

- (a) a heavy chain sequence of SEQ ID NO: 82 and a light chain sequence of SEQ ID NO: 83;
- (b) a heavy chain sequence of SEQ ID NO: 84 and a light chain sequence of SEQ ID NO: 85;
- (c) a heavy chain sequence of SEQ ID NO: 86 and a light chain sequence of SEQ ID NO: 87;
- (d) a heavy chain sequence of SEQ ID NO: 88 and a light chain sequence of SEQ ID NO: 89;
- (e) a heavy chain sequence of SEQ ID NO: 90 and a light chain sequence of SEQ ID NO: 83;
- (f) a heavy chain sequence of SEQ ID NO: 91 and a light chain sequence of SEQ ID NO: 85;
- (g) a heavy chain sequence of SEQ ID NO: 92 and a light chain sequence of SEQ ID NO: 87; or
- (h) a heavy chain sequence of SEQ ID NO: 93 and a light chain sequence of SEQ ID NO: 89.

[0102] In a further aspect of the invention, an anti-latent TGF-beta 1 antibody according to

any of the above embodiments is a monoclonal antibody, including a chimeric, humanized or human antibody. In one embodiment, an anti-latent TGF-beta 1 antibody according to any of the above embodiments is a chimeric antibody. In one embodiment, an anti-latent TGF-beta 1 antibody according to any of the above embodiments is a humanized antibody. In one embodiment, an anti-latent TGF-beta 1 antibody according to any of the above embodiments is a human antibody. In some embodiments, an anti-latent TGF-beta 1 antibody according to any of the above embodiments is a chimeric monoclonal antibody. In some embodiments, an anti-latent TGF-beta 1 antibody according to any of the above embodiments is a humanized monoclonal antibody. In some embodiments, an anti-latent TGF-beta 1 antibody according to any of the above embodiments is a human monoclonal antibody. In another embodiment, the antibody is a full length antibody, e.g., an intact IgG1, IgG2, IgG3 or IgG4 antibody or an intact antibody of other class or isotype as defined herein. In another embodiment, the antibody is, e.g., an IgG1, IgG2, IgG3 or IgG4 antibody or an antibody of other class or isotype as defined herein.

[0103] In one embodiment, an antibody fragment of an anti-latent TGF-beta 1 antibody is, e.g., a Fv, Fab, Fab', scFv, diabody, or F(ab')₂ fragment. In one embodiment, an antibody fragment of an anti-latent TGF-beta 1 antibody includes any antigen binding molecule which comprises a variable heavy chain and/or variable light chain structure of immunoglobulin.

[0104] In a further aspect, an anti-latent TGF-beta 1 antibody according to any of the above embodiments may incorporate any of the features, singly or in combination, as described in Sections 1-7 below:

[0105] 1. Antibody binding activity

In certain embodiments, an antibody provided herein has a dissociation constant (KD) of 1 micromolar or less, 100 nM or less, 10 nM or less, 1 nM or less, 0.1 nM or less, 0.01 nM or less, or 0.001 nM or less (e.g. 10⁻⁸ M or less, e.g. from 10⁻⁸ M to 10⁻¹³ M, e.g., from 10⁻⁹ M to 10⁻¹³ M).

[0106] In one embodiment, binding activity of an antibody is measured by a radiolabeled antigen binding assay (RIA) and represented by KD. In one embodiment, an RIA is performed with the Fab version of an antibody of interest and its antigen. For example, solution binding activity of Fabs for antigen is measured by equilibrating Fab with a minimal concentration of (¹²⁵I)-labeled antigen in the presence of a titration series of unlabeled antigen, then capturing bound antigen with an anti-Fab antibody-coated plate (see, e.g., Chen et al., J. Mol. Biol. 293:865-881(1999)). To establish conditions for the assay, MICROTITER (registered trademark) multi-well plates (Thermo Scientific) are coated overnight with 5 microgram/ml of a capturing anti-Fab antibody (Cappel Labs) in 50 mM sodium carbonate (pH 9.6), and subsequently blocked with 2% (w/v) bovine

serum albumin in PBS for two to five hours at room temperature (approximately 23 degrees Celsius (C)). In a non-adsorbent plate (Nunc #269620), 100 pM or 26 pM [¹²⁵I]-antigen are mixed with serial dilutions of a Fab of interest (e.g., consistent with assessment of the anti-VEGF antibody, Fab-12, in Presta et al., Cancer Res. 57:4593-4599 (1997)). The Fab of interest is then incubated overnight; however, the incubation may continue for a longer period (e.g., about 65 hours) to ensure that equilibrium is reached. Thereafter, the mixtures are transferred to the capture plate for incubation at room temperature (e.g., for one hour). The solution is then removed and the plate washed eight times with 0.1% polysorbate 20 (TWEEN-20 (registered trademark)) in PBS. When the plates have dried, 150 microliter/well of scintillant (MICROSCINT-20 TM; Packard) is added, and the plates are counted on a TOPCOUNTTM gamma counter (Packard) for ten minutes. Concentrations of each Fab that give less than or equal to 20% of maximal binding are chosen for use in competitive binding assays.

[0107] In one embodiment, for measuring binding activity of an antibody, ligand-capturing methods, for example, using BIACORE (registered trademark) T200 or BIACORE (registered trademark) 4000 (GE Healthcare, Uppsala, Sweden), which rely upon surface plasmon resonance analysis methods as the measurement principle, are used. BIACORE (registered trademark) Control Software is used for operation of devices. In one embodiment, amine-coupling kit (GE Healthcare, Uppsala, Sweden) is used according to the manufacturer's instructions to let a molecule for ligand capturing, for example, an anti-tag antibody, an anti-IgG antibody, protein A, etc. fixed onto a sensor chip (GE Healthcare, Uppsala, Sweden) coated with carboxymethyl dextran. The ligand-capturing molecule is diluted with a 10 mM sodium acetate solution at an appropriate pH and is injected at an appropriate flow rate and for an appropriate injection time. Binding activity measurements are measured using a 0.05% polysorbate 20 (in other name Tween (registered trademark)-20)-containing buffer as a measurement buffer, at a flow rate of 10-30 microliter/minute, and at a measurement temperature of preferably at 25 degrees C or 37 degrees C. For the measurement carried out with an antibody captured by the ligand-capturing molecule as a ligand, an antibody is injected to let a target amount of the antibody captured, and then a serial dilution of an antigen and/or an Fc receptor (analyte) prepared using the measurement buffer is injected. For the measurement carried out with an antigen and/or an Fc receptor captured by the ligand-capturing molecule as a ligand, an antigen and/or an Fc receptor is injected to let a target amount thereof captured, and then a serial dilution of an antibody (analyte) prepared using the measurement buffer is injected.

[0108] In one embodiment, the measurement results are analyzed using BIACORE (registered trademark) Evaluation Software. Kinetics parameter calculation is carried

out by fitting sensorgrams of association and dissociation at the same time using a 1:1 binding model, and an association rate (k_{on} or k_a), a dissociation rate (k_{off} or k_d), and an equilibrium dissociation constant (K_D) may be calculated. For the case of weak binding activity, in particular, for the cases where dissociation is fast and kinetics parameters are difficult to calculate, the Steady state model may be used to calculate the equilibrium dissociation constant (K_D). As additional parameters concerning binding activity, "binding amount of analyte per unit ligand amount" may be calculated by dividing a binding amount of analyte (resonance unit: RU) at a specific concentration by an amount of captured ligand.

[0109] 2. Antibody Fragments

In certain embodiments, antibody fragments include, but are not limited to, Fab, Fab', Fab'-SH, $F(ab')_2$, Fv, and scFv fragments, and other fragments described below. For a review of certain antibody fragments, see Hudson et al. Nat. Med. 9:129-134 (2003). For a review of scFv fragments, see, e.g., Pluckthun, in The Pharmacology of Monoclonal Antibodies, vol. 113, Rosenberg and Moore eds., (Springer-Verlag, New York), pp. 269-315 (1994); see also WO 93/16185; and U.S. Patent Nos. 5,571,894 and 5,587,458. For discussion of Fab and $F(ab')_2$ fragments comprising salvage receptor binding epitope residues and having increased in vivo half-life, see U.S. Patent No. 5,869,046.

[0110] Diabodies are antibody fragments with two antigen-binding sites that may be bivalent or bispecific. See, for example, EP 404,097; WO 1993/01161; Hudson et al., Nat. Med. 9:129-134 (2003); and Hollinger et al., Proc. Natl. Acad. Sci. USA 90: 6444-6448 (1993). Triabodies and tetrabodies are also described in Hudson et al., Nat. Med. 9:129-134 (2003).

[0111] Single-domain antibodies are antibody fragments comprising all or a portion of the heavy chain variable domain or all or a portion of the light chain variable domain of an antibody. In certain embodiments, a single-domain antibody is a human single-domain antibody (Domantis, Inc., Waltham, MA; see, e.g., U.S. Patent No. 6,248,516 B1).

[0112] Antibody fragments can be made by various techniques, including but not limited to proteolytic digestion of an intact antibody as well as production by recombinant host cells (e.g. E. coli or phage), as described herein.

[0113] The present invention also relates to antigen-binding molecules which bind to TGF-beta 1, which include, but are not limited to, for example, minibodies (low molecular weight antibodies), and scaffold proteins. In the present invention, any scaffold protein is acceptable as long as it is a peptide that has a stable three-dimensional structure and is capable of binding to at least an antigen. Such peptides include, for example, fragments of antibody variable regions, fibronectin, protein A domain, LDL receptor A domain, lipocalin, and other molecules described in Nygren et al. (Current Opinion in

Structural Biology, (1997) 7:463-469; Journal of Immunol Methods, (2004) 290:3-28), Binz et al. (Nature Biotech. (2005) 23:1257-1266), and Hosse et al. (Protein Science, (2006) 15:14-27). When referring to such an antibody, e.g., "anti-latent TGF-beta 1 antibody" should be replaced with "anti-latent TGF-beta 1 antigen-binding molecule" in the context of the present specification.

[0114] 3. Chimeric and Humanized Antibodies

In certain embodiments, an antibody provided herein is a chimeric antibody. Certain chimeric antibodies are described, e.g., in U.S. Patent No. 4,816,567; and Morrison et al., Proc. Natl. Acad. Sci. USA, 81:6851-6855 (1984)). In one example, a chimeric antibody comprises a non-human variable region (e.g., a variable region derived from a mouse, rat, hamster, rabbit, or non-human primate, such as a monkey) and a human constant region. In a further example, a chimeric antibody is a "class switched" antibody in which the class or subclass has been changed from that of the parent antibody. Antigen-binding fragments of chimeric antibodies may also be provided.

[0115] In certain embodiments, a chimeric antibody is a humanized antibody. Typically, a non-human antibody is humanized to reduce immunogenicity to humans, while retaining the specificity and binding activity of the parental non-human antibody. Generally, a humanized antibody comprises one or more variable domains in which HVRs, e.g., CDRs, (or portions thereof) are derived from a non-human antibody, and FRs (or portions thereof) are derived from human antibody sequences. A humanized antibody optionally will also comprise at least a portion of a human constant region. In some embodiments, some FR residues in a humanized antibody are substituted with corresponding residues from a non-human antibody (e.g., the antibody from which the HVR residues are derived), e.g., to restore or improve antibody specificity or binding activity.

[0116] Humanized antibodies and methods of making them are reviewed, e.g., in Almagro and Fransson, Front. Biosci. 13:1619-1633 (2008), and are further described, e.g., in Riechmann et al., Nature 332:323-329 (1988); Queen et al., Proc. Nat'l Acad. Sci. USA 86:10029-10033 (1989); US Patent Nos. 5, 821,337, 7,527,791, 6,982,321, and 7,087,409; Kashmiri et al., Methods 36:25-34 (2005) (describing specificity determining region (SDR) grafting); Padlan, Mol. Immunol. 28:489-498 (1991) (describing "resurfacing"); Dall'Acqua et al., Methods 36:43-60 (2005) (describing "FR shuffling"); and Osbourn et al., Methods 36:61-68 (2005) and Klimka et al., Br. J. Cancer, 83:252-260 (2000) (describing the "guided selection" approach to FR shuffling).

[0117] Human framework regions that may be used for humanization include but are not limited to: framework regions selected using the "best-fit" method (see, e.g., Sims et al. J. Immunol. 151:2296 (1993)); framework regions derived from the consensus

sequence of human antibodies of a particular subgroup of light or heavy chain variable regions (see, e.g., Carter et al. Proc. Natl. Acad. Sci. USA, 89:4285 (1992); and Presta et al. J. Immunol., 151:2623 (1993)); human mature (somatically mutated) framework regions or human germline framework regions (see, e.g., Almagro and Fransson, Front. Biosci. 13:1619-1633 (2008)); and framework regions derived from screening FR libraries (see, e.g., Baca et al., J. Biol. Chem. 272:10678-10684 (1997) and Rosok et al., J. Biol. Chem. 271:22611-22618 (1996)).

[0118] 4. Human Antibodies

In certain embodiments, an antibody provided herein is a human antibody. Human antibodies can be produced using various techniques known in the art. Human antibodies are described generally in van Dijk and van de Winkel, Curr. Opin. Pharmacol. 5: 368-74 (2001) and Lonberg, Curr. Opin. Immunol. 20:450-459 (2008).

[0119] Human antibodies may be prepared by administering an immunogen to a transgenic animal that has been modified to produce intact human antibodies or intact antibodies with human variable regions in response to antigenic challenge. Such animals typically contain all or a portion of the human immunoglobulin loci, which replace the endogenous immunoglobulin loci, or which are present extrachromosomally or integrated randomly into the animal's chromosomes. In such transgenic mice, the endogenous immunoglobulin loci have generally been inactivated. For review of methods for obtaining human antibodies from transgenic animals, see Lonberg, Nat. Biotech. 23:1117-1125 (2005). See also, e.g., U.S. Patent Nos. 6,075,181 and 6,150,584 describing XENOMOUSE™ technology; U.S. Patent No. 5,770,429 describing HUMAB (registered trademark) technology; U.S. Patent No. 7,041,870 describing K-M MOUSE (registered trademark) technology, and U.S. Patent Application Publication No. US 2007/0061900, describing VELOCIMOUSE (registered trademark) technology). Human variable regions from intact antibodies generated by such animals may be further modified, e.g., by combining with a different human constant region.

[0120] Human antibodies can also be made by hybridoma-based methods. Human myeloma and mouse-human heteromyeloma cell lines for the production of human monoclonal antibodies have been described. (See, e.g., Kozbor J. Immunol., 133: 3001 (1984); Brodeur et al., Monoclonal Antibody Production Techniques and Applications, pp. 51-63 (Marcel Dekker, Inc., New York, 1987); and Boerner et al., J. Immunol., 147: 86 (1991).) Human antibodies generated via human B-cell hybridoma technology are also described in Li et al., Proc. Natl. Acad. Sci. USA, 103:3557-3562 (2006). Additional methods include those described, for example, in U.S. Patent No. 7,189,826 (describing production of monoclonal human IgM antibodies from hybridoma cell lines) and Ni, Xiandai Mianyixue, 26(4):265-268 (2006) (describing human-human hybridomas). Human hybridoma technology (Trioma technology) is also described in

Vollmers and Brandlein, *Histology and Histopathology*, 20(3):927-937 (2005) and Vollmers and Brandlein, *Methods and Findings in Experimental and Clinical Pharmacology*, 27(3):185-91 (2005).

[0121] Human antibodies may also be generated by isolating Fv clone variable domain sequences selected from human-derived phage display libraries. Such variable domain sequences may then be combined with a desired human constant domain. Techniques for selecting human antibodies from antibody libraries are described below.

[0122] 5. Library-Derived Antibodies

Antibodies of the invention may be isolated by screening combinatorial libraries for antibodies with the desired activity or activities. For example, a variety of methods are known in the art for generating phage display libraries and screening such libraries for antibodies possessing the desired binding characteristics. Such methods are reviewed, e.g., in Hoogenboom et al. in *Methods in Molecular Biology* 178:1-37 (O'Brien et al., ed., Human Press, Totowa, NJ, 2001) and further described, e.g., in the McCafferty et al., *Nature* 348:552-554; Clackson et al., *Nature* 352: 624-628 (1991); Marks et al., *J. Mol. Biol.* 222: 581-597 (1992); Marks and Bradbury, in *Methods in Molecular Biology* 248:161-175 (Lo, ed., Human Press, Totowa, NJ, 2003); Sidhu et al., *J. Mol. Biol.* 338(2): 299-310 (2004); Lee et al., *J. Mol. Biol.* 340(5): 1073-1093 (2004); Fellouse, *Proc. Natl. Acad. Sci. USA* 101(34): 12467-12472 (2004); and Lee et al., *J. Immunol. Methods* 284(1-2): 119-132(2004).

[0123] In certain phage display methods, repertoires of VH and VL genes are separately cloned by polymerase chain reaction (PCR) and recombined randomly in phage libraries, which can then be screened for antigen-binding phage as described in Winter et al., *Ann. Rev. Immunol.*, 12: 433-455 (1994). Phage typically display antibody fragments, either as single-chain Fv (scFv) fragments or as Fab fragments. Libraries from immunized sources provide high-binding activity antibodies to the immunogen without the requirement of constructing hybridomas. Alternatively, the naive repertoire can be cloned (e.g., from human) to provide a single source of antibodies to a wide range of non-self and also self antigens without any immunization as described by Griffiths et al., *EMBO J*, 12: 725-734 (1993). Finally, naive libraries can also be made synthetically by cloning unrearranged V-gene segments from stem cells, and using PCR primers containing random sequence to encode the highly variable CDR3 regions and to accomplish rearrangement in vitro, as described by Hoogenboom and Winter, *J. Mol. Biol.*, 227: 381-388 (1992). Patent publications describing human antibody phage libraries include, for example: US Patent No. 5,750,373, and US Patent Publication Nos. 2005/0079574, 2005/0119455, 2005/0266000, 2007/0117126, 2007/0160598, 2007/0237764, 2007/0292936, and 2009/0002360.

[0124] Antibodies or antibody fragments isolated from human antibody libraries are

considered human antibodies or human antibody fragments herein.

[0125] 6. Multispecific Antibodies

In certain embodiments, an antibody provided herein is a multispecific antibody, e.g. a bispecific antibody. Multispecific antibodies are monoclonal antibodies that have binding specificities for at least two different sites. In certain embodiments, one of the binding specificities is for TGF-beta 1 and the other is for any other antigen. In certain embodiments, bispecific antibodies may bind to two different epitopes of TGF-beta 1. Bispecific antibodies may also be used to localize cytotoxic agents to cells which express TGF-beta 1. Bispecific antibodies can be prepared as full length antibodies. Antibody fragments of bispecific antibodies may also be provided.

[0126] Techniques for making multispecific antibodies include, but are not limited to, recombinant co-expression of two immunoglobulin heavy chain-light chain pairs having different specificities (see Milstein and Cuello, Nature 305: 537 (1983)), WO 93/08829, and Traunecker et al., EMBO J. 10: 3655 (1991)), and "knob-in-hole" engineering (see, e.g., U.S. Patent No. 5,731,168). Multi-specific antibodies may also be made by engineering electrostatic steering effects for making antibody Fc-heterodimeric molecules (WO 2009/089004A1); cross-linking two or more antibodies or fragments (see, e.g., US Patent No. 4,676,980, and Brennan et al., Science, 229: 81 (1985)); using leucine zippers to produce bi-specific antibodies (see, e.g., Kostelny et al., J. Immunol., 148(5):1547-1553 (1992)); using "diabody" technology for making bispecific antibody fragments (see, e.g., Hollinger et al., Proc. Natl. Acad. Sci. USA, 90:6444-6448 (1993)); and using single-chain Fv (scFv) dimers (see, e.g. Gruber et al., J. Immunol., 152:5368 (1994)); and preparing trispecific antibodies as described, e.g., in Tutt et al. J. Immunol. 147: 60 (1991).

[0127] Engineered antibodies with three or more functional antigen binding sites, including "Octopus antibodies," are also included herein (see, e.g. US 2006/0025576A1).

[0128] The antibody fragment herein also includes a "Dual Acting Fab" or "DAF" comprising an antigen binding site that binds to TGF-beta 1 as well as another, different antigen (see, US 2008/0069820, for example).

[0129] 7. Antibody Variants

In certain embodiments, amino acid sequence variants of the antibodies provided herein are contemplated. For example, it may be desirable to improve the binding activity and/or other biological properties of the antibody. Amino acid sequence variants of an antibody may be prepared by introducing appropriate modifications into the nucleotide sequence encoding the antibody, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution can be made to arrive at the final

construct, provided that the final construct possesses the desired characteristics, e.g., antigen-binding.

[0130] a) Substitution, Insertion, and Deletion Variants

In certain embodiments, antibody variants having one or more amino acid substitutions are provided. Sites of interest for substitutional mutagenesis include the HVRs and FRs. Conservative substitutions are shown in Table 2 under the heading of "preferred substitutions." More substantial changes are provided in Table 2 under the heading of "exemplary substitutions," and as further described below in reference to amino acid side chain classes. Amino acid substitutions may be introduced into an antibody of interest and the products screened for a desired activity, e.g., retained/improved antigen binding, decreased immunogenicity, or improved ADCC or CDC.

[0131] [Table 2]

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

[0132] Amino acids may be grouped according to common side-chain properties:

(1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile;

- (2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;
- (3) acidic: Asp, Glu;
- (4) basic: His, Lys, Arg;
- (5) residues that influence chain orientation: Gly, Pro;
- (6) aromatic: Trp, Tyr, Phe.

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

- [0133] One type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody (e.g. a humanized or human antibody). Generally, the resulting variant(s) selected for further study will have modifications (e.g., improvements) in certain biological properties (e.g., increased binding activity, reduced immunogenicity) relative to the parent antibody and/or will have substantially retained certain biological properties of the parent antibody. An exemplary substitutional variant is a binding activity matured antibody, which may be conveniently generated, e.g., using phage display-based binding activity maturation techniques such as those described herein. Briefly, one or more HVR residues are mutated and the variant antibodies displayed on phage and screened for a particular biological activity (e.g. binding activity).
- [0134] Alterations (e.g., substitutions) may be made in HVRs, e.g., to improve antibody binding activity. Such alterations may be made in HVR "hotspots," i.e., residues encoded by codons that undergo mutation at high frequency during the somatic maturation process (see, e.g., Chowdhury, *Methods Mol. Biol.* 207:179-196 (2008)), and/or residues that contact antigen, with the resulting variant VH or VL being tested for binding activity. Binding activity maturation by constructing and reselecting from secondary libraries has been described, e.g., in Hoogenboom et al. in *Methods in Molecular Biology* 178:1-37 (O'Brien et al., ed., Human Press, Totowa, NJ, (2001).) In some embodiments of binding activity maturation, diversity is introduced into the variable genes chosen for maturation by any of a variety of methods (e.g., error-prone PCR, chain shuffling, or oligonucleotide-directed mutagenesis). A secondary library is then created. The library is then screened to identify any antibody variants with the desired binding activity. Another method to introduce diversity involves HVR-directed approaches, in which several HVR residues (e.g., 4-6 residues at a time) are randomized. HVR residues involved in antigen binding may be specifically identified, e.g., using alanine scanning mutagenesis or modeling. CDR-H3 and CDR-L3 in particular are often targeted.
- [0135] In certain embodiments, substitutions, insertions, or deletions may occur within one or more HVRs so long as such alterations do not substantially reduce the ability of the antibody to bind antigen. For example, conservative alterations (e.g., conservative sub-

stitutions as provided herein) that do not substantially reduce binding activity may be made in HVRs. Such alterations may, for example, be outside of antigen contacting residues in the HVRs. In certain embodiments of the variant VH and VL sequences provided above, each HVR either is unaltered, or contains no more than one, two or three amino acid substitutions.

[0136] A useful method for identification of residues or regions of an antibody that may be targeted for mutagenesis is called "alanine scanning mutagenesis" as described by Cunningham and Wells (1989) *Science*, 244:1081-1085. In this method, a residue or group of target residues (e.g., charged residues such as arg, asp, his, lys, and glu) are identified and replaced by a neutral or negatively charged amino acid (e.g., alanine or polyalanine) to determine whether the interaction of the antibody with antigen is affected. Further substitutions may be introduced at the amino acid locations demonstrating functional sensitivity to the initial substitutions. Alternatively, or additionally, a crystal structure of an antigen-antibody complex may be analyzed to identify contact points between the antibody and antigen. Such contact residues and neighboring residues may be targeted or eliminated as candidates for substitution. Variants may be screened to determine whether they contain the desired properties.

[0137] Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue. Other insertional variants of the antibody molecule include the fusion of an enzyme (e.g. for ADEPT) or a polypeptide which increases the plasma half-life of the antibody to the N- or C-terminus of the antibody.

[0138] b) Glycosylation variants

In certain embodiments, an antibody provided herein is altered to increase or decrease the extent to which the antibody is glycosylated. Addition or deletion of glycosylation sites to an antibody may be conveniently accomplished by altering the amino acid sequence such that one or more glycosylation sites is created or removed.

[0139] Where the antibody comprises an Fc region, the carbohydrate attached thereto may be altered. Native antibodies produced by mammalian cells typically comprise a branched, biantennary oligosaccharide that is generally attached by an N-linkage to Asn297 of the CH2 domain of the Fc region. See, e.g., Wright et al. *TIBTECH* 15:26-32 (1997). The oligosaccharide may include various carbohydrates, e.g., mannose, N-acetyl glucosamine (GlcNAc), galactose, and sialic acid, as well as a fucose attached to a GlcNAc in the "stem" of the biantennary oligosaccharide structure. In some embodiments, modifications of the oligosaccharide in an antibody of the invention may be made in order to create antibody variants with certain improved

properties.

- [0140] In one embodiment, antibody variants are provided having a carbohydrate structure that lacks fucose attached (directly or indirectly) to an Fc region. For example, the amount of fucose in such antibody may be from 1% to 80%, from 1% to 65%, from 5% to 65% or from 20% to 40%. The amount of fucose is determined by calculating the average amount of fucose within the sugar chain at Asn297, relative to the sum of all glycostructures attached to Asn 297 (e. g. complex, hybrid and high mannose structures) as measured by MALDI-TOF mass spectrometry, as described in WO 2008/077546, for example. Asn297 refers to the asparagine residue located at about position 297 in the Fc region (EU numbering of Fc region residues); however, Asn297 may also be located about +/- 3 amino acids upstream or downstream of position 297, i.e., between positions 294 and 300, due to minor sequence variations in antibodies. Such fucosylation variants may have improved ADCC function. See, e.g., US Patent Publication Nos. US 2003/0157108 (Presta, L.); US 2004/0093621 (Kyowa Hakko Kogyo Co., Ltd). Examples of publications related to "defucosylated" or "fucose-deficient" antibody variants include: US 2003/0157108; WO 2000/61739; WO 2001/29246; US 2003/0115614; US 2002/0164328; US 2004/0093621; US 2004/0132140; US 2004/0110704; US 2004/0110282; US 2004/0109865; WO 2003/085119; WO 2003/084570; WO 2005/035586; WO 2005/035778; WO2005/053742; WO2002/031140; Okazaki et al. J. Mol. Biol. 336:1239-1249 (2004); Yamane-Ohnuki et al. Biotech. Bioeng. 87: 614 (2004). Examples of cell lines capable of producing defucosylated antibodies include Lec13 CHO cells deficient in protein fucosylation (Ripka et al. Arch. Biochem. Biophys. 249:533-545 (1986); US Pat Appl No US 2003/0157108 A1, Presta, L; and WO 2004/056312 A1, Adams et al., especially at Example 11), and knockout cell lines, such as alpha-1,6-fucosyltransferase gene, FUT8, knockout CHO cells (see, e.g., Yamane-Ohnuki et al. Biotech. Bioeng. 87: 614 (2004); Kanda, Y. et al., Biotechnol. Bioeng., 94(4):680-688 (2006); and WO2003/085107).
- [0141] Antibodies variants are further provided with bisected oligosaccharides, e.g., in which a biantennary oligosaccharide attached to the Fc region of the antibody is bisected by GlcNAc. Such antibody variants may have reduced fucosylation and/or improved ADCC function. Examples of such antibody variants are described, e.g., in WO 2003/011878 (Jean-Mairet et al.); US Patent No. 6,602,684 (Umana et al.); and US 2005/0123546 (Umana et al.). Antibody variants with at least one galactose residue in the oligosaccharide attached to the Fc region are also provided. Such antibody variants may have improved CDC function. Such antibody variants are described, e.g., in WO 1997/30087 (Patel et al.); WO 1998/58964 (Raju, S.); and WO 1999/22764 (Raju, S.).

[0142] c) Fc region variants

In certain embodiments, one or more amino acid modifications may be introduced into the Fc region of an antibody provided herein, thereby generating an Fc region variant. The Fc region variant may comprise a human Fc region sequence (e.g., a human IgG1, IgG2, IgG3 or IgG4 Fc region) comprising an amino acid modification (e.g. a substitution) at one or more amino acid positions. In another embodiment, a human Fc variant may comprise a chimeric human Fc region sequence (e.g., a human IgG1/4 or human IgG2/4 Fc region), or a chimeric human Fc region sequence which further comprises an amino acid modification (e.g. a substitution) at one or more amino acid positions.

[0143] In certain embodiments, the invention contemplates an antibody variant that possesses some but not all effector functions, which make it a desirable candidate for applications in which the half life of the antibody in vivo is important yet certain effector functions (such as complement and ADCC) are unnecessary or deleterious. In vitro and/or in vivo cytotoxicity assays can be conducted to confirm the reduction/depletion of CDC and/or ADCC activities. For example, Fc receptor (FcR) binding assays can be conducted to ensure that the antibody lacks Fc gamma R binding (hence likely lacking ADCC activity), but retains FcRn binding ability. The primary cells for mediating ADCC, NK cells, express Fc gamma RIII only, whereas monocytes express Fc gamma RI, Fc gamma RII and Fc gamma RIII. FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, Annu. Rev. Immunol. 9:457-492 (1991). Non-limiting examples of in vitro assays to assess ADCC activity of a molecule of interest is described in U.S. Patent No. 5,500,362 (see, e.g. Hellstrom, I. et al. Proc. Nat'l Acad. Sci. USA 83:7059-7063 (1986)) and Hellstrom, I et al., Proc. Nat'l Acad. Sci. USA 82:1499-1502 (1985); 5,821,337 (see Bruggemann, M. et al., J. Exp. Med. 166:1351-1361 (1987)). Alternatively, non-radioactive assays methods may be employed (see, for example, ACT1™ non-radioactive cytotoxicity assay for flow cytometry (CellTechnology, Inc. Mountain View, CA; and CytoTox 96 (registered trademark) non-radioactive cytotoxicity assay (Promega, Madison, WI). Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed in vivo, e.g., in a animal model such as that disclosed in Clynes et al. Proc. Nat'l Acad. Sci. USA 95:652-656 (1998). C1q binding assays may also be carried out to confirm that the antibody is unable to bind C1q and hence lacks CDC activity. See, e.g., C1q and C3c binding ELISA in WO 2006/029879 and WO 2005/100402. To assess complement activation, a CDC assay may be performed (see, for example, Gazzano-Santoro et al., J. Immunol. Methods 202:163 (1996); Cragg, M.S. et al., Blood 101:1045-1052 (2003); and Cragg, M.S. and M.J.

Glennie, Blood 103:2738-2743 (2004)). FcRn binding and in vivo clearance/half life determinations can also be performed using methods known in the art (see, e.g., Petkova, S.B. et al., Int'l. Immunol. 18(12):1759-1769 (2006)).

[0144] Antibodies with reduced effector function include those with substitution of one or more of Fc region residues 238, 265, 269, 270, 297, 327 and 329 (U.S. Patent No. 6,737,056). Such Fc mutants include Fc mutants with substitutions at two or more of amino acid positions 265, 269, 270, 297 and 327, including the so-called "DANA" Fc mutant with substitution of residues 265 and 297 to alanine (US Patent No. 7,332,581).

[0145] "Reduced effector function" refers to reduction of an effector function of the Fc region of an antibody, which vary with the antibody isotype. Examples of reduced effector functions include: reduction of C1q binding; reduction of complement dependent cytotoxicity (CDC); reduction of Fc receptor binding (for example, reduction of Fc gamma receptor binding); reduction of antibody-dependent cell-mediated cytotoxicity (ADCC); reduction of phagocytosis; reduction of down regulation of cell surface receptors (e.g. B cell receptor); and reduction of B cell activation.

"Reduction" of an effector function/activity refers to an effector function/activity that is 95% or less, preferably 90% or less, 85% or less, 80% or less, 75% or less, more preferably 70% or less, 65% or less, 60% or less, 55% or less, 50% or less, 45% or less, 40% or less, 35% or less, 30% or less, 25% or less, 20% or less, 15% or less, 10% or less, 9% or less, 8% or less, 7% or less, 6% or less, 5% or less, 4% or less, 3% or less, 2% or less, 1% or less of the function/activity of an intact IgG (or an antibody comprising an intact Fc region). For example, the function/activity of the IgG (e.g., Fc gamma receptor binding activity) is preferably reduced by a factor of at least about 10-fold or more, about 50-fold or more, about 100-fold or more as compared with the function/activity (e.g., Fc gamma receptor binding activity) of the intact IgG (or an antibody comprising an intact Fc region).

[0146] Certain antibody variants with increased or decreased binding to FcRs are described. (See, e.g., U.S. Patent No. 6,737,056; WO 2004/056312, and Shields et al., J. Biol. Chem. 9(2): 6591-6604 (2001).)

[0147] In certain embodiments, an antibody variant comprises an Fc region with one or more amino acid substitutions which improve ADCC, e.g., substitutions at positions 298, 333, and/or 334 of the Fc region (EU numbering of residues).

[0148] In some embodiments, alterations are made in the Fc region that result in altered (i.e., either increased or decreased) C1q binding and/or Complement Dependent Cytotoxicity (CDC), e.g., as described in US Patent No. 6,194,551, WO 99/51642, and Idusogie et al. J. Immunol. 164: 4178-4184 (2000).

[0149] Antibodies with increased half lives and increased binding to the neonatal Fc

receptor (FcRn), which is responsible for the transfer of maternal IgGs to the fetus (Guyer et al., J. Immunol. 117:587 (1976) and Kim et al., J. Immunol. 24:249 (1994)), are described in US2005/0014934A1 (Hinton et al.). Those antibodies comprise an Fc region with one or more substitutions therein which increase binding of the Fc region to FcRn. Such Fc variants include those with substitutions at one or more of Fc region residues: 238, 256, 265, 272, 286, 303, 305, 307, 311, 312, 317, 340, 356, 360, 362, 376, 378, 380, 382, 413, 424 or 434, e.g., substitution of Fc region residue 434 (US Patent No. 7,371,826).

[0150] See also Duncan & Winter, Nature 322:738-40 (1988); U.S. Patent No. 5,648,260; U.S. Patent No. 5,624,821; and WO 94/29351 concerning other examples of Fc region variants.

[0151] d) Cysteine engineered antibody variants

In certain embodiments, it may be desirable to create cysteine engineered antibodies, e.g., "thioMAbs," in which one or more residues of an antibody are substituted with cysteine residues. In particular embodiments, the substituted residues occur at accessible sites of the antibody. By substituting those residues with cysteine, reactive thiol groups are thereby positioned at accessible sites of the antibody and may be used to conjugate the antibody to other moieties, such as drug moieties or linker-drug moieties, to create an immunoconjugate, as described further herein. In certain embodiments, any one or more of the following residues may be substituted with cysteine: V205 (Kabat numbering) of the light chain; A118 (EU numbering) of the heavy chain; and S400 (EU numbering) of the heavy chain Fc region. Cysteine engineered antibodies may be generated as described, e.g., in U.S. Patent No. 7,521,541.

[0152] e) Antibody Derivatives

In certain embodiments, an antibody provided herein may be further modified to contain additional nonproteinaceous moieties that are known in the art and readily available. The moieties suitable for derivatization of the antibody include but are not limited to water soluble polymers. Non-limiting examples of water soluble polymers include, but are not limited to, polyethylene glycol (PEG), copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1, 3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), and dextran or poly(n-vinyl pyrrolidone)polyethylene glycol, polypropylene glycol homopolymers, polypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols (e.g., glycerol), polyvinyl alcohol, and mixtures thereof. Polyethylene glycol propionaldehyde may have advantages in manufacturing due to its stability in water. The polymer may be of any molecular weight, and may be branched or unbranched. The number of polymers attached to the antibody may vary, and if more than one

polymer are attached, they can be the same or different molecules. In general, the number and/or type of polymers used for derivatization can be determined based on considerations including, but not limited to, the particular properties or functions of the antibody to be improved, whether the antibody derivative will be used in a therapy under defined conditions, etc.

[0153] In another embodiment, conjugates of an antibody and nonproteinaceous moiety that may be selectively heated by exposure to radiation are provided. In one embodiment, the nonproteinaceous moiety is a carbon nanotube (Kam et al., Proc. Natl. Acad. Sci. USA 102: 11600-11605 (2005)). The radiation may be of any wavelength, and includes, but is not limited to, wavelengths that do not harm ordinary cells, but which heat the nonproteinaceous moiety to a temperature at which cells proximal to the antibody-nonproteinaceous moiety are killed.

[0154] B. Recombinant Methods and Compositions

Antibodies may be produced using recombinant methods and compositions, e.g., as described in U.S. Patent No. 4,816,567. In one embodiment, isolated nucleic acid encoding an anti-latent TGF-beta 1 antibody described herein is provided. Such nucleic acid may encode an amino acid sequence comprising the VL and/or an amino acid sequence comprising the VH of the antibody (e.g., the light and/or heavy chains of the antibody). In a further embodiment, one or more vectors (e.g., expression vectors) comprising such nucleic acid are provided. In a further embodiment, a host cell comprising such nucleic acid is provided. In one such embodiment, a host cell comprises (e.g., has been transformed with): (1) a vector comprising a nucleic acid that encodes an amino acid sequence comprising the VL of the antibody and an amino acid sequence comprising the VH of the antibody, or (2) a first vector comprising a nucleic acid that encodes an amino acid sequence comprising the VL of the antibody and a second vector comprising a nucleic acid that encodes an amino acid sequence comprising the VH of the antibody. In one embodiment, the host cell is eukaryotic, e.g. a Chinese Hamster Ovary (CHO) cell or lymphoid cell (e.g., Y0, NS0, Sp2/0 cell). In one embodiment, a method of making an anti-latent TGF-beta 1 antibody is provided, wherein the method comprises culturing a host cell comprising a nucleic acid encoding the antibody, as provided above, under conditions suitable for expression of the antibody, and optionally recovering the antibody from the host cell (or host cell culture medium).

[0155] For recombinant production of an anti-latent TGF-beta 1 antibody, nucleic acid encoding an antibody, e.g., as described above, is isolated and inserted into one or more vectors for further cloning and/or expression in a host cell. Such nucleic acid may be readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the

heavy and light chains of the antibody).

- [0156] Suitable host cells for cloning or expression of antibody-encoding vectors include prokaryotic or eukaryotic cells described herein. For example, antibodies may be produced in bacteria, in particular when glycosylation and Fc effector function are not needed. For expression of antibody fragments and polypeptides in bacteria, see, e.g., U.S. Patent Nos. 5,648,237, 5,789,199, and 5,840,523. (See also Charlton, *Methods in Molecular Biology*, Vol. 248 (B.K.C. Lo, ed., Humana Press, Totowa, NJ, 2003), pp. 245-254, describing expression of antibody fragments in *E. coli*.) After expression, the antibody may be isolated from the bacterial cell paste in a soluble fraction and can be further purified.
- [0157] In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for antibody-encoding vectors, including fungi and yeast strains whose glycosylation pathways have been "humanized," resulting in the production of an antibody with a partially or fully human glycosylation pattern. See Gerngross, *Nat. Biotech.* 22:1409-1414 (2004), and Li et al., *Nat. Biotech.* 24:210-215 (2006).
- [0158] Suitable host cells for the expression of glycosylated antibody are also derived from multicellular organisms (invertebrates and vertebrates). Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains have been identified which may be used in conjunction with insect cells, particularly for transfection of *Spodoptera frugiperda* cells.
- [0159] Plant cell cultures can also be utilized as hosts. See, e.g., US Patent Nos. 5,959,177, 6,040,498, 6,420,548, 7,125,978, and 6,417,429 (describing PLANTIBODIES™ technology for producing antibodies in transgenic plants).
- [0160] Vertebrate cells may also be used as hosts. For example, mammalian cell lines that are adapted to grow in suspension may be useful. Other examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7); human embryonic kidney line (293 or 293 cells as described, e.g., in Graham et al., *J. Gen Virol.* 36:59 (1977)); baby hamster kidney cells (BHK); mouse sertoli cells (TM4 cells as described, e.g., in Mather, *Biol. Reprod.* 23:243-251 (1980)); monkey kidney cells (CV1); African green monkey kidney cells (VERO-76); human cervical carcinoma cells (HELA); canine kidney cells (MDCK); buffalo rat liver cells (BRL 3A); human lung cells (W138); human liver cells (Hep G2); mouse mammary tumor (MMT 060562); TRI cells, as described, e.g., in Mather et al., *Annals N.Y. Acad. Sci.* 383:44-68 (1982); MRC 5 cells; and FS4 cells. Other useful mammalian host cell lines include Chinese hamster ovary (CHO) cells, including DHFR- CHO cells (Urlaub et al., *Proc. Natl. Acad. Sci. USA* 77:4216 (1980)); and myeloma cell lines such as Y0, NS0 and Sp2/0. For a review of certain mammalian host cell lines suitable for antibody

production, see, e.g., Yazaki and Wu, *Methods in Molecular Biology*, Vol. 248 (B.K.C. Lo, ed., Humana Press, Totowa, NJ), pp. 255-268 (2003).

[0161] C. Assays

Anti-latent TGF-beta 1 antibodies provided herein may be identified, screened for, or characterized for their physical/chemical properties and/or biological activities by various assays known in the art.

[0162] 1. Binding assays and other assays

In one aspect, an antibody of the invention is tested for its antigen binding affinity/activity, e.g., by known methods such as ELISA, Western blot, surface plasmon resonance (e.g. BIACORE (registered trademark)) or a similar technique (e.g. KinExa or OCTET(registered trademark)), etc.

[0163] In another aspect, competition assays may be used to identify an antibody that competes with any anti-latent TGF-beta 1 antibodies described herein, preferably hT0947AE20-SG181, hT0947AE27-SG181, hT0947AE34-SG181, hT0947AE37-SG181, hT0947AE20-SG191, hT0947AE27-SG191, hT0947AE34-SG191, or hT0947AE37-SG191 for binding to latent TGF-beta 1. In certain embodiments, such a competing antibody binds to the same epitope (e.g., a linear or a conformational epitope) that is bound by any anti-latent TGF-beta 1 antibodies described herein, preferably hT0947AE20-SG181, hT0947AE27-SG181, hT0947AE34-SG181, hT0947AE37-SG181, hT0947AE20-SG191, hT0947AE27-SG191, hT0947AE34-SG191, or hT0947AE37-SG191. Detailed exemplary methods for mapping an epitope to which an antibody binds are provided in Morris (1996) "Epitope Mapping Protocols," in *Methods in Molecular Biology* vol. 66 (Humana Press, Totowa, NJ). Methods for mapping an epitope include but not limited to, X-ray crystallography and alanine scanning mutagenesis methods.

[0164] In certain embodiments, when such a competing antibody is present in excess, it blocks (e.g., reduces) the binding of a reference antibody to latent TGF-beta 1 by at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75% or more. In some instances, binding is inhibited by at least 80%, 85%, 90%, 95%, or more. In certain embodiments, such a competing antibody binds to the same epitope (e.g., a linear epitope or a conformational epitope) that is bound by an anti-latent TGF-beta 1 antibody described herein. In further aspects, the reference antibody is hT0947AE20-SG181, hT0947AE27-SG181, hT0947AE34-SG181, hT0947AE37-SG181, hT0947AE20-SG191, hT0947AE27-SG191, hT0947AE34-SG191, or hT0947AE37-SG191.

[0165] In an exemplary competition assay, immobilized latent TGF-beta 1 is incubated in a solution comprising a first labeled antibody (a reference antibody) that binds to latent TGF-beta 1 (e.g., hT0947AE20-SG181, hT0947AE27-SG181, hT0947AE34-SG181,

hT0947AE37-SG181, hT0947AE20-SG191, hT0947AE27-SG191, hT0947AE34-SG191, or hT0947AE37-SG191.) and a second unlabeled antibody that is being tested for its ability to compete with the first antibody for binding to latent TGF-beta 1. The second antibody may be present in a hybridoma supernatant. As a control, immobilized latent TGF-beta 1 is incubated in a solution comprising the first labeled antibody but not the second unlabeled antibody. After incubation under conditions permissive for binding of the first antibody to latent TGF-beta 1, excess unbound antibody is removed, and the amount of label associated with immobilized latent TGF-beta 1 is measured. If the amount of label associated with immobilized latent TGF-beta 1 is substantially reduced in the test sample relative to the control sample, then that indicates that the second antibody is competing with the first antibody for binding to latent TGF-beta 1. See Harlow and Lane (1988) *Antibodies: A Laboratory Manual* ch.14 (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).

[0166] In certain embodiments, binding of an anti-latent TGF-beta 1 antibody to a cell surface latent TGF-beta 1 can be tested by known methods such as ELISA, Western blot, BIACORE, Flow cytometry, etc. For example, cells expressing latent TGF-beta 1 can be brought into contact with either anti-latent TGF-beta 1 antibodies directly conjugated with PE- or APC-, or unconjugated anti-latent TGF-beta 1 antibodies followed by PE- or APC-conjugated secondary antibodies, and the staining of cell surface latent TGF-beta 1 can be detected. See, e.g., Oida et al., *PLoS One*. 2010 Nov 24;5(11):e15523; Su et al, *Hum Mol Genet*. 2015 Jul 15;24(14):4024-36.

[0167] 2. Activity assays

In one aspect, assays are provided for identifying anti-latent TGF-beta 1 antibodies thereof having biological activity. Biological activity may include, e.g., inhibiting activation of latent TGF-beta 1, inhibiting the release of mature TGF-beta 1 from latent TGF-beta 1, inhibiting protease mediated release of mature TGF-beta 1 from latent TGF-beta 1, inhibiting protease mediated release of mature TGF-beta 1 from latent TGF-beta 1 without inhibiting protease mediated cleavage of the LAP region of latent TGF-beta 1, inhibiting protease mediated release of mature TGF-beta 1 from latent TGF-beta 1 without blocking access of a protease to latent TGF-beta 1, inhibiting protease mediated release of mature TGF-beta 1 from latent TGF-beta 1 while allowing a protease to cleave the LAP region of the latent TGF-beta 1, inhibiting protease mediated release of mature TGF-beta 1 from latent TGF-beta 1 without inhibiting or with partially inhibiting integrin mediated TGF-beta 1 activation, etc. Antibodies having such biological activity in vivo and/or in vitro are also provided.

[0168] In certain embodiments, an antibody of the invention is tested for such biological activity.

[0169] In some embodiments, whether a test antibody inhibits activation of latent TGF-beta

1, i.e., inhibits the release of mature TGF-beta 1 from latent TGF-beta 1, is determined by detecting mature TGF-beta 1 using a method known in the art such as electrophoresis, chromatography, immunoblot analysis, an enzyme-linked immunosorbent assay (ELISA), or mass spectrometry, after an activator of latent TGF-beta 1 (e.g., protease, integrin, other non-protease activator, etc.) is contacted with latent TGF-beta 1 in the presence or absence of the test antibody. In one example, an activator can be isolated (e.g., isolated protease or integrin) and/or non-isolated (e.g., mouse, monkey or human PBMC comprising integrin). It is also known that the activation latent TGF-beta 1, i.e., the release of mature TGF-beta 1 from latent TGF-beta 1, also occurs in the absence of an activator (spontaneous activation of latent TGF-beta 1). In some embodiments, whether a test antibody inhibits spontaneous activation of latent TGF-beta 1 is determined by detecting mature TGF-beta 1 using the method described above, after latent TGF-beta 1 is incubated with or without the test antibody. In some embodiments, where a decreased amount of mature TGF-beta 1 is detected in the presence of (or following contact with) the test antibody as compared to the amount detected in the absence of the test antibody, the test antibody is identified as an antibody that can inhibit the activation of latent TGF-beta 1. In an example, the amount of mature TGF-beta 1, either decreased or increased, can be measured in terms of concentration of mature TGF-beta 1 (for example, g/ml, mg/ml, microgram/ml, ng/ml, or pg/ml, etc.). In another example, the amount of mature TGF-beta, either decreased or increased, can be measured in terms of optical density (O.D.) (for example, at a wavelength in mm or nm, etc.) of a label directly or indirectly associated with mature TGF-beta.

[0170] In certain embodiments, inhibition of TGF-beta 1 activation includes at least a 5%, 10%, 15%, 20%, 25%, 30%, 35%, or 40% or greater decrease in the amount of mature TGF-beta 1 in the assay as compared to a negative control under similar conditions. In some embodiments, it refers to the inhibition of TGF-beta 1 activation i.e., the inhibition of the release of mature TGF-beta 1 of at least 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% or greater.

[0171] In some embodiments, whether a test antibody inhibits activation of latent TGF-beta 1, i.e., inhibits the release of mature TGF-beta 1 from latent TGF-beta 1, is also determined by detecting mature TGF-beta 1 activity, for example, the activity of binding to TGF-beta 1 receptor, or the activity of mediating signal transduction in a cell expressing TGF-beta 1 receptor, etc. In some embodiments, binding of mature TGF-beta 1 to TGF-beta 1 receptor can be detected using a receptor binding assay. In some embodiments, the activity of mediating TGF-beta 1 signal transduction can be determined by detecting the activation of the TGF-beta 1/Smad pathway. Cells useful for such an assay can be those that express endogenous TGF-beta 1 receptor or that were generated by transfection of cells with a TGF-beta 1 receptor gene. For example, HEK-Blue™

TGF-beta cell which was used in the working examples described herein, or those that are genetically modified, transiently or stably, to express a transgene encoding TGF-beta 1 receptor can be used. TGF-beta 1 mediated signal transduction can be detected at any level in the signal transduction pathway, for example, by examining phosphorylation of Smad polypeptide, examining expression of a TGF-beta 1 regulated gene including a reporter gene, or measuring proliferation of a TGF-beta 1-dependent cell.

- [0172] In some embodiments, the activity of mediating TGF-beta 1 signal transduction can also be determined by detecting the activation of the TGF-beta 1/Smad pathway, by examining phosphorylation of Smad polypeptide (see, e.g., Fukasawa et. al., *Kidney International*. 65(1):63-74 (2004), and Ganapathy et al., *Molecular Cancer* 26;9:122 (2010)). In other embodiments, the activity of mediating TGF-beta 1 signal transduction can be determined by examining the ability of TGF-beta to inhibit cell migration in "wounded" monolayer cultures of BAE cells, examining the ability of TGF-beta to inhibit cell growth, examining the ability of TGF-beta to suppress plasminogen activator (PA) activity, examining the ability of TGF-beta to upregulate plasminogen activator inhibitor-1 (PAI-1), etc. (see Mazzieri et. al., *Methods in Molecular Biology* 142:13-27(2000))
- [0173] Inhibition of TGF-beta 1 activation can also be detected and/or measured using the methods set forth and exemplified in the working examples. Using assays of these or other suitable types, test antibodies can be screened for those capable of inhibiting the activation of TGF-beta 1. In certain embodiments, inhibition of TGF-beta 1 activation includes at least a 5%, 10%, 15%, 20%, 25%, 30%, 35%, or 40% or greater decrease in TGF-beta 1 activation in the assay as compared to a negative control under similar conditions. In some embodiments, it refers to the inhibition of TGF-beta 1 activation of at least 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% or greater. In certain embodiments, inhibition of TGF-beta 1 activation includes at least a 5%, 10%, 15%, 20%, 25%, 30%, 35%, or 40% or greater decrease in the amount of mature TGF-beta 1 detected in the assay as compared to a negative control under similar conditions. In some embodiments, it refers to the decrease in the amount of mature TGF-beta 1 of at least 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% or greater.
- [0174] In some embodiments, whether a test antibody inhibits the cleavage of the LAP portion of latent TGF-beta 1 is determined by detecting the cleavage product of latent TGF-beta 1 and/or non-cleaved latent TGF-beta 1 using various methods known in the art such as electrophoresis, chromatography, immunoblot analysis, an enzyme-linked immunosorbent assay (ELISA), or mass spectrometry, after a protease is contacted with latent TGF-beta 1 in the presence or absence of the test antibody. For example, where a protein tag (e.g., FLAG-tag, etc.) is added to N-terminal of the LAP region of latent TGF-beta 1, the portion to which the protein tag added is cut off when the

protease mediated cleavage occurs. Therefore, the cleavage product of latent TGF-beta 1 can be detected by detecting latent TGF-beta 1 (or LAP region of latent TGF-beta 1) without the protein tag, and/or the non-cleaved latent TGF-beta 1 can be detected by detecting latent TGF-beta 1 with the protein tag.

[0175] For another example, where a protein tag (e.g., FLAG-tag, etc.) is added to the N-terminal of the LAP region of latent TGF-beta 1, and where the location of cleavage site by a protease is not near the N-terminal of the LAP region of latent TGF-beta 1, the LAP region with the protein tag becomes shortened when the protease mediated cleavage occurs. Therefore, the cleavage product of latent TGF-beta 1 can be detected by detecting latent TGF-beta 1 having a shortened LAP region (or shortened LAP region of latent TGF-beta 1) with the protein tag.

[0176] In some embodiments, where a decreased amount of the cleavage product of latent TGF-beta 1 is detected in the presence of (or following contact with) the test antibody as compared to the amount detected in the absence of the test antibody, the test antibody is identified as an antibody that can inhibit the cleavage of latent TGF-beta 1. Conversely, where the amount of the cleavage product of latent TGF-beta 1 is not significantly decreased in the presence of (or following contact with) the test antibody as compared to the amount detected in the absence of the test antibody, the test antibody is identified as an antibody that does not inhibit the cleavage of latent TGF-beta 1. In some embodiments, where an increased amount of the non-cleaved latent TGF-beta 1 is detected in the presence of (or following contact with) the test antibody as compared to the amount detected in the absence of the test antibody, the test antibody is identified as an antibody that can inhibit the cleavage of latent TGF-beta 1. Conversely, where an amount of non-cleaved latent TGF-beta 1 is not significantly increased in the presence of (or following contact with) the test antibody as compared to the amount detected in the absence of the test antibody, the test antibody is identified as an antibody that does not inhibit the cleavage of latent TGF-beta 1. In certain embodiments, whether a test antibody blocks access of a protease to latent TGF-beta 1 is determined by methods for the detection of protein interactions between the protease and latent TGF-beta 1, e.g., ELISA or surface plasmon resonance (e.g. BIACORE(registered trademark)) or a similar technique (e.g., KinExa or OCTET(registered trademark)). Where a decreased interaction between the protease and latent TGF-beta 1 is detected in the presence of (or following contact with) the test antibody as compared to the interaction detected in the absence of the test antibody, the test antibody is identified as an antibody that can block access of the protease to latent TGF-beta 1.

[0177] In certain embodiments, non-inhibition of the cleavage of latent TGF-beta 1 includes at least a 5%, 10%, 15%, 20%, 25%, 30%, 35%, or 40% or greater increase in the

amount of the cleavage product of latent TGF-beta 1 in the assay as compared to a negative control under similar conditions. In some embodiments, non-inhibition of the cleavage of latent TGF-beta 1 includes at least 50% or less, 45% or less, 40% or less, 35% or less, 30% or less, 25% or less, 20% or less, 15% or less, 10% or less, 5% or less increase in the amount of the non-cleaved latent TGF-beta 1 in the assay as compared to a negative control under similar conditions.

[0178] In some embodiments, an anti-latent TGF-beta 1 antibody may be subjected to other biological activity assays, e.g., in order to evaluate its effectiveness as a therapeutic. Such assays are known in the art and depend on the target antigen and intended use for the antibody. For example, the biological effects of TGF-beta 1 blockade by the anti-latent TGF-beta 1 antibody can be assessed in a Unilateral Ureteral Obstruction (UUO) induced mouse renal fibrosis model (e.g., as described in Chevalier RL et al., Ureteral obstruction as a model of renal interstitial fibrosis and obstructive nephropathy. *Kidney Int.* 2009 Jun;75(11):1145-1152.), and/or a syngeneic tumor model (e.g., as described in Mariathasan S et al., TGF-beta attenuates tumour response to PD-L1 blockade by contributing to exclusion of T cells. *Nature.* 2018 Feb 22;554(7693):544-548.). In further embodiments, the anti-latent TGF-beta 1 antibody may be subject to biological activity assays described herein.

[0179] 3. Methods for screening

In one aspect, a method for screening an antibody of the invention comprises various assays described herein and known in the art. For example, a method for screening an anti-latent TGF-beta 1 antibody comprises:

- (a) contacting a biological sample comprising latent TGF-beta 1 and a protease with a test antibody;
- (b) detecting (i) whether a test antibody inhibits the cleavage of the LAP region of latent TGF-beta 1 and (ii) whether a test antibody inhibits activation of latent TGF-beta 1; and
- (c) selecting the test antibody that inhibits activation of latent TGF-beta 1 without inhibiting protease mediated cleavage of the LAP portion of latent TGF-beta 1.

[0180] Alternatively, rather than steps (b) and (c) above, the method for screening an anti-latent TGF-beta 1 antibody comprises, e.g., steps (b) and (c) below:

- (b) measuring (i) the amount of non-cleaved latent TGF-beta 1 and (ii) the amount of mature TGF-beta 1; and
- (c) selecting the test antibody that inhibits a protease mediated release of mature TGF-beta 1 from latent TGF-beta 1 without inhibiting a protease mediated cleavage of the LAP region of latent TGF-beta 1, if the amount of non-cleaved latent TGF-beta 1 is not significantly increased and the amount of mature-TGF-beta 1 is decreased as compared to when the test antibody is absent.

Alternatively, rather than steps (b) and (c) above, the method for screening an anti-latent TGF-beta 1 antibody comprises, e.g., steps (b) and (c) below:

(b) measuring (i) the amount of cleavage product of latent TGF-beta 1 and (ii) the level of mature TGF-beta 1 activity; and

(c) selecting the test antibody that inhibits a protease mediated activation of latent TGF-beta 1 without inhibiting a protease mediated cleavage of the LAP region of latent TGF-beta 1, if the amount of cleavage product is not significantly decreased and the level of mature-TGF-beta 1 activity is decreased as compared to when the test antibody is absent.

Furthermore, the present invention provides a method for producing an anti-latent TGF-beta 1 antibody, which comprises, e.g., steps (d) and (e) below in addition to steps (a) to (c) above:

(d) obtaining amino acid sequence information of the anti-latent TGF-beta 1 antibody selected in step (c); and

(e) introducing a gene encoding the anti-latent TGF-beta 1 antibody into a host cell.

In one embodiment, the method for producing an anti-latent TGF-beta 1 antibody further comprises, after (e) above: (f) culturing the host cell so that the anti-latent TGF-beta 1 antibody is produced.

[0181] In this context, the term "not significantly increased/decreased", e.g., in the phrases "the amount of non-cleaved latent TGF-beta 1 is not significantly increased" and "the amount of cleavage product (of latent TGF-beta 1) is not significantly decreased" means that the level/degree of the increase/decrease may be zero, or may not be zero but near zero, or may be very low enough to be able to be technically neglected or realistically/substantially considered to be zero by those skilled in the art. For example, in an immunoblotting analysis, when a researcher cannot detect or observe any significant signal/band (or a relatively high or strong signal) for non-cleaved latent TGF-beta 1, it is considered that the amount of non-cleaved latent TGF-beta 1 is "not significantly increased", or the amount of cleavage product (of latent TGF-beta 1) is "not significantly decreased". In addition, the term "not significantly increased/decreased" is interchangeably used with the term "not substantially increased/decreased".

[0182] In some embodiments, whether a test antibody inhibits the cleavage of the LAP region of latent TGF-beta 1, and whether a test antibody inhibits activation of latent TGF-beta 1 can be determined by various assays described herein and known in the art.

[0183] D. Immunoconjugates

The invention also provides immunoconjugates comprising an anti-latent TGF-beta 1 antibody herein conjugated to one or more cytotoxic agents, such as chemotherapeutic agents or drugs, growth inhibitory agents, toxins (e.g., protein toxins, enzymatically

active toxins of bacterial, fungal, plant, or animal origin, or fragments thereof), or radioactive isotopes.

- [0184] In one embodiment, an immunoconjugate is an antibody-drug conjugate (ADC) in which an antibody is conjugated to one or more drugs, including but not limited to a maytansinoid (see U.S. Patent Nos. 5,208,020, 5,416,064 and European Patent EP 0 425 235 B1); an auristatin such as monomethylauristatin drug moieties DE and DF (MMAE and MMAF) (see U.S. Patent Nos. 5,635,483 and 5,780,588, and 7,498,298); a dolastatin; a calicheamicin or derivative thereof (see U.S. Patent Nos. 5,712,374, 5,714,586, 5,739,116, 5,767,285, 5,770,701, 5,770,710, 5,773,001, and 5,877,296; Hinman et al., *Cancer Res.* 53:3336-3342 (1993); and Lode et al., *Cancer Res.* 58:2925-2928 (1998)); an anthracycline such as daunomycin or doxorubicin (see Kratz et al., *Current Med. Chem.* 13:477-523 (2006); Jeffrey et al., *Bioorganic & Med. Chem. Letters* 16:358-362 (2006); Torgov et al., *Bioconj. Chem.* 16:717-721 (2005); Nagy et al., *Proc. Natl. Acad. Sci. USA* 97:829-834 (2000); Dubowchik et al., *Bioorg. & Med. Chem. Letters* 12:1529-1532 (2002); King et al., *J. Med. Chem.* 45:4336-4343 (2002); and U.S. Patent No. 6,630,579); methotrexate; vindesine; a taxane such as docetaxel, paclitaxel, larotaxel, tesetaxel, and ortataxel; a trichothecene; and CC1065.
- [0185] In another embodiment, an immunoconjugate comprises an antibody as described herein conjugated to an enzymatically active toxin or fragment thereof, including but not limited to diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, Aleurites fordii proteins, dianthin proteins, *Phytolacca americana* proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcumin, crotin, saponaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and the tricothecenes.
- [0186] In another embodiment, an immunoconjugate comprises an antibody as described herein conjugated to a radioactive atom to form a radioconjugate. A variety of radioactive isotopes are available for the production of radioconjugates. Examples include ^{211}At , ^{131}I , ^{125}I , ^{90}Y , ^{186}Re , ^{188}Re , ^{153}Sm , ^{212}Bi , ^{32}P , ^{212}Pb and radioactive isotopes of Lu. When the radioconjugate is used for detection, it may comprise a radioactive atom for scintigraphic studies, for example Tc-99m or ^{123}I , or a spin label for nuclear magnetic resonance (NMR) imaging (also known as magnetic resonance imaging, MRI), such as iodine-123 again, iodine-131, indium-111, fluorine-19, carbon-13, nitrogen-15, oxygen-17, gadolinium, manganese or iron.
- [0187] Conjugates of an antibody and cytotoxic agent may be made using a variety of bi-functional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP), succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl

adipimidate HCl), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta et al., Science 238:1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionuclide to the antibody. See WO94/11026. The linker may be a "cleavable linker" facilitating release of a cytotoxic drug in the cell. For example, an acid-labile linker, peptidase-sensitive linker, photolabile linker, dimethyl linker or disulfide-containing linker (Chari et al., Cancer Res. 52:127-131 (1992); U.S. Patent No. 5,208,020) may be used.

[0188] The immunoconjugates or ADCs herein expressly contemplate, but are not limited to such conjugates prepared with cross-linker reagents including, but not limited to, BMPS, EMCS, GMBS, HBVS, LC-SMCC, MBS, MPBH, SBAP, SIA, SIAB, SMCC, SMPB, SMPH, sulfo-EMCS, sulfo-GMBS, sulfo-KMUS, sulfo-MBS, sulfo-SIAB, sulfo-SMCC, and sulfo-SMPB, and SVSB (succinimidyl-(4-vinylsulfone)benzoate) which are commercially available (e.g., from Pierce Biotechnology, Inc., Rockford, IL., U.S.A).

[0189] E. Methods and Compositions for Diagnostics and Detection

In certain embodiments, any of the anti-latent TGF-beta 1 antibodies provided herein is useful for detecting the presence of TGF-beta 1, e.g., latent TGF-beta 1 in a biological sample. The term "detecting" as used herein encompasses quantitative or qualitative detection/measurement. In certain embodiments, a biological sample comprises a cell or tissue, such as serum, whole blood, plasma, biopsy sample, tissue sample, cell suspension, saliva, sputum, oral fluid, cerebrospinal fluid, amniotic fluid, ascites fluid, milk, colostrums, mammary gland secretion, lymph, urine, sweat, lacrimal fluid, gastric fluid, synovial fluid, peritoneal fluid, ocular lens fluid and mucus.

[0190] In one embodiment, an anti-latent TGF-beta 1 antibody for use in a method of diagnosis or detection is provided. In a further aspect, a method of detecting the presence of TGF-beta 1, e.g., latent TGF-beta 1 in a biological sample is provided. For example, the method of detecting the presence of latent TGF-beta 1 comprises:

- (a) contacting a biological sample with an anti-latent TGF-beta 1 antibody of the present invention described herein under conditions permissive for binding of the anti-latent TGF-beta 1 antibody to latent TGF-beta 1; and
- (b) detecting whether a complex is formed between the anti-latent TGF-beta 1 antibody and latent TGF-beta 1.

- [0191] Such a method may be an in vitro or in vivo method. In one embodiment, an anti-latent TGF-beta 1 antibody is used to select subjects eligible for therapy with an anti-latent TGF-beta 1 antibody, e.g., where TGF-beta 1, e.g., latent TGF-beta 1 is a biomarker for selection of patients. That is, the anti-latent TGF-beta 1 antibody is useful as a diagnostic agent in targeting TGF-beta 1.
- [0192] More specifically, the anti-latent TGF-beta 1 antibody is useful for the diagnosis of fibrosis, preferably myocardial fibrosis, renal fibrosis, ocular fibrosis, myelofibrosis, liver fibrosis, and lung/pulmonary fibrosis. Anti-latent TGF-beta 1 antibodies of the invention are also useful for the diagnosis of cancer.
- [0193] In some embodiments, the present invention provides a method of inhibiting release of mature TGF-beta 1 from latent TGF-beta 1 without inhibiting a cleavage of the LAP region of latent TGF-beta 1 mediated by protease in a biological sample, comprising contacting the biological sample containing latent TGF-beta 1 with the anti-latent TGF-beta 1 antibody of the present invention under conditions permissive for binding of the antibody to latent TGF-beta 1.
- [0194] In certain embodiments, e.g., for the detection/diagnosis purposes, labeled anti-latent TGF-beta 1 antibodies are provided. Labels include, but are not limited to, labels or moieties that are detected directly (such as fluorescent, chromophoric, electron-dense, chemiluminescent, and radioactive labels), as well as moieties, such as enzymes or ligands, that are detected indirectly, e.g., through an enzymatic reaction or molecular interaction. Exemplary labels include, but are not limited to, the radioisotopes ^{32}P , ^{14}C , ^{125}I , ^3H , and ^{131}I , fluorophores such as rare earth chelates or fluorescein and its derivatives, rhodamine and its derivatives, dansyl, umbelliferone, luciferases, e.g., firefly luciferase and bacterial luciferase (US Patent No. 4,737,456), luciferin, 2,3-dihydrophthalazinediones, horseradish peroxidase (HRP), alkaline phosphatase, beta-galactosidase, glucoamylase, lysozyme, saccharide oxidases, e.g., glucose oxidase, galactose oxidase, and glucose-6-phosphate dehydrogenase, heterocyclic oxidases such as uricase and xanthine oxidase, coupled with an enzyme that employs hydrogen peroxide to oxidize a dye precursor such as HRP, lactoperoxidase, or microperoxidase, biotin/avidin, spin labels, bacteriophage labels, stable free radicals, and the like.
- [0195] F. Pharmaceutical Formulations
Pharmaceutical formulations of an anti-latent TGF-beta 1 antibody as described herein are prepared by mixing such an antibody having the desired degree of purity with one or more optional pharmaceutically acceptable carriers (Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions. Pharmaceutically acceptable carriers are generally nontoxic to recipients at the dosages and concentrations employed, and include, but are

not limited to: buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride; benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants such as polyethylene glycol (PEG). Exemplary pharmaceutically acceptable carriers herein further include interstitial drug dispersion agents such as soluble neutral-active hyaluronidase glycoproteins (sHASEGP), for example, human soluble PH-20 hyaluronidase glycoproteins, such as rHuPH20 (HYLENEX (registered trademark), Baxter International, Inc.). Certain exemplary sHASEGPs and methods of use, including rHuPH20, are described in US Patent Publication Nos. 2005/0260186 and 2006/0104968. In one aspect, a sHASEGP is combined with one or more additional glycosaminoglycanases such as chondroitinases.

- [0196] Exemplary lyophilized antibody formulations are described in US Patent No. 6,267,958. Aqueous antibody formulations include those described in US Patent No. 6,171,586 and WO2006/044908, the latter formulations including a histidine-acetate buffer.
- [0197] In one aspect, the invention provides pharmaceutical formulations comprising an anti-latent TGF-beta 1 antibody for treatment of fibrosis, preferably myocardial fibrosis, renal fibrosis, ocular fibrosis, myelofibrosis, liver fibrosis, and lung/pulmonary fibrosis. The invention also provides pharmaceutical formulations comprising an anti-latent TGF-beta 1 antibody for treatment of cancer.
- [0198] The formulation herein may also contain more than one active ingredients as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. For example, it may be desirable to further provide immune checkpoint inhibitors.
- [0199] Active ingredients may be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions.

Such techniques are disclosed in Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980).

[0200] Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, e.g. films, or microcapsules.

[0201] The formulations to be used for in vivo administration are generally sterile. Sterility may be readily accomplished, e.g., by filtration through sterile filtration membranes.

[0202] G. Therapeutic Methods and Compositions

Any of the anti-latent TGF-beta 1 antibodies provided herein may be used in therapeutic methods. In one aspect, an anti-latent TGF-beta 1 antibody for use as a medicament is provided. In further aspects, an anti-latent TGF-beta 1 antibody for use in treating cancer or fibrosis (such as renal fibrosis, liver fibrosis, and lung/pulmonary fibrosis), etc., is provided. In further aspects, an anti-latent TGF-beta 1 antibody for use in treating fibrosis is provided. In further aspects, an anti-latent TGF-beta 1 antibody for use in treating renal fibrosis is provided. In further aspects, an anti-latent TGF-beta 1 antibody for use in treating liver fibrosis is provided. In further aspects, an anti-latent TGF-beta 1 antibody for use in treating lung/pulmonary fibrosis is provided. In certain embodiments, an anti-latent TGF-beta 1 antibody for use in a method of treatment is provided. In certain embodiments, the invention provides an anti-latent TGF-beta 1 antibody for use in a method of treating an individual having cancer or fibrosis (such as renal fibrosis, liver fibrosis, and lung/pulmonary fibrosis), etc., comprising administering to the individual an effective amount of the anti-latent TGF-beta 1 antibody. In certain embodiments, the invention provides an anti-latent TGF-beta 1 antibody for use in a method of treating an individual having fibrosis, comprising administering to the individual an effective amount of the anti-latent TGF-beta 1 antibody. In certain embodiments, the invention provides an anti-latent TGF-beta 1 antibody for use in a method of treating an individual having renal fibrosis, comprising administering to the individual an effective amount of the anti-latent TGF-beta 1 antibody. In certain embodiments, the invention provides an anti-latent TGF-beta 1 antibody for use in a method of treating an individual having liver fibrosis, comprising administering to the individual an effective amount of the anti-latent TGF-beta 1 antibody. In certain embodiments, the invention provides an anti-latent TGF-beta 1 antibody for use in a method of treating an individual having lung/pulmonary fibrosis, comprising administering to the individual an effective amount of the anti-latent TGF-beta 1 antibody. In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, e.g., as described below. In further embodiments, the invention provides an anti-

latent TGF-beta 1 antibody for use in inhibiting protease mediated activation of latent TGF-beta 1. In certain embodiments, the invention provides an anti-latent TGF-beta 1 antibody for use in a method of inhibiting protease mediated activation of latent TGF-beta 1 in an individual comprising administering to the individual an effective of the anti-latent TGF-beta 1 antibody to inhibit protease mediated activation of latent TGF-beta 1. An "individual" according to any of the above embodiments is preferably a human.

[0203] In a further aspect, the invention provides for the use of an anti-latent TGF-beta 1 antibody in the manufacture or preparation of a medicament. In one embodiment, the medicament is for treatment of cancer or fibrosis (such as renal fibrosis, liver fibrosis, and lung/pulmonary fibrosis), etc. In one embodiment, the medicament is for treatment of fibrosis. In one embodiment, the medicament is for treatment of renal fibrosis. In one embodiment, the medicament is for treatment of liver fibrosis. In one embodiment, the medicament is for treatment of lung/pulmonary fibrosis. In a further embodiment, the medicament is for use in a method of treating cancer or fibrosis (such as renal fibrosis, liver fibrosis, and lung/pulmonary fibrosis), etc., comprising administering to an individual having cancer or fibrosis (such as renal fibrosis, liver fibrosis, and lung/pulmonary fibrosis), etc., an effective amount of the medicament. In a further embodiment, the medicament is for use in a method of treating fibrosis, comprising administering to an individual having fibrosis, an effective amount of the medicament. In a further embodiment, the medicament is for use in a method of treating renal fibrosis, comprising administering to an individual having renal fibrosis, an effective amount of the medicament. In a further embodiment, the medicament is for use in a method of treating liver fibrosis, comprising administering to an individual having liver fibrosis, an effective amount of the medicament. In a further embodiment, the medicament is for use in a method of treating lung/pulmonary fibrosis, comprising administering to an individual having lung/pulmonary fibrosis, an effective amount of the medicament. In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, e.g., as described below. In a further embodiment, the medicament is for inhibiting protease mediated activation of latent TGF-beta 1. In a further embodiment, the medicament is for use in a method of inhibiting protease mediated activation of latent TGF-beta 1 in an individual comprising administering to the individual an amount effective of the medicament to inhibit protease mediated activation of latent TGF-beta 1. An "individual" according to any of the above embodiments may be a human.

[0204] In a further aspect, the invention provides a method for treating a cancer or fibrosis (such as renal fibrosis, liver fibrosis, and lung/pulmonary fibrosis), etc. In a further aspect, the invention provides a method for treating fibrosis. In a further aspect, the

invention provides a method for treating renal fibrosis. In a further aspect, the invention provides a method for treating liver fibrosis. In a further aspect, the invention provides a method for treating lung/pulmonary fibrosis. In one embodiment, the method comprises administering to an individual having such cancer or fibrosis (such as renal fibrosis, liver fibrosis, and lung/pulmonary fibrosis), etc., an effective amount of an anti-latent TGF-beta 1 antibody. In one embodiment, the method comprises administering to an individual having such fibrosis, an effective amount of an anti-latent TGF-beta 1 antibody. In one embodiment, the method comprises administering to an individual having such renal fibrosis, an effective amount of an anti-latent TGF-beta 1 antibody. In one embodiment, the method comprises administering to an individual having such liver fibrosis, an effective amount of an anti-latent TGF-beta 1 antibody. In one embodiment, the method comprises administering to an individual having such lung/pulmonary fibrosis, an effective amount of an anti-latent TGF-beta 1 antibody. In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, as described below. In some embodiments, the administration of the antibody and the agent is concomitant. An "individual" according to any of the above embodiments may be a human.

[0205] In a further aspect, the invention provides a method for inhibiting protease mediated activation of latent TGF-beta 1 in an individual. In one embodiment, the method comprises administering to the individual an effective amount of an anti-latent TGF-beta 1 antibody to inhibit protease mediated activation of latent TGF-beta 1. In one embodiment, an "individual" is a human.

[0206] In a further aspect, the invention provides pharmaceutical formulations comprising any of the anti-latent TGF-beta 1 antibodies provided herein, e.g., for use in any of the above therapeutic methods. In one embodiment, a pharmaceutical formulation comprises any of the anti-latent TGF-beta 1 antibodies provided herein and a pharmaceutically acceptable carrier. In another embodiment, a pharmaceutical formulation comprises any of the anti-latent TGF-beta 1 antibodies provided herein and at least one additional therapeutic agent, e.g., as described below.

[0207] An antibody of the invention (and any additional therapeutic agent) can be administered by any suitable means, including parenteral, intrapulmonary, and intranasal, and, if desired for local treatment, intralesional administration. Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous administration. Dosing can be by any suitable route, e.g. by injections, such as intravenous or subcutaneous injections, depending in part on whether the administration is brief or chronic. Various dosing schedules including but not limited to single or multiple administrations over various time-points, bolus administration, and pulse infusion are contemplated herein.

- [0208] Antibodies of the invention would be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disorder being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the agent, the method of administration, the scheduling of administration, and other factors known to medical practitioners. The antibody need not be, but is optionally formulated with one or more agents currently used to prevent or treat the disorder in question. The effective amount of such other agents depends on the amount of antibody present in the formulation, the type of disorder or treatment, and other factors discussed above.
- [0209] For the prevention or treatment of disease, the appropriate dosage of an antibody of the invention (when used alone or in combination with one or more other additional therapeutic agents) will depend on the type of disease to be treated, the type of antibody, the severity and course of the disease, whether the antibody is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the antibody, and the discretion of the attending physician. The antibody is suitably administered to the patient at one time or over a series of treatments.
- [0210] A formulation (e.g., pharmaceutical formulation) containing an antibody of the present disclosure may be administered by parenteral administration routes, e.g., injection such as subcutaneous injection, intravenous injection, intramuscular injection, etc., transdermal, transmucosal, nasal, and pulmonary routes. In some embodiments, the formulation is administered by subcutaneous injection. In some embodiments, the formulation is for use in subcutaneous administration, e.g., subcutaneous injection.
- [0211] In some embodiments, the formulation of the present disclosure has a viscosity of less than 70 mPa-s (millipascal second), less than 60 mPa-s, less than 50 mPa-s, less than 40 mPa-s, less than 30 mPa-s, less than 20 mPa-s, less than 15 mPa-s. In some embodiments, the viscosity is 3 mPa-s to 8 mPa-s. In some embodiments, the viscosity is 3 mPa-s to 7 mPa-s. In some embodiments, the viscosity is 3 mPa-s to 6 mPa-s. In some embodiments, the viscosity is 3 mPa-s to 5 mPa-s. In some embodiments, the viscosity is 3 mPa-s to 4 mPa-s. In some embodiments, the viscosity is 3 mPa-s to 3.5 mPa-s. The viscosity of the formulation may be measured by a rotational viscometer method using a cone-plate type viscometer, e.g., according to the Japanese Pharmacopoeia, General Tests, 2.53 Viscosity Determination.
- [0212] In some embodiments, the concentration of an antibody in the formulation is 50 to 350 mg/ml. In some embodiments, the concentration of an antibody in the formulation is 100 to 300 mg/ml. In some embodiments, the concentration of an antibody in the formulation is 100 to 250 mg/ml. In some embodiments, the concentration of an

antibody in the formulation is 100 to 200 mg/ml.

[0213] It is understood that any of the articles of manufacture described herein may include an immunoconjugate of the invention in place of or in addition to an anti-latent TGF-beta 1 antibody.

[0214] IV. ARTICLES OF MANUFACTURE. KITS

A. Articles of Manufacture

In another aspect of the invention, an article of manufacture containing materials useful for the treatment, prevention and/or diagnosis of any of the disorders described above (e.g., fibrosis, such as renal fibrosis, liver fibrosis, and lung/pulmonary fibrosis, or cancer) is provided. The article of manufacture comprises a container and a label on or a package insert associated with the container. Suitable containers include, for example, bottles, vials, syringes, IV solution bags, etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is by itself or combined with another composition effective for treating, preventing and/or diagnosing the condition (e.g., fibrosis, such as renal fibrosis, liver fibrosis, and lung/pulmonary fibrosis, or cancer) and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active ingredient in the composition is an antibody or immunoconjugate of the invention. The label or package insert indicates that the composition is used for treating the condition of choice (e.g., fibrosis, such as renal fibrosis, liver fibrosis, and lung/pulmonary fibrosis, or cancer). Moreover, the article of manufacture may comprise (a) a first container with a composition contained therein, wherein the composition comprises an antibody/immunoconjugate of the invention; and (b) a second container with a composition contained therein, wherein the composition comprises a further cytotoxic or otherwise therapeutic agent. The article of manufacture in this embodiment of the invention may further comprise a package insert indicating that the compositions can be used to treat a particular condition (e.g., fibrosis, such as renal fibrosis, liver fibrosis, and lung/pulmonary fibrosis, or cancer). Alternatively, or additionally, the article of manufacture may further comprise a second (or third) container comprising a pharmaceutically-acceptable buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

[0215] It is understood that any of the above articles of manufacture may include an immunoconjugate of the invention in place of or in addition to an anti-latent TGF-beta 1 antibody.

[0216] B. Kits

The present disclosure provides kits for use in a method for the treatment, prevention and/or diagnosis of the disorders described herein, in particular, treatment of an individual having fibrosis (such as renal fibrosis, liver fibrosis, and lung/pulmonary fibrosis) or cancer, which contain an anti-latent TGF-beta 1 antibody, an immunoconjugate comprising the anti-latent TGF-beta 1 antibody, an isolated nucleic acid encoding the anti-latent TGF-beta 1 antibody, or a vector comprising the nucleic acid of the present disclosure, or produced by a method of the present disclosure. The kits may additionally contain any therapeutic agents, such as an immune checkpoint inhibitor including an anti-PD-L1 antibody. The kits may be packaged with an additional pharmaceutically acceptable carrier or medium disclosed herein, or instruction manual describing how to use the kits, etc. As with the article of manufacture described herein, the kit may contain materials useful for the treatment of fibrosis (such as renal fibrosis, liver fibrosis, and lung/pulmonary fibrosis) or cancer; a container and a label on or a package insert associated with the container; a composition which is by itself or combined with another composition effective for treating fibrosis (such as renal fibrosis, liver fibrosis, and lung/pulmonary fibrosis) or cancer; a sterile access port, etc. The kits may further comprise a label or package insert indicating that the compositions can be used to treat fibrosis (such as renal fibrosis, liver fibrosis, and lung/pulmonary fibrosis) or cancer. Alternatively, or additionally, the kits may further comprise a second (or third) container comprising a pharmaceutically-acceptable buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution. The kits may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

[0217] < pH-dependent binding of anti-latent TGF-beta 1 antibodies >

Herein, "acidic pH" refers to pH that may be selected, for example, from pH 4.0 to pH 6.5. In one embodiment, acidic pH refers to, but is not limited to, pH 4.0, pH 4.1, pH 4.2, pH 4.3, pH 4.4, pH 4.5, pH 4.6, pH 4.7, pH 4.8, pH 4.9, pH 5.0, pH 5.1, pH 5.2, pH 5.3, pH 5.4, pH 5.5, pH 5.6, pH 5.7, pH 5.8, pH 5.9, pH 6.0, pH 6.1, pH 6.2, pH 6.3, pH 6.4, or pH 6.5. In a specific embodiment, the term acidic pH refers to the pH 5.8.

[0218] Herein, "neutral pH" refers to pH selected, for example, from 6.7 to pH 10.0. In one embodiment, neutral pH refers to, but is not limited to, pH 6.7, pH 6.8, pH 6.9, pH 7.0, pH 7.1, pH 7.2, pH 7.3, pH 7.4, pH 7.5, pH 7.6, pH 7.7, pH 7.8, pH 7.9, pH 8.0, pH 8.1, pH 8.2, pH 8.3, pH 8.4, pH 8.5, pH 8.6, pH 8.7, pH 8.8, pH 8.9, pH 9.0, pH 9.5, or pH 10.0. In a specific embodiment, the term neutral pH refers to the pH 7.4.

[0219] In alternative aspect, an anti-latent TGF-beta 1 antibody of the present disclosure is an anti-latent TGF-beta 1 antibody that binds to latent TGF-beta 1 in a pH-dependent

manner. In the context of the present disclosure, an anti-latent TGF-beta 1 antibody that binds to latent TGF-beta 1 in a pH-dependent manner refers to an antibody whose binding affinity for latent TGF-beta 1 at an acidic pH has been reduced as compared to the binding affinity for latent TGF-beta 1 at a neutral pH. For example, pH-dependent anti-latent TGF-beta 1 antibodies include antibodies that have a higher affinity for latent TGF-beta 1 at a neutral pH than at an acidic pH. In one embodiment, an anti-latent TGF-beta 1 antibody of the present disclosure has an affinity for latent TGF-beta 1 at a neutral pH that is at least 2 times, 3 times, 5 times, 10 times, 15 times, 20 times, 25 times, 30 times, 35 times, 40 times, 45 times, 50 times, 55 times, 60 times, 65 times, 70 times, 75 times, 80 times, 85 times, 90 times, 95 times, 100 times, 200 times, 400 times, 1000 times, 10000 times or more greater than the affinity at an acidic pH.

In one embodiment, an anti-latent TGF-beta 1 antibody that binds to latent TGF-beta 1 in a pH-dependent manner refers to an antibody whose binding affinity for latent TGF-beta 1 at pH 5.8 has been reduced as compared to the binding affinity for latent TGF-beta 1 at pH 7.4. For example, pH-dependent anti-latent TGF-beta 1 antibodies include antibodies that have a higher affinity for latent TGF-beta 1 at pH 7.4 than at pH 5.8. In one embodiment, an anti-latent TGF-beta 1 antibody of the present disclosure has an affinity for latent TGF-beta 1 at pH 7.4 that is at least 2 times, 3 times, 5 times, 10 times, 15 times, 20 times, 25 times, 30 times, 35 times, 40 times, 45 times, 50 times, 55 times, 60 times, 65 times, 70 times, 75 times, 80 times, 85 times, 90 times, 95 times, 100 times, 200 times, 400 times, 1000 times, 10000 times or more greater than the affinity at pH 5.8.

The binding affinity can be measured using, without particular limitations, surface plasmon resonance methods (such as BIACORE (registered trademark)). When using BIACORE (registered trademark) measurement, in some embodiments, the following buffer and temperature conditions are preferably used: 20 mM ACES, 150 mM NaCl, 0.05% Tween-20, and 0.005% NaN₃. The association rate constant (kon) and dissociation rate constant (koff) can be calculated using the BIACORE (registered trademark) T200 Evaluation Software (GE Healthcare) based on a simple one-to-one Langmuir binding model by fitting the association and dissociation sensorgrams simultaneously. The equilibrium dissociation constant (KD) is calculated as a ratio of koff/kon. To screen for antibodies whose binding affinity varies depending on pH, without particular limitations, ELISA, kinetic exclusion assay (KinExA™), and others as well as surface plasmon resonance methods (such as BIACORE (registered trademark)) can be used. The pH-dependent latent TGF-beta 1-binding ability refers to the property to bind to latent TGF-beta 1 in a pH-dependent manner. Meanwhile, whether an antibody is capable of binding to latent TGF-beta 1 multiple times can be assessed by the methods described in, e.g., WO2009/125825.

- [0220] In one embodiment, it is preferable that an anti-latent TGF-beta 1 antibody of the present disclosure has a small dissociation constant (KD) for latent TGF-beta 1 at a neutral pH. In one embodiment, the dissociation constant of an antibody of the present disclosure for latent TGF-beta 1 at a neutral pH is, for example, 0.3 nM or less, but is not limited thereto. In one embodiment, the dissociation constant of an antibody of the present disclosure for latent TGF-beta 1 at a neutral pH is, for example, 0.1 nM or less, but is not limited thereto. In one embodiment, the dissociation constant of an antibody of the present disclosure for latent TGF-beta 1 at a neutral pH is, for example, 0.03 nM or less, but is not limited thereto.
- [0221] In one embodiment, it is preferable that an anti-latent TGF-beta 1 antibody of the present disclosure has a small dissociation constant (KD) for latent TGF-beta 1 at pH 7.4. In one embodiment, the dissociation constant of an antibody of the present disclosure for latent TGF-beta 1 at pH 7.4 is, for example, 0.3 nM or less, but is not limited thereto. In one embodiment, the dissociation constant of an antibody of the present disclosure for latent TGF-beta 1 at pH 7.4 is, for example, 0.1 nM or less, but is not limited thereto. In one embodiment, the dissociation constant of an antibody of the present disclosure for latent TGF-beta 1 at pH 7.4 is, for example, 0.03 nM or less, but is not limited thereto.
- [0222] In one embodiment, it is preferable that an anti-latent TGF-beta 1 antibody of the present disclosure has a large dissociation constant (KD) for latent TGF-beta 1 at an acidic pH. In one embodiment, the dissociation constant of an antibody of the present disclosure for latent TGF-beta 1 at an acidic pH is, for example, 3 nM or more, but is not limited thereto. In one embodiment, the dissociation constant of an antibody of the present disclosure for latent TGF-beta 1 at an acidic pH is, for example, 10 nM or more, but is not limited thereto. In one embodiment, the dissociation constant of an antibody of the present disclosure for latent TGF-beta 1 at an acidic pH is, for example, 30 nM or more, but is not limited thereto.
- [0223] In one embodiment, it is preferable that an anti-latent TGF-beta 1 antibody of the present disclosure has a large dissociation constant (KD) for latent TGF-beta 1 at pH 5.8. In one embodiment, the dissociation constant of an antibody of the present disclosure for latent TGF-beta 1 at pH 5.8 is, for example, 3 nM or more, but is not limited thereto. In one embodiment, the dissociation constant of an antibody of the present disclosure for latent TGF-beta 1 at pH 5.8 is, for example, 10 nM or more, but is not limited thereto. In one embodiment, the dissociation constant of an antibody of the present disclosure for latent TGF-beta 1 at pH 5.8 is, for example, 30 nM or more, but is not limited thereto.
- [0224] In some embodiments, the dissociation constant (KD) for human latent TGF-beta 1 at pH 5.8 is 2.0×10^{-7} M or more, 3.0×10^{-7} M or more, 4.0×10^{-7} M or more, 5.0×10^{-7}

M or more.

In some embodiments, the dissociation constant (KD) for human latent TGF-beta 1 at pH 5.8 is 2.0×10^{-7} M to 3.0×10^{-7} M, 2.0×10^{-7} M to 4.0×10^{-7} M, or 2.0×10^{-7} M to 5.0×10^{-7} M.

- [0225] In one embodiment, it is preferable that the binding affinity of an anti-latent TGF-beta 1 antibody of the present disclosure for latent TGF-beta 1 is greater at a neutral pH than at an acidic pH.
- [0226] In one embodiment, the dissociation constant ratio between acidic pH and neutral pH, [KD (acidic pH)/KD (neutral pH)], of an anti-latent TGF-beta 1 antibody of the present disclosure is, for example, 30 or more, but is not limited thereto. In one embodiment, the dissociation constant ratio between acidic pH and neutral pH, [KD (acidic pH)/KD (neutral pH)], of an anti-latent TGF-beta 1 antibody of the present disclosure is, for example, 3 or more, 5 or more, 10 or more, 50 or more, 90 or more, 100 or more, 150 or more, 200 or more, for example, 90, 100, 150, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 5000, 5500, 6000, 6500, 7000, 7500, 8000, 8500, 9000, or 9500, but is not limited thereto.
- [0227] In one embodiment, the dissociation constant ratio between pH 5.8 and pH 7.4, [KD (pH 5.8)/KD (pH 7.4)], of an anti-latent TGF-beta 1 antibody of the present disclosure is 30 or more, but is not limited thereto. In one embodiment, the dissociation constant ratio between pH 5.8 and pH 7.4, [KD (pH 5.8)/KD (pH 7.4)], of an antibody of the present disclosure is, for example, 3 or more, 5 or more, 10 or more, 50 or more, 90 or more, 100 or more, 150 or more, 200 or more, for example, 90, 100, 150, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 5000, 5500, 6000, 6500, 7000, 7500, 8000, 8500, 9000, or 9500, but is not limited thereto.
- In some embodiments, the dissociation constant ratio between pH 5.8 and pH 7.4, [KD (pH 5.8)/KD (pH 7.4)], of an antibody of the present disclosure for human latent TGF-beta-1 is, for example, 100 or more, 120 or more, 130 or more, or 160 or more.
- In some embodiments, the dissociation constant ratio between pH 5.8 and pH 7.4, [KD (pH 5.8)/KD (pH 7.4)], of an antibody of the present disclosure for human latent TGF-beta-1 is, for example, 100 to 170, 120 to 170, 130 to 170, or 160 to 170.
- [0228] In one embodiment, it is preferable that an anti-latent TGF-beta 1 antibody of the present disclosure has a large dissociation rate constant (koff) at an acidic pH. In one embodiment, the dissociation rate constant of an antibody of the present disclosure at an acidic pH is, for example, 0.003 (1/s) or more, but is not limited thereto. In one embodiment, the dissociation rate constant of an antibody of the present disclosure at an acidic pH is, for example, 0.005 (1/s) or more, but is not limited thereto. In one embodiment, the dissociation rate constant of an antibody of the present disclosure at an acidic pH is, for example, 0.01 (1/s) or more, but is not limited thereto.

[0229] In one embodiment, it is preferable that an anti-latent TGF-beta 1 antibody of the present disclosure has a large dissociation rate constant (k_{off}) at pH 5.8. In one embodiment, the dissociation rate constant of an antibody of the present disclosure at pH 5.8 is, for example, 0.003 (1/s) or more, but is not limited thereto. In one embodiment, the dissociation rate constant of an antibody of the present disclosure at pH 5.8 is, for example, 0.005 (1/s) or more, but is not limited thereto. In one embodiment, the dissociation rate constant of an antibody of the present disclosure at pH 5.8 is, for example, 0.01 (1/s) or more, but is not limited thereto.

Examples

[0230] The following are examples of methods and compositions of the invention. It is understood that various other embodiments may be practiced, given the general description provided above.

[0231] **EXAMPLE 1: Expression and purification of latent TGF-beta 1**

The sequences used for expression and purification were: FLAG (registered trademark)-tagged human latent TGF-beta 1 (SEQ ID NO: 64) and FLAG (registered trademark)-tagged mouse latent TGF-beta 1 (SEQ ID NO: 65), and FLAG (registered trademark)-tagged cynomolgus monkey latent TGF-beta 1 (SEQ ID NO: 66). Each of these FLAG (registered trademark)-tagged latent TGF-beta 1 has, from its N-terminus to C-terminus, a signal sequence derived from rat serum albumin (SEQ ID NO: 67), a FLAG (registered trademark)-tag, and a sequence of latent TGF-beta 1. The Cys residue at the thirtieth (30th) position in each of these FLAG (registered trademark)-tagged latent TGF-beta 1 was substituted with Ser, which corresponds to 'C33S mutation' (see, e.g., Yoshinaga K, et al. Perturbation of transforming growth factor (TGF)-beta 1 association with latent TGF-beta binding protein yields inflammation and tumors. Proc Natl Acad Sci USA. 2008;105(48):18758-18763).

FLAG (registered trademark)-tagged human latent TGF-beta 1 (hereinafter called "human latent TGF-beta 1 (SLC)" or "human latent TGF-beta 1"), FLAG (registered trademark)-tagged mouse latent TGF-beta 1 (hereinafter called "mouse latent TGF-beta 1 (SLC)" or "mouse latent TGF-beta 1"), or FLAG (registered trademark)-tagged cynomolgus monkey latent TGF-beta 1 (hereinafter called "monkey latent TGF-beta 1 (SLC)" or "monkey latent TGF-beta 1") was co-expressed transiently using FreeStyle293 (trademark)-F or Expi293F (trademark) cell line (Thermo Fisher Scientific) along with a plasmid expressing human Furin. Conditioned media expressing human, mouse or monkey latent TGF-beta 1 (SLC) was applied to a column packed with anti-FLAG (registered trademark) M2 affinity resin (Sigma), and latent TGF-beta 1 (SLC) was eluted with a FLAG (registered trademark) peptide (Sigma). Fractions containing human, mouse or monkey latent TGF-beta 1 (SLC) were collected

and subsequently subjected to a SUPERDEX (registered trademark) 200 gel filtration column (GE healthcare) equilibrated with 1x PBS. Fractions containing human, mouse or monkey latent TGF-beta 1 (SLC) were then pooled and stored at -80 degrees C.

[0232] Example 2. Humanization and optimization of anti-TGF-beta 1 antibody

(2-1) Humanization

A parent anti-TGF-beta antibody TBA0947, chimeric antibody, was humanized as follows. First, variable regions of the heavy and light chains of humanized antibodies were designed using variable regions of TBA0947 and human germline frameworks. Then, the polynucleotides of each designed heavy variable region and light chain variable region were cloned into expression vectors containing the heavy chain constant region SG181 sequence (SEQ ID NO: 22) and the light chain constant region SK1 sequence (SEQ ID NO: 25), respectively. Humanized antibodies were transiently expressed in FreeStyle 293-F Cells (Thermo Fisher Scientific), and BIACORE (registered trademark) analysis was carried out. A humanized antibody that showed at least similar BIACORE (registered trademark) binding activity as the parent antibody was selected.

[0233] (2-2) Optimization

One anti-human latent TGF-beta 1 antibody hT0947AE07-SG191 was developed from TBA0947 by improving antigen binding affinity/activity. Although its preferable antigen binding affinity/activity, the hT0947AE07-SG191 showed fast clearance in cynomolgus monkey PK study (FIGS. 14-1 and 14-6). For the PK study in cynomolgus monkey, human IgG1 based heavy chain constant region named SG191 (SEQ ID NO 23) was used as it shows enhanced human FcRn-binding to improve PK profile.

To further improve its pharmacokinetic profile, the pH dependent antibody T0947H0053H0156-SG181/T0947L001L0049-SK1 was developed from the hT0947AE07-SG181 by histidine scanning of its heavy chain CDRs and light chain CDRs. pH dependent binding was evaluated by BIACORE (registered trademark) analysis. In BIACORE (registered trademark) analysis, human IgG1 based heavy chain constant region named SG181 (SEQ ID NO 22) was used for stable capture on to BIACORE (registered trademark) sensor chip. While the pH dependent antibody showed dissociation of human latent TGF-beta 1 at pH5.8 in BIACORE (registered trademark) analysis, the binding affinity to human latent TGF-beta 1 at pH7.4 is not strong enough with KD 1.34E-08 M (FIG. 2). Therefore, antibody variants were designed to improve affinity towards human TGF-beta 1 while maintaining pH dependent characteristics.

[0234] First, T0947H0053H0156-SG181/T0947L001L0049-SK1 was subjected to comprehensive mutagenesis of all residues in its heavy chain CDRs. Each amino acid was substituted with 18 other naturally occurring amino acids, excluding the original amino

acid and cysteine. In total, 648 of antibody variants were designed through the comprehensive mutagenesis. The designed antibody variants were transiently expressed in Expi293 (trademark) cells (Thermo Fisher Scientific) and purified from culture supernatants. All antibodies produced were evaluated in BIACORE (registered trademark) analysis towards human and mouse latent TGF-beta 1 and in vitro neutralization assay of human and mouse latent TGF-beta 1 against plasmin (PLN) as well as antibody expression titer.

- [0235] Successive rounds of design of mutation combinations in CDRs were conducted to identify the preferable amino acid sequences in CDRs by utilizing data for each variant evaluated in vitro experiments by the BIACORE (registered trademark) and neutralization assay for latent TGF-beta 1 as mentioned above. All designed antibodies were produced by transient expression in Expi293 cells and evaluated in BIACORE (registered trademark) analysis towards human and mouse latent TGF-beta 1 and neutralization assay of human latent TGF-beta 1 and mouse latent TGF-beta 1 against PLN as well as antibody expression titer.
- [0236] In the 1st round, 40 antibody variants were designed and generated. For each generated antibody, pH dependent binding to human latent TGF-beta 1 and mouse latent TGF-beta 1 was assessed by BIACORE (registered trademark). Based on the BIACORE (registered trademark) results and neutralization activity, antibodies with combined mutations were designed for the 2nd round.
- [0237] From the 2nd to 6th rounds, 80 antibody variants were designed and tested in each round. Although 480 antibody variants were assessed in total, KD at pH7.4 for human latent TGF-beta 1 could not be sufficiently improved while maintaining pH dependent binding characteristics. Thus, to explore more antibody mutations, T0947H0053H1184-SG181/T0947L001L0083-SK1 was selected from all the antibody variants evaluated through 1st to 6th rounds of antibody design, and another comprehensive mutagenesis of its heavy chain CDRs was conducted. Then, 450 antibody variants with single mutation from T0947H0053H1184-SG181/T0947L001L0083-SK1 were evaluated by BIACORE (registered trademark) and in vitro TGF-beta 1 neutralization assay. The potential antibody mutations were used to combine in the further rounds of antibody design.
- [0238] In the 7th and 8th rounds, 80 antibodies were designed in each round and tested for BIACORE (registered trademark) and neutralization activity. In 9th and 10th rounds, 120 antibodies were designed in each round and tested for BIACORE (registered trademark) and neutralization activity. In 11th to 13th rounds, 80 antibodies were designed in each round and tested for Biacore and neutralization activity.
- [0239] After the 13th round, hT0947AE20-SG181, hT0947AE22-SG181, hT0947AE23-SG181, hT0947AE24-SG181, hT0947AE25-SG181 and

hT0947AE26-SG181 were selected from all the antibody variants designed and tested thorough 1st to 13th rounds and comprehensive mutagenesis based on BIACORE (registered trademark) binding characteristics and neutralization activity. Through the antibody optimization process, KD of those antibodies was clearly improved from the parent pH-dependent anti human latent TGF-beta 1 antibody T0947H0053H0156-SG181/T0947L001L0049-SK1, and those antibodies showed strong neutralization activity toward human latent TGF-beta 1 against PLN (FIG. 1). These results suggest that antibodies potentially having in vivo efficacy could be generated through the antibody optimization. However, KD of those antibodies for human latent TGF-beta 1 at pH7.4 were 2.5 - 5-fold greater than the non-pH dependent anti-human latent TGF-beta 1 antibody hT0947AE07-SG181 (FIGS. 2 and 3) which suggests affinity of those antibodies still can be improved. Therefore, additional rounds of antibody engineering were applied.

[0240] As an effort to design antibodies which have better antigen binding affinity/activity toward human latent TGF-beta 1, the method to evaluate BIACORE (registered trademark) results utilized for the next rounds of antibody design was modified. For the antibodies designed in the 1st to 13th rounds, the BIACORE (registered trademark) results were evaluated based on both KD at neutral pH and pH dependency to latent human TGF-beta 1. Whereas, for the antibodies designed in the 14th to 17th rounds, BIACORE (registered trademark) results were evaluated based on KD at neutral pH towards human latent TGF-beta 1.

[0241] After the 13th rounds, hT0947AE22-SG181 which showed fast dissociation from human latent TGF-beta 1 at pH5.8 was selected for another comprehensive mutagenesis on its heavy and light chain CDRs. 1134 variants were produced for in vitro characterization. By using the in vitro characterization results for antibodies generated through comprehensive mutagenesis, additional rounds of antibody design and characterization were conducted until the 17th round. In 14th to 17th rounds, 150, 96, 160 and 180 antibody variants were designed and produced, respectively. In these 14th to 17th rounds, antibody optimization was conducted to improve affinity in KD values evaluated in BIACORE (registered trademark) rather than pH dependent binding properties. In addition, AC-SINS and PEG-SPR were carried out for all the antibody variants designed in 14th to 17th rounds to find antibody variants with preferred physicochemical properties because antibodies in previous rounds got increased hydrophobicity. Antibodies were designed to maintain AC-SINS and PEG-SPR data to the similar level as clinical antibodies which are already approved by FDA. As shown in FIG. 3, from 14th to 17th rounds, KD for human latent TGF-beta 1 could be improved while maintaining pH dependent binding characteristics. From those all the antibodies generated through the successive rounds of those antibody design and in vitro charac-

terization process, pH dependent anti-latent TGF-beta 1 antibodies
hT0947AE20-SG181, hT0947AE22-SG181, hT0947AE23-SG181,
hT0947AE24-SG181, hT0947AE25-SG181, hT0947AE26-SG181,
hT0947AE27-SG181, hT0947AE34-SG181 and hT0947AE37-SG181 were selected
from all the antibody variants evaluated through all the rounds of antibody design.

[0242] (2-3) Amino acid sequence of optimized antibody

The amino acid sequences of anti-latent TGF-beta 1 antibodies are listed in Table 3.

[0243]

[Table 3]

Antibody name	VH name	VH SEQ ID NO	CH name	CH SEQ ID NO	VL name	VL SEQ ID NO	CL name	CL SEQ ID NO	Remark
hT0947AE07- SG181	T0947H0 053H0066	1	SG181	22	T0947L00 1L0049	12	SK1	25	Human IgG1
T0947H0053H0 156- SG181/T0947L 001L0049-SK1	T0947H0 053H0156	2	SG181	22	T0947L00 1L0049	12	SK1	25	Human IgG1
T0947H0053H1 184- SG181/T0947L 001L0083-SK1	T0947H0 053H1184	3	SG181	22	T0947L00 1L0083	13	SK1	25	Human IgG1
hT0947AE20- SG181	T0947H0 053H1185	4	SG181	22	T0947L00 1L0127	16	SK1	25	Human IgG1
hT0947AE22- SG181	T0947H0 053H1222	5	SG181	22	T0947L00 1L0109	15	SK1	25	Human IgG1
hT0947AE23- SG181	T0947H0 053H2151	7	SG181	22	T0947L00 1L0226	18	SK1	25	Human IgG1
hT0947AE24- SG181	T0947H0 053H2158	8	SG181	22	T0947L00 1L0096	14	SK1	25	Human IgG1
hT0947AE25- SG181	T0947H0 053H1890	6	SG181	22	T0947L00 1L0083	13	SK1	25	Human IgG1
hT0947AE26- SG181	T0947H0 053H1890	6	SG181	22	T0947L00 1L0290	19	SK1	25	Human IgG1
hT0947AE27- SG181	T0947H0 053H3061	9	SG181	22	T0947L00 1L0936	21	SK1	25	Human IgG1
hT0947AE34- SG181	T0947H0 053H3306	11	SG181	22	T0947L00 1L0215	17	SK1	25	Human IgG1
hT0947AE37- SG181	T0947H0 053H3254	10	SG181	22	T0947L00 1L0386	20	SK1	25	Human IgG1

hT0947AE07-SG191	T0947H0053H0066	1	SG191	23	T0947L001L0049	12	SK1	25	Human IgG1
hT0947AE20-SG191	T0947H0053H1185	4	SG191	23	T0947L001L0127	16	SK1	25	Human IgG1
hT0947AE22-SG191	T0947H0053H1222	5	SG191	23	T0947L001L0109	15	SK1	25	Human IgG1
hT0947AE23-SG191	T0947H0053H2151	7	SG191	23	T0947L001L0226	18	SK1	25	Human IgG1
hT0947AE24-SG191	T0947H0053H2158	8	SG191	23	T0947L001L0096	14	SK1	25	Human IgG1
hT0947AE25-SG191	T0947H0053H1890	6	SG191	23	T0947L001L0083	13	SK1	25	Human IgG1
hT0947AE26-SG191	T0947H0053H1890	6	SG191	23	T0947L001L0290	19	SK1	25	Human IgG1
hT0947AE27-SG191	T0947H0053H3061	9	SG191	23	T0947L001L0936	21	SK1	25	Human IgG1
hT0947AE34-SG191	T0947H0053H3306	11	SG191	23	T0947L001L0215	17	SK1	25	Human IgG1
hT0947AE37-SG191	T0947H0053H3254	10	SG191	23	T0947L001L0386	20	SK1	25	Human IgG1
hT0947AE07-mF18	T0947H0053H0066	1	mF18	24	T0947L001L0049	12	mk1	26	Mouse IgG1
hT0947AE20-mF18	T0947H0053H1185	4	mF18	24	T0947L001L0127	16	mk1	26	Mouse IgG1
hT0947AE22-mF18	T0947H0053H1222	5	mF18	24	T0947L001L0109	15	mk1	26	Mouse IgG1
hT0947AE27-mF18	T0947H0053H3061	9	mF18	24	T0947L001L0936	21	mk1	26	Mouse IgG1
hT0947AE34-mF18	T0947H0053H3306	11	mF18	24	T0947L001L0215	17	mk1	26	Mouse IgG1
hT0947AE37-mF18	T0947H0053H3254	10	mF18	24	T0947L001L0386	20	mk1	26	Mouse IgG1

[0244] The heavy-chain HVR (CDR) sequences and framework (FR) sequences of each antibody are shown in Table 4-1.

[0245]

[Table 4-1]

Antibody name	VH name	HVR1	HVR2	HVR3	FR1	FR2	FR3	FR4
		SEQ	SEQ	SEQ	SEQ	SEQ	SEQ	SEQ
		ID NO	ID NO	ID NO	ID NO	ID NO	ID NO	ID NO
hT0947AE07	T0947H0053H0066	27	30	34	41	44	45	46
T0947H0053H0156/ T0947L001L0049	T0947H0053H0156	27	30	35	41	44	45	47
T0947H0053H1184/ T0947L001L0083	T0947H0053H1184	28	30	36	41	44	45	47
hT0947AE20	T0947H0053H1185	29	30	36	42	44	45	47
hT0947AE22	T0947H0053H1222	29	30	37	43	44	45	47
hT0947AE23	T0947H0053H2151	29	30	38	43	44	45	47
hT0947AE24	T0947H0053H2158	27	30	37	43	44	45	47
hT0947AE25	T0947H0053H1890	28	31	36	43	44	45	47
hT0947AE26	T0947H0053H1890	28	31	36	43	44	45	47
hT0947AE27	T0947H0053H3061	28	32	37	43	44	45	47
hT0947AE34	T0947H0053H3306	27	30	39	43	44	45	47
hT0947AE37	T0947H0053H3254	27	33	40	43	44	45	47

[0246] The light-chain HVR (CDR) sequences and framework (FR) sequences of each antibody are shown in Table 4-2.

[0247]

[Table 4-2]

Antibody name	VL name	HVR1	HVR2	HVR3	FR1	FR2	FR3	FR4
		SEQ	SEQ	SEQ	SEQ	SEQ	SEQ	SEQ
		ID NO	ID NO	ID NO	ID NO	ID NO	ID NO	ID NO
hT0947AE07	T0947L001L0049	48	52	55	60	61	62	63
T0947H0053H0156/ T0947L001L0049	T0947L001L0049	48	52	55	60	61	62	63
T0947H0053H1184/ T0947L001L0083	T0947L001L0083	49	52	55	60	61	62	63
hT0947AE20	T0947L001L0127	49	52	56	60	61	62	63
hT0947AE22	T0947L001L0109	48	52	56	60	61	62	63
hT0947AE23	T0947L001L0226	48	53	58	60	61	62	63
hT0947AE24	T0947L001L0096	48	54	55	60	61	62	63
hT0947AE25	T0947L001L0083	49	52	55	60	61	62	63
hT0947AE26	T0947L001L0290	49	52	59	60	61	62	63
hT0947AE27	T0947L001L0936	50	52	56	60	61	62	63
hT0947AE34	T0947L001L0215	48	53	56	60	61	62	63
hT0947AE37	T0947L001L0386	51	52	56	60	61	62	63

[0248] (2-4) In vitro characterization for antibody screening during antibody optimization
 BIACORE (registered trademark) screening assay for binding affinity and pH dependent interactions of anti-latent TGF-beta 1 antibody towards recombinant human latent TGF-beta 1

Binding affinity of anti-latent TGF-beta 1 antibody towards recombinant human latent TGF-beta 1 were measured using BIACORE (registered trademark) T200 instrument (GE Healthcare). For the BIACORE (registered trademark) screening, antibodies with human IgG1 constant regions (SG181, SEQ ID NO: 22 and SK1, SEQ ID NO: 25) were prepared. A mouse anti-human Fc (GE Healthcare) was immobilized onto all flow cells of a CM4 sensor chip using amine coupling kit (GE Healthcare). Antibodies were captured onto the anti-human Fc sensor surfaces to around 20RU capture level, then recombinant human latent TGF-beta 1 was injected over the flow cell. All antibodies and analytes were prepared in ACES pH 7.4 containing 20 mM ACES, 150 mM NaCl, 0.05% Tween 20, 0.005% NaN₃. Sensor surface was re-generated each cycle with 3M MgCl₂. Binding affinity were determined by processing and fitting the data to 1:1 binding model using BIACORE (registered trademark) T200 Evaluation software, version 3.0 (GE Healthcare).

[0249] pH dependent interaction screening of anti-latent TGF-beta 1 antibodies towards recombinant human latent TGF-beta 1 was determined by a modified BIACORE

(registered trademark) assay described above. Briefly, an additional dissociation phase at pH 5.8 was integrated into the BIACORE (registered trademark) assay immediately after dissociation phase at pH 7.4. Dissociation rate at pH 5.8 buffer was determined by processing and fitting data using Scrubber 2.0 (BioLogic Software) curve fitting software.

[0250] Affinity-capture self-interaction nanoparticle spectroscopy (AC-SINS)

For the AC-SINS assay, antibodies with human IgG1 constant regions (SG181, SEQ ID NO: 22 and SK1, SEQ ID NO: 25) were prepared. In AC-SINS assay, 0.025 mg/mL concentration of the anti-latent TGF-beta 1 antibodies were added to gold nanoparticles (Ted Pella Inc. #15705) pre-coated with 80% of polyclonal goat anti-human IgG Fc (Jackson ImmunoResearch #109-005-098) as capturing and 20% of goat non-specific antibody (Jackson ImmunoResearch #005-000-003). Antibodies were incubated with the pre-coated gold nanoparticles for 2 hours at room temperature and the wavelength shift was measured using SpectraMax (registered trademark) M2 (Molecular Devices).

[0251] Polyethylene glycol (PEG)-induced antibody precipitation assay

For the PEGSPR assay, antibodies with human IgG1 constant regions (SG181, SEQ ID NO 22: and SK1, SEQ ID NO: 25) were prepared. In PEG-SPR assay, equal volume of 0.05 mg/mL concentration of the anti-latent TGF-beta 1 antibodies were added to 25% PEG solution. The mixtures were mixed on shaker at 700rpm for 1 min, then incubated at room temperature for 2hrs. Samples were transferred to Multi-ScreenHTS GV Filter Plate and centrifuged at 2500rpm for 5min. The flowthrough that contains soluble samples were analyzed with BIACORE (registered trademark) 8K+ instrument (GE Healthcare). Protein A/G was immobilized onto all flow cells of a CM5 sensor chip using amine coupling kit (GE Healthcare). Assay temperature was set at 25 degrees C, and running buffer consist of ACES pH 7.4 containing 20 mM ACES, 150 mM NaCl, 0.05% Tween 20, 0.005% NaN₃. Samples were injected onto the protein A/G sensor surfaces at 10uL/min for 30s. Sensor surface was regenerated each cycle with 10mM Glycine-HCl pH 1.5. Binding response of each sample was analyzed using BIACORE (registered trademark) 8K Evaluation software, version 1.1.1.7442 (GE Healthcare).

Solubility of each sample was analyzed by normalizing the binding response in PEG to the PBS.

[0252] Latent TGF-beta 1 neutralization assay against plasmin (PLN)

For the latent TGF-beta 1 neutralization assay, antibodies with human IgG1 constant regions (SG181, SEQ ID NO: 22 and SK1, SEQ ID NO: 25) were prepared. Mouse latent TGF-beta 1 (mSLC) and human latent TGF-beta 1 (hSLC), which were prepared in Example 1, were each incubated with human plasmin (Calbiochem), with or without

the presence of anti-latent TGF-beta 1 antibodies at 37 degree C for 1 hour. Antibodies were pre-incubated with mouse or human latent TGF-beta 1 (SLC) for 30 minutes at room temperature before incubating with plasmin. Anti-KLH antibody IC17-hIgG1 (Heavy chain SEQ ID NO: 68, Light chain SEQ ID NO: 69) was used as a negative control. Plasmin-mediated latent TGF-beta 1 activation and antibody mediated inhibition were analyzed by mature TGF-beta 1 ELISA (Human TGF-beta 1 Quantikine (registered trademark) ELISA Kit, R&D systems) according to manufacturer's procedure.

[0253] Example 3. Immunogenicity assessment of anti-latent TGF-beta 1 antibodies

The immunogenic potential of the antibodies was evaluated by using as an indicator the proportion of IL-2 secreting CD4⁺ T cells before active proliferation is exhibited as described in WO2018/124005 (Kubo C. et al). Specifically, CD8 CD25^{low} peripheral blood mononuclear cells (PBMCs) were prepared from human PBMCs, and the cells were cultured for 67 hours in the presence of the antibodies.

[0254] FIG. 4 shows the results of determining the proportions of IL-2-secreting cells in the cultured cell populations. The frequencies of positive donors for hT0947AE20-SG191, hT0947AE27-SG191, hT0947AE34-SG191 and hT0947AE37-SG191 were similar to or slightly higher compared to that for negative control. On the other hand, the frequencies of positive donors for hT0947AE22-SG191, hT0947AE23-SG191, hT0947AE24-SG191, hT0947AE25-SG191 and hT0947AE26-SG191 were higher than that for negative control. These results suggested that hT0947AE20-SG191, hT0947AE27-SG191, hT0947AE34-SG191 and hT0947AE37-SG191 were considered to have low immunogenic potential.

[0255] Example 4. BIACORE (registered trademark) binding affinity evaluation of anti-latent TGF-beta 1 antibodies toward recombinant human, cyno, and mouse latent TGF-beta 1

For BIACORE (registered trademark) affinity evaluation, antibodies with human IgG1 constant regions (SG191, SEQ ID NO: 23 and SK1, SEQ ID NO: 25) were prepared. Binding affinity of anti-latent TGF-beta 1 antibodies (hT0947AE07-SG191, hT0497AE20-SG191, hT0497AE27-SG191, hT0497AE34-SG191 and hT0497AE37-SG191) towards latent TGF-beta 1 of human, cyno, and mouse were measured using BIACORE (registered trademark) 8K instrument (GE Healthcare). A mouse anti-human Fc (GE Healthcare) was immobilized onto all flow cells of a CM5 sensor chip using amine coupling kit (GE Healthcare). Antibodies were captured onto the anti-human Fc sensor surfaces to around 20RU capture level, then recombinant latent TGF-beta 1 of human, cyno, and mouse was injected over the flow cell. All antibodies and analytes were prepared in ACES pH 7.4 containing 20 mM ACES, 150 mM NaCl, 0.05% Tween 20, 0.005% NaN₃. Sensor surface was regenerated each cycle

with 3M MgCl₂. Binding affinity were determined by processing and fitting the data to 1:1 binding model using BIAcore (registered trademark) 8K Evaluation software, version 1.1.1.7442 (GE Healthcare).

[0256] Binding affinity of anti-latent TGF-beta 1 antibodies towards latent TGF-beta 1 of human, cyno, and mouse at pH7.4 and pH5.8 are shown in FIGS. 5 and 6, respectively. pH dependent anti-latent TGF-beta 1 antibodies showed dissociation at acidic pH 5.8 with kd 3.93E-02 to 6.54E-02 as shown in FIG. 6 while the antibody hT0947AE07-SG191 showed slower dissociation with kd 1.49E-03 at pH 5.8.

[0257] FIG. 7 shows the ratios (pH 5.8/pH 7.4) of koff and KD (which were calculated from the results of FIGS. 5 and 6) for hT0947AE07-SG191, hT0947AE20-SG191, hT0947AE27-SG191, hT0947AE34-SG191, and hT0947AE37-SG191.

[0258] Example 5. Characterization of anti-latent TGF-beta 1 antibody

(5-1) Anti-latent TGF-beta 1 antibody bound to cell surface latent TGF-beta 1

For the FACS assay, antibodies with human IgG1 constant regions (SG191, SEQ ID NO: 23 and SK1, SEQ ID NO: 25) were prepared. Binding activities of anti-latent TGF-beta 1 antibodies (hT0947AE07-SG191, hT0497AE20-SG191, hT0497AE27-SG191, hT0497AE34-SG191 and hT0497AE37-SG191) to cell surface latent TGF-beta 1 were tested by FACS using Ba/F3 cells expressing mouse latent TGF-beta 1 or FreeStyle™ 293-F cells expressing human latent TGF-beta 1 (ThermoFisher). The anti-latent TGF-beta 1 antibodies (10 microgram/mL each) were incubated with each cell line for 30 minutes at 4 degree C and washed with FACS buffer (2% PBS, 2mM EDTA in PBS). Anti-KLH antibody having human IgG1 Fc region, IC17dK-SG181 (Heavy chain SEQ ID NO: 70, Light chain SEQ ID NO: 71), which does not bind to mouse latent TGF-beta 1 nor human latent TGF-beta 1, was used as a negative control antibody. Goat F(ab')₂ anti-Human IgG, Mouse ads-PE (Southern Biotech, Cat. 2043-09) was then added and incubated for 30 minutes at 4 degree C and washed with FACS buffer. Data acquisition was performed on an FACS Verse (Becton Dickinson), followed by analysis and calculation of Mean Fluorescence Intensity (MFI), using FlowJo (trademark) software (Tree Star) and GraphPad Prism software (GraphPad). As shown in FIG. 8, all anti-latent TGF-beta 1 antibodies bound to mouse cell surface latent TGF-beta 1 expressed on Ba/F3 cells and human cell surface latent TGF-beta 1 expressed on FreeStyle™ 293-F cells.

[0259] (5-2) Anti-latent TGF-beta 1 antibody inhibited spontaneous latent TGF-beta 1 activation

For the latent TGF-beta 1 neutralization assay, antibodies with human IgG1 constant regions (SG191, SEQ ID NO: 23 and SK1, SEQ ID NO: 25) were prepared. Mouse latent TGF-beta 1 (mSLC) and human latent TGF-beta 1 (hSLC), which were prepared in Example 1, were each incubated with or without the presence of one of the anti-

latent TGF-beta 1 antibodies (hT0947AE07-SG191, hT0497AE20-SG191, hT0497AE27-SG191, hT0497AE34-SG191 and hT0497AE37-SG191) at 37 degree C for 1 hour. The anti-KLH antibody, IC17dk-SG181 (Heavy chain SEQ ID NO: 70, Light chain SEQ ID NO: 71), was used as a negative control. Spontaneous latent TGF-beta 1 activation and antibody mediated inhibition of the spontaneous latent TGF-beta 1 activation were analyzed by mature TGF-beta 1 ELISA (Human TGF-beta 1 Quantikine (registered trademark) ELISA Kit, R&D systems) according to manufacturer's procedure.

As shown in FIG. 9, spontaneous activation of latent TGF-beta 1 was suppressed by anti-latent TGF-beta 1 antibodies.

[0260] (5-3) Anti-latent TGF-beta 1 antibody inhibited plasmin (PLN)-mediated latent TGF-beta 1 activation

For the latent TGF-beta 1 neutralization assay, antibodies with human IgG1 constant regions (SG191, SEQ ID NO: 23 and SK1, SEQ ID NO: 25) were prepared. Mouse latent TGF-beta 1 (mSLC) and human latent TGF-beta 1 (hSLC), which were prepared in Example 1, were each incubated with human plasmin (Calbiochem), with or without the presence of one of the anti-latent TGF-beta 1 antibodies (hT0947AE07-SG191, hT0497AE20-SG191, hT0497AE27-SG191, hT0497AE34-SG191 and hT0497AE37-SG191) at 37 degree C for 1 hour. Antibodies were pre-incubated with mouse or human latent TGF-beta 1 (SLC) for 30 minutes at room temperature before incubating with plasmin. Anti-KLH antibody, IC17dk-SG181 (Heavy chain SEQ ID NO: 70, Light chain SEQ ID NO: 71), was used as a negative control. Plasmin-mediated latent TGF-beta 1 activation and antibody mediated inhibition were analyzed by mature TGF-beta 1 ELISA (Human TGF-beta 1 Quantikine (registered trademark) ELISA Kit, R&D systems) according to manufacturer's procedure. As shown in FIG. 10, plasmin-mediated latent TGF-beta 1 activation was suppressed by anti-latent TGF-beta 1 antibodies.

[0261] (5-4) Anti-latent TGF-beta 1 antibody inhibited plasma kallikrein (PLK)-mediated latent TGF-beta 1 activation

For the latent TGF-beta 1 neutralization assay, antibodies with human IgG1 constant regions (SG191, SEQ ID NO: 23 and SK1, SEQ ID NO: 25) were prepared. Mouse latent TGF-beta 1 (mSLC) and human latent TGF-beta 1 (hSLC), which were prepared in Example 1, were each incubated with human kallikrein (Enzyme Research Laboratories), with or without the presence of one of the anti-latent TGF-beta 1 antibodies (hT0947AE07-SG191, hT0497AE20-SG191, hT0497AE27-SG191, hT0497AE34-SG191 and hT0497AE37-SG191) at 37 degree C for 2 hours. Antibodies were pre-incubated with mouse or human latent TGF-beta 1 (SLC) for 30 minutes at room temperature before incubating with kallikrein. Anti-KLH antibody,

IC17dk-SG181 (Heavy chain SEQ ID NO 70, Light chain SEQ ID NO 71), was used as a negative control. Kallikrein-mediated latent TGF-beta 1 activation and antibody mediated inhibition was analyzed by mature TGF-beta 1 ELISA (Human TGF-beta 1 Quantikine (registered trademark) ELISA Kit, R&D systems) according to manufacturer's procedure. As shown in FIG. 11, kallikrein-mediated latent TGF-beta 1 activation was suppressed by anti-latent TGF-beta 1 antibodies.

[0262] (5-5) Anti-latent TGF-beta 1 antibody inhibited MMP2 and MMP9-mediated human latent TGF-beta 1 activation

For the latent TGF-beta 1 neutralization assay, antibodies with human IgG1 constant regions (SG191, SEQ ID NO: 23 and SK1, SEQ ID NO: 25) were prepared. Human latent TGF-beta 1 (SLC), which were prepared in Example 1, was incubated with activated metalloproteinase 2 (MMP2) or MMP9 (R&D systems), with or without the presence of one of the anti-latent TGF-beta 1 antibodies (hT0947AE07-SG191, hT0497AE20-SG191, hT0497AE27-SG191, hT0497AE34-SG191 and hT0497AE37-SG191) at 37 degree C for 2 hours. Antibodies were pre-incubated with human latent TGF-beta 1 (SLC) for 30 minutes at room temperature before incubating with MMP2 or MMP9. Anti-KLH antibody, IC17dk-SG181 (Heavy chain SEQ ID NO: 70, Light chain SEQ ID NO: 71), was used as a negative control. MMP2 and MMP9-mediated human latent TGF-beta 1 activation and antibody mediated inhibition was analyzed by mature TGF-beta 1 ELISA (Human TGF-beta 1 Quantikine (registered trademark) ELISA Kit, R&D systems) according to the manufacturer's procedure. As shown in FIG. 11, both MMP2-mediated human latent TGF-beta 1 activation and MMP9-mediated human latent TGF-beta 1 activation were suppressed by anti-latent TGF-beta 1 antibodies.

[0263] (5-6) Anti-latent TGF-beta 1 antibody had less impact on inhibiting integrin-mediated latent TGF-beta 1 activation in mouse PBMC

Mouse PBMC and HEK-Blue™ TGF-beta cell co-culture assay was conducted to detect integrin-mediated latent TGF-beta 1 activation. Mouse PBMC was isolated from mouse blood by using Histopaque-1083 density gradient medium (Sigma-Aldrich). HEK-Blue™ TGF-beta cells (Invivogen), which express Smad3/4-binding elements (SBE)-inducible SEAP reporter genes, allow the detection of bioactive TGF-beta 1 (both mouse TGF-beta 1 and human TGF-beta 1) by monitoring the activation of Smad3/4. Active TGF-beta 1 stimulates the production of SEAP and its secretion into cell supernatant. The quantity of SEAP secreted was assessed by using QUANTI-Blue™ reagent (Invivogen).

[0264] HEK-Blue™ TGF-beta cells were maintained in DMEM medium (Gibco) supplemented with 10% fetal bovine serum, 50 U/mL streptomycin, 50 microgram/mL penicillin, 100 microgram/mL Normocin (trademark), 30 microgram/mL of

Blasticidin, 200 microgram/mL of HygroGold (trademark) and 100 microgram/mL of Zeocin (registered trademark). During functional assay, the medium for cells was changed to assay medium (RPMI1640 with 10% FBS) and seeded to 96-well plate. Then, one of the anti-latent TGF-beta 1 antibodies (hT0947AE07-SG191, hT0947AE20-SG191, hT0947AE34-SG191, or hT0947AE37-SG191) and mouse PBMC were applied to the wells and incubated together with HEK-Blue™ TGF-beta cells overnight. Then the cell supernatant was mixed with QUANTI-Blue™ and the optical density (OD) at 620 nm was measured in a colorimetric plate reader. RGD peptide (GRRGDLATIH (SEQ ID NO: 101), GenScript) is known to bind to integrins and serve as a decoy integrin ligand to suppress integrin-mediated TGF-beta 1 activation. Therefore, RGD peptide was used as a positive control. Furthermore, RGE control peptide (GRRGELATIH (SEQ ID NO: 102), GenScript), which is known not to serve as a decoy integrin ligand, was used as a negative control. Anti-KLH antibody, IC17dk-SG181 (Heavy chain SEQ ID NO: 70, Light chain SEQ ID NO: 71), was used as a negative control. Anti-mature TGF-beta antibody, GC1008-F1332m (Heavy chain SEQ ID NO: 76, Light chain SEQ ID NO: 77), was used as a positive control. F1332m is a human IgG1 heavy chain constant region which includes amino acid substitutions to reduce effector function.

[0265] As shown in FIG. 12, anti-latent TGF-beta 1 antibodies had less impact on inhibiting integrin-mediated TGF-beta 1 activation in mouse PBMC compared to those of positive controls.

[0266] (5-7) Anti-latent TGF-beta 1 antibody had less impact on inhibiting integrin-mediated latent TGF-beta 1 activation in Detroit 562

Detroit 562 and HEK-Blue™ TGF-beta cell co-culture assay was conducted to detect integrin-mediated latent TGF-beta 1 activation. Detroit 562 was purchased from ATCC (Virginia, US). HEK-Blue™ TGF-beta cells (Invivogen), which express Smad3/4-binding elements (SBE)-inducible SEAP reporter genes, allow the detection of bioactive TGF-beta 1 (both mouse TGF-beta 1 and human TGF-beta 1) by monitoring the activation of Smad3/4. Active TGF-beta 1 stimulates the production of SEAP and its secretion into cell supernatant. The quantity of SEAP secreted was assessed by using QUANTI-Blue™ reagent (Invivogen).

[0267] HEK-Blue™ TGF-beta cells were maintained in DMEM medium (Gibco) supplemented with 10% fetal bovine serum, 50 U/mL streptomycin, 50 microgram/mL penicillin, 100 microgram/mL Normocin (trademark), 30 microgram/mL of Blasticidin, 200 microgram/mL of HygroGold (trademark) and 100 microgram/mL of Zeocin (registered trademark). During functional assay, the medium for cells was changed to assay medium (RPMI1640 with 10% FBS) and seeded to 96-well plate. Then one of the anti-latent TGF-beta 1 antibodies (hT0947AE07-SG191,

hT0947AE20-SG191, hT0947AE27-SG191, hT0947AE34-SG191, or hT0947AE37-SG191) and Detroit 562 were applied to the wells and incubated together with HEK-Blue™ TGF-beta cells overnight. Then the cell supernatant was mixed with QUANTI-Blue™ and the optical density (OD) at 620 nm was measured in a colorimetric plate reader. RGD peptide (GRRGDLATIH (SEQ ID NO: 101), GenScript) is known to bind to integrins and serve as a decoy integrin ligand to suppress integrin-mediated TGF-beta 1 activation. Therefore, RGD peptide was used as a positive control. Furthermore, RGE control peptide (GRRGELATIH (SEQ ID NO: 102), GenScript), which is known not to serve as a decoy integrin ligand, was used as a negative control. Anti-KLH antibody IC17-SG105 (Heavy chain SEQ ID NO: 74, Light chain SEQ ID NO: 75), was used as a negative control. Anti-mature TGF-beta 1 antibody, 21D1-F1332m (Heavy chain SEQ ID NO: 78, Light chain SEQ ID NO: 79), and Anti-integrin avb6 antibody, 3G9-mF18 (Heavy chain SEQ ID NO: 80, Light chain SEQ ID NO: 81), were used as a positive control. F1332m is a human IgG1 heavy chain constant region which includes amino acid substitutions to reduce effector function.

As shown in FIG. 13, anti-latent TGF-beta 1 antibodies had less impact on inhibiting integrin-mediated TGF-beta 1 activation in Detroit 562 compared to those of positive controls.

[0268] Example 6. Pharmacokinetics of the anti-latent TGF-beta 1 antibody

(6-1) PK Test of pH-Dependent Antibody Using Cynomolgus Monkeys

The pharmacokinetics in cynomolgus monkeys was evaluated for anti-human latent TGF-beta 1 antibodies (hT0947AE07-SG191, hT0947AE20-SG191, hT0947AE27-SG191, hT0947AE34-SG191 and hT0947AE37-SG191). HT0947AE07-SG191 was administered to cynomolgus monkeys by single-dose intravenous administration at 0.4 and 2 mg/kg on day 0. The other antibodies were administered to cynomolgus monkeys by single-dose intravenous administration at 0.8 and 2 or 4 mg/kg on day 0. Anti-CD40 antibody was administered to cynomolgus monkeys by intravenous administration at 5, 0.5, 0.5, 0.5 and 5 mg/kg on days 0, 7, 14, 21 and 29, respectively. Blood samples were collected over time. And collected blood was immediately centrifuged for 5 minutes at 1700xg and 4 degrees C to obtain plasma. The separated plasma was stored in a freezer set to -70 degrees C or lower until measurements were carried out.

The measurement of concentration in cynomolgus monkey plasma was carried out by ELISA.

[0269] As shown in FIGS. 14-1 to 14-6, pH dependent anti-human latent TGF-beta 1 antibodies hT0947AE20-SG191, hT0947AE27-SG191, hT0947AE34-SG191 and hT0947AE37-SG191 showed slower clearance compared to non-pH dependent

hT0947AE07-SG191.

[0270] (6-2) PK Test of pH-Dependent Antibody Using Mice

The pharmacokinetics in mice was evaluated for anti-TGF-beta 1 antibodies (hT0947AE04-SG181 (Heavy chain SEQ ID NO: 103, Light chain SEQ ID NO: 104), hT0947AE07-SG191, hT0947AE20-SG181, hT0947AE27-SG181, hT0947AE34-SG181 and hT0947AE37-SG181). The antibodies were administered to mice by single-dose caudal vein administration at 2 and 10 mg/kg, and blood samples were collected over time. Collected blood was immediately centrifuged for 15 minutes at 15,000 rpm and 4 degrees C to obtain plasma. The separated plasma was stored in a freezer set to -20 degrees C or lower until measurements were carried out.

The measurement of concentration in mice plasma was carried out by ELISA.

[0271] As shown in FIGS. 14-7 to 14-10, pH dependent anti-human latent TGF-beta 1 antibodies hT0947AE20-SG181, hT0947AE27-SG181, hT0947AE34-SG181 and hT0947AE37-SG181 showed slower clearance compared to non-pH dependent antibodies, hT0947AE04-SG181 and hT0947AE07-SG191.

[0272] Example 7. In vivo efficacy of anti-latent TGF-beta 1 antibodies in UUO induced mouse renal fibrosis model

The in vivo efficacy of monoclonal antibodies hT0947AE20-SG191, hT0947AE27-SG191, hT0947AE34-SG191, and hT0947AE37-SG191 were evaluated in Unilateral Ureteral Obstruction (UUO) mouse model which is known to induce a progressive renal fibrosis.

[0273] (7-1) Establishment of UUO induced mouse renal fibrosis model

The in vivo efficacies of monoclonal antibodies hT0947AE20-SG191, hT0947AE27-SG191, hT0947AE34-SG191, and hT0947AE37-SG191 were evaluated in Unilateral Ureteral Obstruction (UUO) mouse model which induces a progressive renal fibrosis.

C57BL/6J mice male mice of 7 weeks of age were purchased from Charles river (Kanagawa, Japan) and were acclimated for 1 weeks before the start of treatments. All mice were maintained on a regular diurnal lighting cycle (12:12 light:dark) with free access to food (CE-2, CLEA Japan, Tokyo, Japan) and water.

[0274] UUO surgery was operated under isoflurane anesthetized condition. The left side of the abdomen was shaved and a vertical incision was made through the skin. A second incision was made through the peritoneum and that skin was also retracted to reveal the kidney. Using forceps, the kidney was brought to the surface and the left ureter was tied with surgical silk, twice, below the kidney. The ligated kidney was placed gently back into its correct anatomical position then peritoneum and skin were sutured. Analgesic agent was added to reduce animal affliction. In the sham operated group, peritoneum and skin were only incised and sutured.

[0275] (7-2) Evaluation of in vivo efficacy

All monoclonal antibodies were administered by subcutaneous injection one day after UUO surgery. Anti-KLH antibody, IC17-mF18 (Heavy chain SEQ ID NO: 72, Light chain SEQ ID NO: 73), was used as a negative control in this study. The sham operated group was administered Anti-KLH antibody (IC17-mF18). The animals were weighed and then killed by exsanguination under isoflurane anesthesia 7 days after operation. Blood samples were collected from the heart cavities or the postcaval vein transferred into tubes with heparin, and centrifuged (10,000g, 10 min, 4 degrees C) to prepare plasma samples. The kidney was quickly removed. Part of the kidney tissue was snap-frozen in liquid nitrogen or on dry ice for molecular analyses.

[0276] The hydroxyproline contents in kidney, which is one of the amino acids included in collagen, was measured to evaluate the extramatrix deposition to the tissue. Wet kidney tissues were dried up at 110 degree C for overnight and weighed. Then, 6N HCl (100 microliter/1mg dry tissue) was added to the dried tissue and boiled at 110 degree C for overnight. Samples were cleaned up by the filter and 10 microliter of each sample was plated into the 96-well plate. The plate with samples was dried out at 60 degree C and hydroxyproline was measured using hydroxyproline assay kit (BioVision) or mass spectrometry analysis. The results of this experiment are shown in FIG. 15. Significant increase in hydroxyproline content was observed in disease induced kidney, and all antibodies (hT0947AE20-SG191, hT0947AE27-SG191, hT0947AE34-SG191, and hT0947AE37-SG191) inhibited kidney fibrosis. Data are presented as mean +/- standard error of the mean (SEM). Statistical analysis was performed using analysis of Student's t-test or Dunnett's multiple comparison test. When P values were <0.05, differences were considered significant.

[0277] Example 8. In vivo efficacy of anti-latent TGF-beta 1 antibodies in aGBM model

The in vivo efficacy of monoclonal antibodies hT0947AE07-mF18, hT0947AE20-mF18, hT0947AE34-mF18 and hT0947AE37-mF18 were evaluated in anti-GBM nephrotoxic serum induced glomerular nephritis model (aGBM mouse model) which is known to cause glomerulosclerosis.

[0278] (8-1) Establishment of aGBM mouse model

Seven-weeks-old female C57BL/6JJcl mice were pre-sensitized by subcutaneously injection with sheep IgG. After five days pre-sensitization, nephrotoxic serum (NTS) containing anti-GBM antibody was injected intravenously for 3 days. All mice were maintained on a regular diurnal lighting cycle (12:12 light:dark) with free access to food (CE-2, CLEA Japan, Tokyo, Japan) and water.

[0279] (8-2) Evaluation of in vivo efficacy

All monoclonal antibodies were administered 30 mg/kg by subcutaneous injection 2 times a week started from one day before the NTS injection. Anti-KLH antibody,

IC17-mF18 (Heavy chain SEQ ID NO: 72, Light chain SEQ ID NO: 73), was used as a negative control in this study. Kidneys were collected 14 days after the first NTS injection. To analyze the glomerulosclerosis, a classifier (DenseNet AI V2 (Plugin)) of Halo AI was used to create the glomerular classification model by training of annotated sclerotic and non-sclerotic glomeruli in some mice and then was applied for all mice.

The results of this experiment are shown in FIG. 16. Significant increase in glomerulosclerosis was observed in disease control mice and all anti-latent TGF-beta 1 antibodies suppressed the number of sclerotic glomeruli. Data are presented as mean +/- standard error of the mean (SEM). Statistical analysis was performed using analysis of Student's t-test or Dunnett's multiple comparison test. When P values were <0.05, differences were considered significant.

[0280] Example 9. In vivo efficacy of anti-latent TGF-beta 1 antibodies in Alport mouse model

The in vivo efficacy of monoclonal antibodies hT0947AE20-mF18 and hT0947AE37-mF18 were evaluated in Col4a3 knockout mouse model (Alport mouse model) which is known to cause progressive kidney dysfunction.

[0281] (9-1) Establishment of Col4a3 knockout mice

A Col4a3 gene knockout mouse strain was established by Zinc Finger Nuclease (ZFN)-mediated gene editing. A pair of ZFNs were designed to induce mutations in exon 48, which encodes the first part of the NC1 domain, a functional domain of the normal collagen chain. All mice were maintained on a regular diurnal lighting cycle (12:12 light:dark) with free access to food (CE-2, CLEA Japan, Tokyo, Japan) and water.

[0282] (9-2) Evaluation of in vivo efficacy

All monoclonal antibodies were administered by subcutaneous injection 2 times a week from age of 14 weeks to 20 weeks. Anti-KLH antibody, IC17dk-mF18 (Heavy chain SEQ ID NO: 72, Light chain SEQ ID NO: 73), was used as a negative control in this study. To analyze the progress of kidney disease in Alport mice, blood was collected at 14, 16, 18, and 20 weeks old from the jugular vein, and 21 weeks from abdominal portion of the vena cava under isoflurane anesthesia transferred into tubes with heparin, and centrifuged (10,000g, 10 min, 4 degrees C) to prepare plasma samples. Biochemical parameters in plasma were measured by using a TBA-120FR autoanalyzer (Canon Medical Systems Corporation, Tochigi, Japan).

[0283] The results of this experiment are shown in FIG. 17. Significant increase in plasma creatinine was observed in disease control mice and both hT0947AE20-mF18 and hT0947AE37-mF18 showed amelioration of renal function. Data are presented as mean +/- standard error of the mean (SEM). Statistical analysis was performed using

analysis of Student's t-test or Dunnett's multiple comparison test. When P values were <0.05, differences were considered significant.

[0284] Example 10. Viscosity of pH dependent anti-latent TGF-beta 1 antibodies

For viscosity measurement, antibodies with human IgG1 constant regions (SG191, SEQ ID NO: 23 and SK1, SEQ ID NO: 25) were prepared. Anti-latent TGF-beta 1 antibodies (hT0497AE07-SG191, hT0497AE20-SG191, hT0497AE27-SG191, hT0497AE34-SG191 and hT0497AE37-SG191) were prepared at 150 mg/mL in a formulation buffer (20mM His, 150mM Arg, 162.1mM Asp, 20 mM Met, pH6.0). The viscosity of mAb solutions was measured at 23 degrees C by an electromagnetic spinning (EMS) method using an EMS viscometer (Kyoto Electronics Manufacturing, Kyoto, Japan). An aluminium ball was put in a glass vial containing the samples and the viscosity of each sample solution was obtained at rotation speeds at 1000 rpm. The viscosity of the liquid sample can be calculated from the rotational speed of the aluminium ball measured by using the flash lamp and the CCD video camera.

[0285] Viscosity of anti-latent TGF-beta 1 antibodies at 150 mg/mL antibody concentration are shown in FIG. 18. pH dependent antibodies hT0947AE20-SG191, hT0947AE27-SG191 and hT0947AE37-SG191 showed lower viscosity compared to the antibody hT0947AE07-SG191, which indicates that those pH dependent antibodies are preferable to develop antibody drug product at high concentrations for sub-cutaneous administration.

[0286] Example 11. Heat accelerated stability study of pH dependent anti-latent TGF-beta 1 antibodies

Anti-latent TGF-beta 1 antibodies (hT0497AE20-SG191, hT0497AE34-SG191 and hT0497AE37-SG191) were concentrated to 130 mg/mL using the Amicon (registered trademark) Ultra 15 mL 30K (Millipore #UFC903024) in a formulation buffer (20mM His, 150mM Arg, 162.1mM Asp, 20 mM Met, 0.5 mg/mL PS80, pH6.0). Samples were kept at 40 degrees C, time points were taken at day 14, and 28. Samples were then analyzed by size exclusion chromatography (SEC; Phenomenex #00H-4788-K0) with running buffer containing 50 mM sodium phosphate, 300 mM sodium chloride, pH 7.0.

The result of SEC analysis is shown in FIG. 19. hT0497AE34-SG191 and hT0497AE37-SG191 did not show significant increase of high molecular weight species (HMW) even after treatment at 40 degrees C for 4 weeks while hT0497AE20-SG191 showed increase of HMW after treatment at 40 degrees C for 2 and 4 weeks.

[0287] Example 12. In vivo efficacy of anti-latent TGF-beta 1 antibodies in CDAHFD mouse model

The in vivo efficacy of monoclonal antibody hT0947AE37-SG191 was evaluated in

CDAHFD (choline-deficient, L-amino acid-defined, high-fat diet) induced mouse NASH/liver fibrosis model.

- [0288] (12-1) Establishment of CDAHFD induced mouse NASH/liver fibrosis model
C57BL/6JmsSlc mice male mice of 5 weeks of age were purchased from Japan SLC (Shizuoka, Japan) and were acclimated for 1 week before the start of treatments. All mice were maintained on a regular diurnal lighting cycle (12:12 light:dark) with free access to food and water. The test diet, a choline-deficient, L-amino acid-defined, high-fat diet (CDAHFD; #A06071302, Research Diets New Brunswick, NJ, USA) was fed to mice for 2 weeks or 8 weeks. During the study, 3 groups were fed CDAHFD (n=10), and 1 group (n=5) was fed CRF-1 (Oriental Yeast, Tokyo, Japan) as a normal control group.
- [0289] (12-2) Evaluation of anti-fibrotic effect
All monoclonal antibodies were administered by subcutaneous injection 2 days before sacrifice. Anti-KLH antibody, IC17dk, was used as a negative control in this study. The animals were weighed and then killed by exsanguination under isoflurane anesthesia on week 2 or week 8 after feeding CDAHFD. Blood samples were collected from the heart cavities or the postcaval vein transferred into tubes with EDTA-3K, and centrifuged (10,000g, 10 min, 4 degrees C) to prepare plasma samples. The liver was quickly removed and weighed. Part of the liver tissue was snap-frozen in liquid nitrogen or on dry ice for molecular analyses.
- [0290] Total RNA was extracted from liver tissues using a TRIzol (trademark) RNA Isolation Reagents (ThermoFisher), and cDNA was synthesized using a Transcriptor Universal cDNA Master (Roche Diagnostics). Gene expression was measured using the 7900HT Fast Real-Time PCR System (ThermoFisher). Primers and Taq-Man probes for genes were purchased from Applied Biosystems. Mouse glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as the endogenous reference for each sample. Relative mRNA expression values were calculated using double delta Ct analysis.
- [0291] The results of this experiment are shown in FIG. 20. Significant increase in collagen type 1 alpha 1 mRNA was observed in disease control mice and hT0947AE37-SG191 (AE37) showed reduction. Data are presented as mean +/- standard error of the mean (SEM). Statistical analysis was performed using analysis of Student's t-test or Dunnett's multiple comparison test. When P values were <0.05, differences were considered significant.
- [0292] Example 13. In vivo efficacy of anti-latent TGF-beta 1 antibodies in BLM mouse model
The in vivo efficacy of monoclonal antibodies hT0947AE37-SG191 was evaluated in bleomycin induced lung fibrosis model.

- [0293] (13-1) Establishment of bleomycin induced mouse lung fibrosis model
C57BL/6J mice male mice of 8 weeks of age were purchased from THE JACKSON LABORATORY JAPAN, INC (Kanagawa, Japan) and were acclimated for 1 week before the start of treatments. All mice were maintained on a regular diurnal lighting cycle (12:12 light:dark) with free access to food CE-2 (CLEA Japan, Tokyo, Japan) and water. Bleomycin (Nihonkayaku, Tokyo, Japan) was administered subcutaneously in total 10 shots (5 days consecutively administration, 2 days of withdrawal, and following 5days consecutively administration).
- [0294] (13-2) Evaluation of in vivo efficacy
All monoclonal antibodies were administered by subcutaneous injection once a week at day 14 and 21 after disease induction. Vehicle or anti-KLH antibody, IC17dk, was used as a negative control in this study. The animals were weighed and then killed by exsanguination under isoflurane anesthesia on day 28 after disease induction. Blood samples were collected from the heart cavities or the postcaval vein transferred into tubes with heparin, and centrifuged (10,000g, 10 min, 4 degrees C) to prepare plasma samples. The lung was quickly removed and weighed. Part of the lung tissue was snap-frozen in liquid nitrogen or on dry ice for molecular analyses.
- [0295] The hydroxyproline contents in lung, which is one of the amino acids included in collagen, was measured to evaluate the extramatrix deposition to the tissue. Wet lung tissues were dried up by freeze dryer for overnight and weighed. Then, 6N HCl (100 microliter/1mg dry tissue) was added to the dried tissue and boiled at 110 degree C for overnight. Samples were cleaned up by the filter and measured using hydroxyproline assay kit (QuickZyme) or mass spectrometry analysis.
- [0296] Total RNA was extracted from lung tissues using RNeasy Mini Kit (Qiagen), and cDNA was synthesized using a Transcriptor Universal cDNA Master (Roche Diagnostics). Gene expression was measured using the 7900HT Fast Real-Time PCR System (ThermoFisher). Primers and Taq-Man probes for genes were purchased from Applied Biosystems. Mouse mitochondrial ribosomal protein L19 (MRPL19) was used as the endogenous reference for each sample. Relative mRNA expression values were calculated using double delta Ct analysis.
- [0297] The results of this experiment are shown in FIG. 21. Significant increase in collagen type 1 alpha 1 mRNA, Serpine1 mRNA, and hydroxyproline content was observed in disease induced lung and hT0947AE37-SG191 (AE37) showed reduction. Data are presented as mean +/- standard error of the mean (SEM). Statistical analysis was performed using analysis of Student's t-test or Dunnett's multiple comparison test. When P values were <0.05, differences were considered significant.
- [0298] The disclosure of Intl. Publ. Nos. WO2019/163927 and WO2021/039945, are herein incorporated in their entirety by reference.

The disclosure of Japanese Patent Appl. No. 2023-019304 is herein incorporated in its entirety by reference.

[0299] Although the present invention is described herein in some detail by way of illustration and example for purposes of clarity of understanding, the descriptions and examples should not be construed as limiting the scope of the invention. The disclosures of all patent and scientific literature cited herein are expressly incorporated in their entirety by reference.

Industrial Applicability

[0300] The present disclosure provides cross-species, humanized and optimized anti-latent TGF-beta 1 antibodies which inhibit a protease mediated activation of latent TGF-beta 1 without inhibiting integrin mediated activation of latent TGF-beta 1 or with partially inhibiting integrin mediated activation of latent TGF-beta 1, or which inhibit a protease mediated activation of latent TGF-beta 1 but have less impact on inhibiting integrin mediated activation of latent TGF-beta 1 activation. Further, the present disclosure provides anti-TGF-beta 1 antibodies which have pH-dependent binding affinity towards latent TGF-beta 1. The anti-latent TGF-beta 1 antibodies are expected to be useful for treating TGF-beta 1-related diseases such as fibrosis and cancer. For example, renal fibrosis, liver fibrosis, and lung fibrosis may be treated using the anti-latent TGF-beta 1 antibodies.

Claims

[Claim 1]

An anti-latent TGF-beta 1 antibody comprising:

- (a) HVR-H1, HVR-H2 and HVR-H3 comprising the amino acid sequences of SEQ ID NO: 29, 30 and 36, respectively, and HVR-L1, HVR-L2 and HVR-L3 comprising the amino acid sequences of SEQ ID NO: 49, 52 and 56, respectively;
- (b) HVR-H1, HVR-H2 and HVR-H3 comprising the amino acid sequences of SEQ ID NO: 28, 32 and 37, respectively, and HVR-L1, HVR-L2 and HVR-L3 comprising the amino acid sequences of SEQ ID NO: 50, 52 and 56, respectively;
- (c) HVR-H1, HVR-H2 and HVR-H3 comprising the amino acid sequences of SEQ ID NO: 27, 30 and 39, respectively, and HVR-L1, HVR-L2 and HVR-L3 comprising the amino acid sequences of SEQ ID NO: 48, 53 and 56, respectively; or
- (d) HVR-H1, HVR-H2 and HVR-H3 comprising the amino acid sequences of SEQ ID NO: 27, 33 and 40, respectively, and HVR-L1, HVR-L2 and HVR-L3 comprising the amino acid sequences of SEQ ID NO: 51, 52 and 56, respectively.

[Claim 2]

The anti-latent TGF-beta 1 antibody of claim 1, comprising:

- (a) (i) a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 4, (ii) a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 16, or (iii) a VH sequence as in (i) and a VL sequence as in (ii);
- (b) (i) a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 9, (ii) a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 21, or (iii) a VH sequence as in (i) and a VL sequence as in (ii);
- (c) (i) a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 11, (ii) a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 17, or (iii) a VH sequence as in (i) and a VL sequence as in (ii); or
- (d) (i) a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 10, (ii) a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 20, or (iii) a VH sequence as in (i) and a VL sequence as in (ii).

[Claim 3]

The anti-latent TGF-beta 1 antibody of claim 1, comprising:

- (a) a VH sequence of SEQ ID NO: 4 and a VL sequence of SEQ ID

- NO: 16;
- (b) a VH sequence of SEQ ID NO: 9 and a VL sequence of SEQ ID NO: 21;
- (c) a VH sequence of SEQ ID NO: 11 and a VL sequence of SEQ ID NO: 17; or
- (d) a VH sequence of SEQ ID NO: 10 and a VL sequence of SEQ ID NO: 20.
- [Claim 4] The anti-latent TGF-beta 1 antibody of claim 1, which is a human, humanized, or chimeric antibody.
- [Claim 5] The anti-latent TGF-beta 1 antibody of claim 1, which is an IgG antibody.
- [Claim 6] The anti-latent TGF-beta 1 antibody of claim 1, wherein the anti-latent TGF-beta 1 antibody comprises a modified IgG1 Fc region having reduced effector function compared with a wild type IgG1 Fc region.
- [Claim 7] The anti-latent TGF-beta 1 antibody of claim 1, wherein the anti-latent TGF beta 1 antibody binds to latent TGF-beta 1 in a pH-dependent manner.
- [Claim 8] An anti-latent TGF-beta 1 antibody comprising:
- (a) a heavy chain sequence of SEQ ID NO: 82 and a light chain sequence of SEQ ID NO: 83;
- (b) a heavy chain sequence of SEQ ID NO: 84 and a light chain sequence of SEQ ID NO: 85;
- (c) a heavy chain sequence of SEQ ID NO: 86 and a light chain sequence of SEQ ID NO: 87;
- (d) a heavy chain sequence of SEQ ID NO: 88 and a light chain sequence of SEQ ID NO: 89;
- (e) a heavy chain sequence of SEQ ID NO: 90 and a light chain sequence of SEQ ID NO: 83;
- (f) a heavy chain sequence of SEQ ID NO: 91 and a light chain sequence of SEQ ID NO: 85;
- (g) a heavy chain sequence of SEQ ID NO: 92 and a light chain sequence of SEQ ID NO: 87; or
- (h) a heavy chain sequence of SEQ ID NO: 93 and a light chain sequence of SEQ ID NO: 89.
- [Claim 9] An immunoconjugate comprising the anti-latent TGF beta-1 antibody of claim 1 and a cytotoxic agent.
- [Claim 10] An isolated nucleic acid encoding the anti-latent TGF beta-1 antibody of claim 1.

- [Claim 11] A vector comprising the nucleic acid of claim 10.
- [Claim 12] A host cell comprising the nucleic acid of claim 10.
- [Claim 13] A method of producing an anti-latent TGF-beta 1 antibody comprising culturing the host cell of claim 12 so that the antibody is produced.
- [Claim 14] An immunoconjugate comprising the anti-latent TGF beta-1 antibody of claim 8 and a cytotoxic agent.
- [Claim 15] An isolated nucleic acid encoding the anti-latent TGF beta-1 antibody of claim 8.
- [Claim 16] A vector comprising the nucleic acid of claim 15.
- [Claim 17] A host cell comprising the nucleic acid of claim 15.
- [Claim 18] A method of producing an anti-latent TGF-beta 1 antibody comprising culturing the host cell of claim 17 so that the antibody is produced.
- [Claim 19] A pharmaceutical formulation comprising the anti-latent TGF-beta 1 antibody of claim 1, and a pharmaceutically acceptable carrier.
- [Claim 20] A pharmaceutical formulation comprising the anti-latent TGF-beta 1 antibody of claim 8, and a pharmaceutically acceptable carrier.
- [Claim 21] A pharmaceutical formulation comprising the immunoconjugate of claim 9, and a pharmaceutically acceptable carrier.
- [Claim 22] A pharmaceutical formulation comprising the immunoconjugate of claim 14, and a pharmaceutically acceptable carrier.
- [Claim 23] The pharmaceutical formulation of claim 19 for use in treatment of fibrosis or cancer.
- [Claim 24] The pharmaceutical formulation of claim 20 for use in treatment of fibrosis or cancer.
- [Claim 25] The pharmaceutical formulation of claim 21 for use in treatment of fibrosis or cancer.
- [Claim 26] The pharmaceutical formulation of claim 22 for use in treatment of fibrosis or cancer.
- [Claim 27] A method of treating fibrosis or cancer comprising administering to a subject in need thereof a therapeutically effective amount of the antibody of claim 1.
- [Claim 28] A method of treating fibrosis or cancer comprising administering to a subject in need thereof a therapeutically effective amount of the antibody of claim 8.
- [Claim 29] A method of treating fibrosis or cancer comprising administering to a subject in need thereof a therapeutically effective amount of the immunoconjugate of claim 9.
- [Claim 30] A method of treating fibrosis or cancer comprising administering to a

subject in need thereof a therapeutically effective amount of the immunoconjugate of claim 14.