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(54) Title: HUMAN ANTIBODIES TO GREM 1

(57) Abstract: The present invention provides antibodies that bind to human gremlin-1 (GREM1), and methods of use. According to certain embodiments of the invention, the antibodies are fully human antibodies that bind to GREM1. The antibodies of the invention are useful for inhibiting or neutralizing GREM1 activity, thus providing a means of treating a GREM1 -related disease or disorder such as fibrosis and cancer. In some embodiments, the antibodies of the present invention are used in treating at least one symptom or complication of fibrosis of the liver, lungs or kidney.

HUMAN ANTIBODIES TO GREM1

FIELD OF THE INVENTION

[001] The present invention is related to human antibodies and antigen-binding fragments of human antibodies that specifically bind to human gremlin-1 (GREM1), and therapeutic and diagnostic methods of using those antibodies.

STATEMENT OF RELATED ART

[002] Fibrosis is a scarring process that is a common feature of chronic organ injury. It is characterized by elevated activity of transforming growth factor-beta (TGF-β) resulting in increased and altered deposition of extracellular matrix and other fibrosis-associated proteins.

[003] Bone morphogenetic proteins (BMPs) are phylogenetically conserved signaling molecules that belong to the TGF-β superfamily and are involved in growth, development and differentiation of various cell types (Yanagita, M., 2009, Biofactors DOI: 10:1002/biof.15). The biological responses to BMPs are negatively regulated by BMP antagonists that can directly associate with BMPs and inhibit receptor binding. Human gremlin-1 (GREM1), a member of the cysteine knot superfamily, is an antagonist for BMP signaling (Hsu, D.R., et al 1998, Mol. Cell 1: 673-683). It binds to BMP2, BMP4 and BMP7. GREM1 blocks BMP signaling, which is thought to be anti-fibrotic in many tissues by blocking the binding of BMP to its receptor.

[004] The expression of GREM1 in normal adult kidney, liver and lung is very low. However, GREM1 expression is increased in both mouse models of fibrosis and human fibrotic diseases such as diabetic nephropathy and pulmonary fibrosis (Koli et al., 2006, Am. J. Pathol. 169: 61-71; Farkas, et al., 2011, Am. J. Respir. Cell. Mol. Biol. 44: 870-878; Lappin, et al., 2002, Nephrol. Dial. Transplant. 17: 65-67). Increased GREM1 expression leads to a reduction in anti-fibrotic BMP signaling. Increased GREM1 expression also correlates with increased serum creatinine levels and tubulointerstitial fibrosis scores in these diseases (Dolan, V., et al 2005, Am. J. Kidney Dis. 45: 1034-9). In several fibrosis models, such as lung and kidney fibrosis, the expression of GREM1 is greatly increased while BMP signaling is decreased (Myllarniemi, et al., 2008, Am. J. Respir. Crit. Care Med. 177: 321-329). Administration of BMP7 can decrease fibrosis in some models of kidney disease, but does not protect against bleomycin-induced lung or skin fibrosis (Weiskirchen, et al., 2009, 14: 4992-5012).

[005] Mice heterozygous for GREM1 show some protection against fibrosis in an experimental model of diabetic nephropathy (Zhang, et al., 2009, BBRC 383: 1-3). These mice show no difference in the onset, severity and progression of diabetes as measured by weight loss and hyperglycemia. They do, however, have attenuated fibrotic structural changes in the kidney and reduced changes in kidney function.

[006] GREM1 may thus serve as a potential therapeutic target for the treatment of fibrotic diseases. There is a need to develop specific GREM1 inhibitors in fibrosis treatment which do

not have any side-effects.

[007] In addition, GREM1 is an agonist of the major proangiogenic receptor vascular endothelial growth factor receptor-2 (VEGFR-2) and may play an oncogenic role especially in carcinomas of the uterine cervix, lung, ovary, kidney, breast, colon, pancreas, and sarcoma (Namkoong et al 2006, BMC Cancer 6: 74 doi:10.1186/1471-2407-6-74; Mitola et al 2010; Blood 116: 3677-3680). Heparan sulfate (HS) and heparin, glycosaminoglycans (GAGs) known for their anticoagulant effects, have been shown to bind to GREM1. GREM1 binds to heparin and activates VEGFR-2 in a BMP-independent manner (Chiodelli et al 2011; Arterioscler. Thromb. Vasc. Biol. 31: e116-e127).

[008] Anti-GREM1 polyclonal and monoclonal antibodies are available commercially (for example, from Sigma-Aldrich, Abnova Corporation, Novus Biologicals, Genway). US 6432410 discloses the nucleotide and protein sequences of human, mouse, xenopus and chick GREM1 and deletion mutants thereof. US20090203041 discloses GREM1 peptide sequences for use as BMP4 inhibitors. Kim et al disclose GREM1 antibodies which inhibit GREM1 in a manner independent of BMP or VEGFR-2 in PLoS One 7(4): e35100. doi:10.1371/journal.pone.0035100 and in WO2013137686. US 7744873 discloses methods for treating glaucoma by administering a GREM1 antagonist, wherein the antagonist is an antibody that binds GREM1. Methods of treatment and formulations for glaucoma or cancer using GREM1 antagonists including antibodies have been described in EP1440159B1, EP1777519A1, EP2053135A1, and US 20090041757.

BRIEF SUMMARY OF THE INVENTION

[009] The invention provides fully human monoclonal antibodies (mAbs) and antigen-binding fragments thereof that bind specifically to human GREM1. Such antibodies may be useful to neutralize the activity of GREM1 or to block binding of GREM1 to a bone morphogenetic protein (BMP) such as BMP2, BMP4 or BMP7. In certain other embodiments, the antibodies may be useful to neutralize the activity or block binding to heparin or heparan sulfate. The antibodies may act to halt the progression, or to lessen the severity of a fibrosis-associated condition or disease, or reduce the number, the duration, or the severity of disease recurrence, or ameliorate at least one symptom associated with fibrosis or cancer. Such antibodies may be used alone or in conjunction with a second agent useful for treating fibrosis or cancer. In certain embodiments, the antibodies specific for GREM1, may be given therapeutically in conjunction with a second agent to lessen the severity of the fibrosis-associated condition or cancer, or to reduce the number, the duration, or the severity of disease recurrence, or ameliorate at least one symptom associated with the fibrosis-associated condition or cancer. In certain embodiments, the antibodies may be used prophylactically as stand-alone therapy to protect patients who are at risk for developing a fibrosis-associated condition or disease. For example, certain patient populations may be at risk for developing a fibrosis condition or disease, including elderly

patients, or patients with family history, or patients with problems of alcohol or drug abuse, or patients who have chronic and/or concomitant underlying medical conditions such as diabetes, metabolic disorders, liver injury, renal injury or lung injury that may pre-dispose them to fibrosis. Other at-risk patient populations include individuals exposed to chemicals such as asbestos or other pollutants, or smokers. Any of these patient populations may benefit from treatment with the antibodies of the invention, when given alone or in conjunction with a second agent.

[010] The antibodies of the present invention may be used to treat fibrosis in lungs, liver, kidney, skin, heart, gut or muscle of a patient. In other embodiments, the antibodies of the invention may be used to treat cancer such as carcinoma of the uterine cervix, lung, ovary, kidney, breast, colon, or pancreas. The antibodies can be full-length (for example, an IgG1 or IgG4 antibody) or may comprise only an antigen-binding portion (for example, a Fab, $F(ab')_2$ or scFv fragment), and may be modified to affect functionality, *e.g.,* to eliminate residual effector functions (Reddy *et al.,* (2000), J. Immunol. 164:1925-1933).

[011] Accordingly, in a first aspect, the invention provides an isolated fully human monoclonal antibody or antigen-binding fragment thereof that binds to human GREM1.

[012] In one embodiment, the human monoclonal antibody binds to GREM1 of SEQ ID NO: 594 or SEQ ID NO: 595.

[013] In one embodiment, the isolated antibody or antigen-binding fragment thereof blocks GREM1 binding to BMP2, BMP4, BMP7 or heparin.

[014] In one embodiment, the isolated human antibody or antigen-binding fragment thereof binds to GREM1 with a K_D equal to or less than 10^{-7} M as measured by surface plasmon resonance.

[015] In one embodiment, the isolated antibody or antigen-binding fragment thereof exhibits one or more properties selected from the group consisting of: (a) binds GREM1 at 37°C with a binding dissociation equilibrium constant (K_D) of less than about 275nM as measured by surface plasmon resonance; (b) binds to GREM1 at 37°C with a dissociative half-life (t½) of greater than about 3 minutes as measured by surface plasmon resonance; (c) binds GREM1 at 25°C with a K_D of less than about 280nM as measured by surface plasmon resonance; (d) binds to GREM1 at 25°C with a t½ of greater than about 2 minutes as measured by surface plasmon resonance; (e) blocks GREM1 binding to BMP4 with an IC_{50} of less than about 1.9nM as measured in a competition ELISA assay at 25°C; (f) blocks GREM1-mediated inhibition of BMP signaling and promotes cell differentiation; and (g) blocks GREM1 binding to heparin.

[016] In one embodiment, the isolated human antibody or antigen-binding fragment thereof which binds to GREM1 comprises three heavy chain complementarity determining regions (CDRs) (HCDR1, HCDR2 and HCDR3) contained within any one of the heavy chain variable region (HCVR) sequences selected from the group consisting of SEQ ID NOs: 2, 18, 34, 50, 66, 82, 98, 114, 130, 146, 162, 178, 194, 210, 226, 242, 258, 274, 290, 306, 322, 338, 354, 370, 386, 402, 418, 434, 450, 466, 482, 498, 514, 530, 546, 562, and 578; and three light chain

CDRs (LCDR1, LCDR2 and LCDR3) contained within any one of the light chain variable region (LCVR) sequences selected from the group consisting of SEQ ID NOs: 10, 26, 42, 58, 74, 90, 106, 122, 138, 154, 170, 186, 202, 218, 234, 250, 266, 282, 298, 314, 330, 346, 362, 378, 394, 410, 426, 442, 458, 474, 490, 506, 522, 538, 554, 570, and 586. Methods and techniques for identifying CDRs within HCVR and LCVR amino acid sequences are well known in the art and can be used to identify CDRs within the specified heavy chain variable region(s) (HCVR) and/or light chain variable region(s) (LCVR) amino acid sequences disclosed herein. Exemplary conventions that can be used to identify the boundaries of CDRs include, *e.g.*, the Kabat definition, the Chothia definition, and the AbM definition. In general terms, the Kabat definition is based on sequence variability, the Chothia definition is based on the location of the structural loop regions, and the AbM definition is a compromise between the Kabat and Chothia approaches. *See*, *e.g.*, Kabat, "Sequences of Proteins of Immunological Interest," National Institutes of Health, Bethesda, Md. (1991); Al-Lazikani *et al.*, (1997), *J. Mol. Biol. 273*:927-948; and Martin *et al.*, (1989), *Proc. Natl. Acad. Sci. USA 86*:9268-9272. Public databases are also available for identifying CDR sequences within an antibody.

[017] In one embodiment, the isolated human antibody or antigen-binding fragment thereof which binds to GREM1 comprises a HCVR having an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 18, 34, 50, 66, 82, 98, 114, 130, 146, 162, 178, 194, 210, 226, 242, 258, 274, 290, 306, 322, 338, 354, 370, 386, 402, 418, 434, 450, 466, 482, 498, 514, 530, 546, 562, and 578.

[018] In one embodiment, the isolated human antibody or antigen-binding fragment thereof which binds to GREM1 comprises a LCVR having an amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 26, 42, 58, 74, 90, 106, 122, 138, 154, 170, 186, 202, 218, 234, 250, 266, 282, 298, 314, 330, 346, 362, 378, 394, 410, 426, 442, 458, 474, 490, 506, 522, 538, 554, 570 and 586.

[019] In one embodiment, the isolated human antibody or antigen-binding fragment thereof which binds to GREM1 comprises (a) a HCVR having an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 18, 34, 50, 66, 82, 98, 114, 130, 146, 162, 178, 194, 210, 226, 242, 258, 274, 290, 306, 322, 338, 354, 370, 386, 402, 418, 434, 450, 466, 482, 498, 514, 530, 546, 562, and 578; and (b) a LCVR having an amino acid sequence selected from the group consisting of SEQ ID NO: 10, 26, 42, 58, 74, 90, 106, 122, 138, 154, 170, 186, 202, 218, 234, 250, 266, 282, 298, 314, 330, 346, 362, 378, 394, 410, 426, 442, 458, 474, 490, 506, 522, 538, 554, 570 and 586.

[020] In one embodiment, the isolated human antibody or antigen-binding fragment thereof which binds to GREM1 comprises :

(a) a HCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 4, 20, 36, 52, 68, 84, 100, 116, 132, 148, 164, 180, 196, 212, 228, 244, 260, 276,

292, 308, 324, 340, 356, 372, 388, 404, 420, 436, 452, 468, 484, 500, 516, 532, 548, 564, and 580;

(b) a HCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 6, 22, 38, 54, 70, 86, 102, 118, 134, 150, 166, 182, 198, 214, 230, 246, 262, 278, 294, 310, 326, 342, 358, 374, 390, 406, 422, 438, 454, 470, 486, 502, 518, 534, 550, 566 and 582;

(c) a HCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 8, 24, 40, 56, 72, 88, 104, 120, 136, 152, 168, 184, 200, 216, 232, 248, 264, 280, 296, 312, 328, 344, 360, 376, 392, 408, 424, 440, 456, 472, 488, 504, 520, 536, 552, 568 and 584;

(d) a LCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 12, 28, 44, 60, 76, 92, 108, 124, 140, 156, 172, 188, 204, 220, 236, 252, 268, 284, 300, 316, 332, 348, 364, 380, 396, 412, 428, 444, 460, 476, 492, 508, 524, 540, 556, 572 and 588;

(e) a LCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 14, 30, 46, 62, 78, 94, 110, 126, 142, 158, 174, 190, 206, 222, 238, 254, 270, 286, 302, 318, 334, 350, 366, 382, 398, 414, 430, 446, 462, 478, 494, 510, 526, 542, 558, 574, and 590; and

(f) a LCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 16, 32, 48, 64, 80, 96, 112, 128, 144, 160, 176, 192, 208, 224, 240, 256, 272, 288, 304, 320, 336, 352, 368, 384, 400, 416, 432, 448, 464, 480, 496, 512, 528, 544, 560, 576 and 592.

[021] In one embodiment, the isolated human antibody or antigen-binding fragment thereof which binds to GREM1 comprises a HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NOs: 2/10, 18/26, 34/42, 50/58, 66/74, 82/90, 98/106, 114/122, 130/138, 146/154, 162/170, 178/186, 194/202, 210/218, 226/234, 242/250, 258/266, 274/282, 290/298, 306/314, 322/330, 338/346, 354/362, 370/378, 386/394, 402/410, 418/426, 434/442, 450/458, 466/474, 482/490, 498/506, 514/52, 530/538, 546/554, 562/570, and 578/586.

[022] In one embodiment, the invention provides a fully human monoclonal antibody or antigen-binding fragment thereof that binds to GREM1, wherein the antibody or fragment thereof exhibits one or more of the following characteristics: (i) comprises a HCVR having an amino acid sequence selected from the group consisting of SEQ ID NO: 2, 18, 34, 50, 66, 82, 98, 114, 130, 146, 162, 178, 194, 210, 226, 242, 258, 274, 290, 306, 322, 338, 354, 370, 386, 402, 418, 434, 450, 466, 482, 498, 514, 530, 546, 562, and 578, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; (ii) comprises a LCVR having an amino acid sequence selected from the group consisting of SEQ ID NO: 10, 26, 42, 58, 74, 90, 106, 122, 138, 154, 170, 186, 202, 218, 234, 250, 266, 282, 298, 314, 330, 346, 362, 378, 394, 410, 426, 442, 458, 474, 490, 506, 522, 538, 554, 570, and 586, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; (iii) comprises a HCDR3 domain having an amino acid sequence

selected from the group consisting of SEQ ID NO: 8, 24, 40, 56, 72, 88, 104, 120, 136, 152, 168, 184, 200, 216, 232, 248, 264, 280, 296, 312, 328, 344, 360, 376, 392, 408, 424, 440, 456, 472, 488, 504, 520, 536, 552, 568, and 584, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and a LCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 16, 32, 48, 64, 80, 96, 112, 128, 144, 160, 176, 192, 208, 224, 240, 256, 272, 288, 304, 320, 336, 352, 368, 384, 400, 416, 432, 448, 464, 480, 496, 512, 528, 544, 560, 576, and 592, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; (iv) comprises a HCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 4, 20, 36, 52, 68, 84, 100, 116, 132, 148, 164, 180, 196, 212, 228, 244, 260, 276, 292, 308, 324, 340, 356, 372, 388, 404, 420, 436, 452, 468, 484, 500, 516, 532, 548, 564, and 580, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a HCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 6, 22, 38, 54, 70, 86, 102, 118, 134, 150, 166, 182, 198, 214, 230, 246, 262, 278, 294, 310, 326, 342, 358, 374, 390, 406, 422, 438, 454, 470, 486, 502, 518, 534, 550, 566, and 582, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a LCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 12, 28, 44, 60, 76, 92, 108, 124, 140, 156, 172, 188, 204, 220, 236, 252, 268, 284, 300, 316, 332, 348, 364, 380, 396, 412, 428, 444, 460, 476, 492, 508, 524, 540, 556, 572, and 588, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and a LCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 14, 30, 46, 62, 78, 94, 110, 126, 142, 158, 174, 190, 206, 222, 238, 254, 270, 286, 302, 318, 334, 350, 366, 382, 398, 414, 430, 446, 462, 478, 494, 510, 526, 542, 558, 574, and 590, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; (v) binds to GREM1 with a K_D equal to or less than 10^{-7} M as measured by surface plasmon resonance.

[023] In a second aspect, the invention provides an isolated antibody or antigen-binding fragment thereof that competes for specific binding to human GREM1 with an antibody or antigen-binding fragment comprising the complementarity determining regions (CDRs) of a heavy chain variable region (HCVR), wherein the HCVR has an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 18, 34, 50, 66, 82, 98, 114, 130, 146, 162, 178, 194, 210, 226, 242, 258, 274, 290, 306, 322, 338, 354, 370, 386, 402, 418, 434, 450, 466, 482, 498, 514, 530, 546, 562, and 578; and the CDRs of a light chain variable region (LCVR), wherein the LCVR has an amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 26, 42, 58, 74, 90, 106, 122, 138, 154, 170, 186, 202, 218, 234, 250, 266, 282, 298, 314, 330, 346, 362, 378, 394, 410, 426, 442, 458, 474, 490, 506, 522, 538, 554, 570, and 586.

[024] In one embodiment, the invention provides an isolated antibody or antigen-binding fragment thereof that binds the same epitope on human GREM1 as an antibody or antigen-binding fragment comprising the CDRs of a heavy chain variable region (HCVR), wherein the HCVR has an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 18, 34, 50, 66, 82, 98, 114, 130, 146, 162, 178, 194, 210, 226, 242, 258, 274, 290, 306, 322, 338, 354, 370, 386, 402, 418, 434, 450, 466, 482, 498, 514, 530, 546, 562, and 578; and the CDRs of a light chain variable region (LCVR), wherein the LCVR has an amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 26, 42, 58, 74, 90, 106, 122, 138, 154, 170, 186, 202, 218, 234, 250, 266, 282, 298, 314, 330, 346, 362, 378, 394, 410, 426, 442, 458, 474, 490, 506, 522, 538, 554, 570, and 586.

[025] In one embodiment, the invention provides for an isolated antibody or antigen-binding fragment thereof that blocks binding of human GREM1 to any one of BMP2, BMP4, BMP7 or heparin, the antibody comprising the complementarity determining regions (CDRs) of a heavy chain variable region (HCVR), wherein the HCVR has an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 18, 34, 50, 66, 82, 98, 114, 130, 146, 162, 178, 194, 210, 226, 242, 258, 274, 290, 306, 322, 338, 354, 370, 386, 402, 418, 434, 450, 466, 482, 498, 514, 530, 546, 562, and 578; and the CDRs of a light chain variable region (LCVR), wherein the LCVR has an amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 26, 42, 58, 74, 90, 106, 122, 138, 154, 170, 186, 202, 218, 234, 250, 266, 282, 298, 314, 330, 346, 362, 378, 394, 410, 426, 442, 458, 474, 490, 506, 522, 538, 554, 570, and 586.

[026] In one embodiment, the invention provides a fully human monoclonal antibody or antigen-binding fragment thereof that binds to GREM1, wherein the antibody or fragment thereof exhibits one or more of the following characteristics: (i) comprises a HCVR having an amino acid sequence selected from the group consisting of SEQ ID NO: 2, 18, 34, 50, 66, 82, 98, 114, 130, 146, 162, 178, 194, 210, 226, 242, 258, 274, 290, 306, 322, 338, 354, 370, 386, 402, 418, 434, 450, 466, 482, 498, 514, 530, 546, 562, and 578, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; (ii) comprises a LCVR having an amino acid sequence selected from the group consisting of SEQ ID NO: 10, 26, 42, 58, 74, 90, 106, 122, 138, 154, 170, 186, 202, 218, 234, 250, 266, 282, 298, 314, 330, 346, 362, 378, 394, 410, 426, 442, 458, 474, 490, 506, 522, 538, 554, 570, and 586, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; (iii) comprises a HCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 8, 24, 40, 56, 72, 88, 104, 120, 136, 152, 168, 184, 200, 216, 232, 248, 264, 280, 296, 312, 328, 344, 360, 376, 392, 408, 424, 440, 456, 472, 488, 504, 520, 536, 552, 568, and 584, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and a LCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 16, 32, 48, 64, 80, 96, 112, 128, 144, 160, 176, 192, 208, 224, 240, 256, 272, 288, 304, 320, 336,

352, 368, 384, 400, 416, 432, 448, 464, 480, 496, 512, 528, 544, 560, 576, and 592, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; (iv) comprises a HCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 4, 20, 36, 52, 68, 84, 100, 116, 132, 148, 164, 180, 196, 212, 228, 244, 260, 276, 292, 308, 324, 340, 356, 372, 388, 404, 420, 436, 452, 468, 484, 500, 516, 532, 548, 564, and 580, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a HCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 6, 22, 38, 54, 70, 86, 102, 118, 134, 150, 166, 182, 198, 214, 230, 246, 262, 278, 294, 310, 326, 342, 358, 374, 390, 406, 422, 438, 454, 470, 486, 502, 518, 534, 550, 566, and 582, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a LCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 12, 28, 44, 60, 76, 92, 108, 124, 140, 156, 172, 188, 204, 220, 236, 252, 268, 284, 300, 316, 332, 348, 364, 380, 396, 412, 428, 444, 460, 476, 492, 508, 524, 540, 556, 572, and 588, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and a LCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 14, 30, 46, 62, 78, 94, 110, 126, 142, 158, 174, 190, 206, 222, 238, 254, 270, 286, 302, 318, 334, 350, 366, 382, 398, 414, 430, 446, 462, 478, 494, 510, 526, 542, 558, 574, and 590, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; (v) binds to GREM1 with a K_D equal to or less than 10^{-7} M as measured by surface plasmon resonance; (vi) blocks GREM1 binding to one of BMP2, BMP4 or BMP7; (vii) blocks GREM1-inhibition of BMP signaling and promotes cell differentiation; and (viii) blocks GREM1 binding to heparin.

[027] In a third aspect, the invention provides nucleic acid molecules encoding anti-GREM1 antibodies or fragments thereof. Recombinant expression vectors carrying the nucleic acids of the invention, and host cells into which such vectors have been introduced, are also encompassed by the invention, as are methods of producing the antibodies by culturing the host cells under conditions permitting production of the antibodies, and recovering the antibodies produced.

[028] In one embodiment, the invention provides an antibody or fragment thereof comprising a HCVR encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1, 17, 33, 49, 65, 81, 97, 113, 129, 145, 161, 177, 193, 209, 225, 241, 257, 273, 289, 305, 321, 337, 353, 369, 385, 401, 417, 433, 449, 465, 481, 497, 513, 529, 545, 561, and 577, or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% homology thereof.

[029] In one embodiment, the antibody or fragment thereof further comprises a LCVR encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO: 9, 25, 41, 57, 73,

89, 105, 121, 137, 153, 169, 185, 201, 217, 233, 249, 265, 281, 297, 313, 329, 345, 361, 377, 393, 409, 425, 441, 457, 473, 489, 505, 521, 537, 553, 569, and 585, or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% homology thereof.

[030] In one embodiment, the invention provides an antibody or antigen-binding fragment of an antibody comprising a HCDR3 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO: 7, 23, 39, 55, 71, 87, 103, 119, 135, 151, 167, 183, 199, 215, 231, 247, 263, 279, 295, 311, 327, 343, 359, 375, 391, 407, 423, 439, 455, 471, 487, 503, 519, 535, 551, 567, and 583, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and a LCDR3 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO: 15, 31, 47, 63, 79, 95, 111, 127, 143, 159, 175, 191, 207, 223, 239, 255, 271, 287, 303, 319, 335, 351, 367, 383, 399, 415, 431, 447, 463, 479, 495, 511, 527, 543, 559, 575, and 591, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[031] In one embodiment, the invention provides an antibody or fragment thereof further comprising a HCDR1 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO: 3, 19, 35, 51, 67, 83, 99, 115, 131, 147, 163, 179, 195, 211, 227, 243, 259, 275, 291, 307, 323, 339, 355, 371, 387, 403, 419, 435, 451, 467, 483, 499, 515, 531, 547, 563, and 579, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a HCDR2 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO: 5, 21, 37, 53, 69, 85, 101, 117, 133, 149, 165, 181, 197, 213, 229, 245, 261, 277, 293, 309, 325, 341, 357, 373, 389, 405, 421, 437, 453, 469, 485, 501, 517, 533, 549, 565, and 581, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a LCDR1 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO: 11, 27, 43, 59, 75, 91, 107, 123, 139, 155, 171, 187, 203, 219, 235, 251, 267, 283, 299, 315, 331, 347, 363, 379, 395, 411, 427, 443, 459, 475, 491, 507, 523, 539, 555, 571, and 587, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and a LCDR2 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NO: 13, 29, 45, 61, 77, 93, 109, 125, 141, 157, 173, 189, 205, 221, 237, 253, 269, 285, 301, 317, 333, 349, 365, 381, 397, 413, 429, 445, 461, 477, 493, 509, 525, 541, 557, 573, and 589, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[032] In some embodiments, the antibody or antigen-binding fragment thereof to human GREM1, as described herein may be linked to a detectable label such as a radionuclide label or a MRI-detectable label.

[033] In a fourth aspect, the invention provides a pharmaceutical composition comprising an isolated fully human monoclonal antibody or antigen-binding fragment thereof that binds to

GREM1 and a pharmaceutically acceptable carrier or diluent. In one embodiment, the invention provides a pharmaceutical composition comprising an isolated fully human monoclonal antibody or antigen-binding fragment thereof that binds specifically to the secreted form of human GREM1 and a pharmaceutically acceptable carrier or diluent. In one embodiment, the invention provides a pharmaceutical composition comprising an isolated fully human monoclonal antibody or antigen-binding fragment thereof that binds specifically to the membrane-associated form of GREM1 (mature GREM1 protein) and a pharmaceutically acceptable carrier or diluent.

[034] In one embodiment, the pharmaceutical composition comprises a fully human monoclonal antibody that binds to GREM1 having any one or more of the characteristics described herein. The antibody that binds to GREM1 binds with a K_D equal to or less than 10^{-7}M.

[035] In one embodiment, the composition comprises an antibody that binds to human GREM1 and has a HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NOs: 2/10, 18/26, 34/42, 50/58, 66/74, 82/90, 98/106, 114/122, 130/138, 146/154, 162/170, 178/186, 194/202, 210/218, 226/234, 242/250, 258/266, 274/282, 290/298, 306/314, 322/330, 338/346, 354/362, 370/378, 386/394, 402/410, 418/426, 434/442, 450/458, 466/474, 482/490, 498/506, 514/522, 530/538, 546/554, 562/570, and 578/586.

[036] In one embodiment, the invention features a composition, which is a combination of an antibody or antigen-binding fragment of an antibody of the invention, and a second therapeutic agent.

[037] The second therapeutic agent may be a small molecule drug, a protein/polypeptide, an antibody, a nucleic acid molecule, such as an anti-sense oligonucleotide, or a siRNA. The second therapeutic agent may be synthetic or naturally derived.

[038] The second therapeutic agent may be any agent that is advantageously combined with the antibody or fragment thereof of the invention, for example, an anti-fibrotic drug such as pirfenidone, an antibiotic, an anti-inflammatory drug, a non-steroidal anti-inflammatory drug (NSAID), a cytotoxic agent, a chemotherapeutic agent, a corticosteroid such as prednisone, an endothelin receptor antagonist such as Bosentan, macitentan or ambrisentan, a nutritional supplement, an anti-hypertensive agent, an antioxidant, a vascular endothelial growth factor (VEGF) antagonist [*e.g.,* a "VEGF-Trap" such as aflibercept or other VEGF-inhibiting fusion protein as set forth in US 7,087,411, or an anti-VEGF antibody or antigen binding fragment thereof (*e.g.*, bevacizumab, or ranibizumab)], another antibody that binds to GREM1, or an antibody against a chemokine such as TGF-β, or against an cytokine such as IL-1, anti-LOXL2, anti-avb6integrin, a galectin-3 targeting drug, imatinib or any other PDGFR antagonist and anti-AOC3 drugs.

[039] In certain embodiments, the second therapeutic agent may be an agent that helps to counteract or reduce any possible side effect(s) associated with the antibody or antigen-binding fragment of an antibody of the invention, if such side effect(s) should occur.

[040] It will also be appreciated that the antibodies and pharmaceutically acceptable

compositions of the present invention can be employed in combination therapies, that is, the antibodies and pharmaceutically acceptable compositions can be administered concurrently with, prior to, or subsequent to, one or more other desired therapeutics or medical procedures. The particular combination of therapies (therapeutics or procedures) to employ in a combination regimen will take into account compatibility of the desired therapeutics and/or procedures and the desired therapeutic effect to be achieved. It will also be appreciated that the therapies employed may achieve a desired effect for the same disorder (for example, an antibody may be administered concurrently with another agent used to treat the same disorder), or they may achieve different effects (*e.g.,* control of any adverse effects). As used herein, additional therapeutic agents which are normally administered to treat or prevent a particular disease, or condition, are appropriate for the disease, or condition, being treated. When multiple therapeutics are co-administered, dosages may be adjusted accordingly, as is recognized in the pertinent art.

[041] A fifth aspect of the invention involves a method for treating a disease or disorder associated with increased GREM1 expression, such as fibrosis or cancer. In certain embodiments, invention provides a method for treating a patient suffering from cancer, or for treating at least one symptom or complication associated with cancer, or halting the progression of cancer, the method comprising administering to the patient an effective amount of an antibody or an antigen-binding fragment thereof that binds to human GREM1; or a pharmaceutical composition comprising an effective amount of an antibody or an antigen-binding fragment thereof that binds to GREM1, such that the cancer-associated condition or disease is either prevented, or lessened in severity and/or duration, or at least one symptom or complication associated with the condition or disease is prevented, or ameliorated, or that the frequency and/or duration of, or the severity of cancer is reduced.

[042] In certain embodiments, invention provides a method for treating a patient suffering from fibrosis, or for treating at least one symptom or complication associated with fibrosis, or halting the progression of fibrosis, or for treating a patient at risk for developing fibrosis, the method comprising administering to the patient an effective amount of an antibody or an antigen-binding fragment thereof that binds to GREM1; or a pharmaceutical composition comprising an effective amount of an antibody or an antigen-binding fragment thereof that binds to GREM1, such that the fibrosis-associated condition or disease is either prevented, or lessened in severity and/or duration, or at least one symptom or complication associated with the condition or disease is prevented, or ameliorated, or that the frequency and/or duration of, or the severity of fibrosis is reduced. In one embodiment, the antibody is administered therapeutically (administered after fibrosis has been established and given throughout the course of the condition) to a patient suffering from fibrosis-associated condition or disease, or suffering from at least one symptom or complication associated with the condition or disease. In one embodiment, the antibody is administered prophylactically (administered prior to

development of the condition) to a patient at risk for developing fibrosis-associated condition or disease, or at risk for developing at least one symptom or complication associated with fibrosis. For example, such "patients at risk for developing fibrosis" include the elderly, or patients with a family history, or smokers, or patients who have some underlying medical condition that may pre-dispose them to acquiring fibrosis such as diabetes, or patients exposed to asbestos, wood, metal dust or chemicals, viral infections, certain medications, or cigarette smoke or patients with chronic liver injuries like viral hepatitis, parasitic infection, metabolic or autoimmune diseases, congenital abnormalities and drug and alcohol abuse. Other patients at risk for developing fibrosis include patients with chronic kidney disease, acute kidney injury, chronic hypertension, heart failure, kidney transplant, scleroderma, exposure to radiocontrast agent, chronic allergy, chronic asthma or lung transplant.

[043] In another embodiment, the at least one symptom or complication associated with the fibrosis-associated condition or disease is selected from the group consisting of shortness of breath, persistent dry hacking cough, pain, weight loss, nausea, loss of appetite, fluid accumulation in abdomen, swelling in legs, fatigue, pulmonary hypertension, hyperglycemia, renal injury, urinary tract infection, liver damage, loss of liver function, loss of renal function, hypertension, decrease in quality of life, reduced life expectancy and relapse of a condition or disease associated with fibrosis. In some embodiments, the disease or condition associated with fibrosis may be present in liver, kidney, lungs, skin, gut or muscle. In another embodiment, the fibrosis-associated condition or disease is selected from the group comprising pulmonary fibrosis, pulmonary hypertension, idiopathic pulmonary fibrosis, renal fibrosis, liver fibrosis, ischemic renal injury, tubulointerstitial fibrosis, diabetic nephropathy, nephrosclerosis, and nephrotoxicity.

[044] Embodiments of the invention relate to methods of protecting against progressive tissue damage, or inhibiting or reducing tissue degeneration in a patient suffering from fibrosis, the methods comprising administering to the patient an effective amount of an antibody or an antigen-binding fragment thereof that binds to GREM1; or a pharmaceutical composition comprising an effective amount of an antibody or an antigen-binding fragment thereof that binds to GREM1, such that the tissue in the patient is protected from progressive damage or tissue degeneration is inhibited or reduced in a patient suffering from fibrosis. In some embodiments of the invention, the tissue affected by fibrotic damage is lungs, wherein the fibrotic disease may be one of pulmonary fibrosis, pulmonary hypertension or idiopathic pulmonary fibrosis. In one embodiment, the tissue affected by fibrotic damage may be liver. In some embodiments, the tissue affected by fibrotic damage may be kidney, wherein the fibrotic disease may comprise one of renal fibrosis, ischemic renal injury, tubulointerstitial fibrosis, diabetic nephropathy, nephrosclerosis, or nephrotoxicity.

[045] In some embodiments, the invention includes methods of treating cancer or inhibiting tumor growth, tumor cell proliferation or tumor metastasis, the methods comprising

administering an isolated antibody or antigen-binding fragment thereof of the present invention that binds to GREM1. In certain embodiments, the invention includes methods for inhibiting angiogenesis, the methods comprising administering an isolated antibody or antigen-binding fragment thereof of the present invention that binds to GREM1.

[046] In one embodiment, the pharmaceutical composition comprising the antibodies of the invention is administered to the patient in combination with a second therapeutic agent.

[047] In another embodiment, the second therapeutic agent is selected from the group consisting of an anti-fibrotic agent such as pirfenidone, an anti-inflammatory drug, a NSAID, a corticosteroid such as prednisone, a nutritional supplement, a vascular endothelial growth factor (VEGF) antagonist [*e.g.,* a "VEGF-Trap" such as aflibercept or other VEGF-inhibiting fusion protein as set forth in US 7,087,411, or an anti-VEGF antibody or antigen binding fragment thereof (*e.g.*, bevacizumab, or ranibizumab)], an antibody to a cytokine such as IL-1, IL-6, IL-13, IL-4, IL-17, IL-25, IL-33 or TGF-β, and any other palliative therapy useful for ameliorating at least one symptom associated with a fibrosis-associated condition or cancer. In some embodiments, the second therapeutic agent may be administered to manage or treat at least one complication associated with fibrosis or cancer.

[048] In embodiments of the invention, the antibody or antigen-binding fragment thereof or the pharmaceutical composition comprising the antibody is administered subcutaneously, intravenously, intradermally, orally or intramuscularly.

[049] In some embodiments, the antibody or antigen-binding fragment thereof is administered at doses of about 0.1 mg/kg of body weight to about 100 mg/kg of body weight, more specifically about 20 mg/kg of body weight to about 50 mg/kg of body weight.

[050] In related embodiments, the invention includes the use of an isolated anti-GREM1 antibody or antigen binding portion of an antibody of the invention in the manufacture of a medicament for the treatment of a disease or disorder related to or caused by GREM1 activity. In one embodiment, the invention includes an isolated anti-GREM1 antibody or antigen-binding fragment thereof for use in promoting BMP signaling or cell differentiation. In one embodiment, the invention includes an isolated anti-GREM1 antibody or antigen-binding fragment thereof for use in inhibiting heparin-mediated angiogenesis. In one embodiment, the invention includes the use of an anti-GREM1 antibody of the invention in the manufacture of a medicament for treating a patient suffering from or at risk of developing fibrosis. In one embodiment, the invention includes the use of an anti-GREM1 antibody of the invention in the manufacture of a medicament for treating a patient suffering from cancer.

[051] A sixth aspect of the invention provides for methods of predicting prognosis of fibrosis in a patient suffering from a condition or disease selected from the group comprising of pulmonary fibrosis, idiopathic pulmonary fibrosis, pulmonary hypertension, renal fibrosis, hepatic fibrosis and diabetic nephropathy, the method comprising reacting a GREM1 protein from the patient

with an antibody or antigen-binding fragment of the invention, wherein binding with human GREM1 indicates poor prognosis.

[052] In one embodiment, the invention features a method of predicting poor survival in a patient suffering from fibrosis, the method comprising reacting a GREM1 protein from the patient with an isolated antibody of the invention as described herein, wherein binding with GREM1 indicates poor survival.

[053] In one embodiment, the tissue or cell sample containing a GREM1 protein from a patient is obtained from the patient's blood, serum, plasma, or biopsy of a tissue, such as liver, lung or kidney.

[054] In a related embodiment, the invention features a method of diagnosing fibrosis in a tissue or monitoring fibrotic activity in a subject suspected of suffering from fibrosis, the method comprising administering an antibody or antigen-binding fragment of the invention linked to a detectable label such as a radionuclide or a MRI-detectable label and imaging the subject upon such administration, wherein GREM1 binding and detection in the image indicates fibrosis.

[055] In one embodiment, the fibrosis is idiopathic pulmonary fibrosis. In some embodiments, the fibrosis is selected from the group comprising pulmonary hypertension, diabetic nephropathy, renal fibrosis, liver fibrosis, and tubulointerstitial fibrosis. In some embodiments, the fibrotic activity is detected in lungs or kidney or liver.

[056] Other embodiments will become apparent from a review of the ensuing detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

[057] Figure 1 describes the structural features of GREM1 protein (From Wordinger, R.J., et al 2008, Exp. Eye Res. 87: 78-79). The predicted positions of structural features are shown. Signal Seq., signal sequence (positions 1–24); DAN, cysteine-rich motif (positions 69–184); , glycosylation site (position 42); *, phosphorylation sites (positions 6, 29, 44, 47, 55, 66, 76, 77, 88, 102 and 151); , PKC specific eukaryotic protein phosphorylation site (position 165); NLS, nuclear localization signal sequences (positions 145, 166, 163, 164).

DETAILED DESCRIPTION

[058] Before the present methods are described, it is to be understood that this invention is not limited to particular methods, and experimental conditions described, as such methods and conditions may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

[059] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein

can be used in the practice or testing of the present invention, preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference in their entirety.

Definitions

[060] The term "bone morphogenetic protein" or "BMP" refers to the group of growth factors which function as pivotal morphogenetic signals, orchestrating tissue architecture throughout the body. They were originally discovered by their ability to induce the formation of bone and cartilage. However, BMPs have a variety of different functions during embryonic development. They are also involved in body patterning and morphogenesis cascades. BMPS have been found to be essential in organ homeostasis. Further, BMPs play important roles in the pathophysiology of several diseases including osteoporosis, arthritis, pulmonary hypertension and kidney diseases. BMPs and their involvement in disease processes have been reviewed by Weiskirchen, R., et al in Front. Biosci. 2009, 14: 4992-5012. Twenty BMPs have been discovered so far, of which BMP2 to BMP7 belong to the transforming growth factor beta superfamily.

[061] The term "GREM1" refers to human gremlin-1, a member of the cysteine knot superfamily. The amino acid sequence of human GREM1 is provided in GenBank as accession number NP_037504 and is also referred to herein as SEQ ID NO: 594. GREM1 is encoded by the nucleic acid provided herein as SEQ ID NO: 593, and is also found in GenBank as accession number NM_013372. GREM1 is a highly conserved 184 aa protein which has been mapped to chromosome 15q13-q15. The protein contains a signal peptide (aa 1 – 24) and a predicted glycosylation site (at aa 42). In addition, the protein contains a cysteine-rich region and a cysteine knot motif (aa 94-184) whose structure is shared by members of the transforming growth factor-beta (TGF-β) superfamily. GREM1 exists in both secreted and cell-associated (e.g. membrane associated) forms. GREM1 is also known as gremlin1, cysteine knot superfamily 1 – BMP antagonist 1 (CKTSF1B1), DAN domain family member 2 (DAND2), Down-regulated in Mos-transformed cells protein (DRM), gremlin, GREMLIN, Gremlin-1 precursor, Increased in high glucose protein 2 (IHG-2), MGC126660, Proliferation-inducing gene 2 protein (PIG2), or Gremlin 1-like protein. GREM1 is an antagonist of bone morphogenetic proteins (BMPs). It binds to BMPs and inhibits their binding to their receptors. The interplay between GREM1 and BMPs fine-tunes the level of available BMPs and affects developmental and disease processes. GREM1 can bind to and inhibit BMP-2, BMP-4 and BMP-7. GREM1 has been found to be up regulated in fibrotic diseases, especially of the kidney, lung and liver.

[062] The term "fibrosis", as used herein refers to the formation of excess fibrous connective tissue in an organ or tissue in a reparative or reactive process. This is as opposed to formation of fibrous tissue as a normal constituent of an organ or tissue. Scarring is confluent fibrosis that obliterates the architecture of the underlying organ or tissue. Fibrosis can affect many organs in

the body. The following table shows some examples of fibrosis along with the affected organ:

Type of fibrosis	Organ affected
Pulmonary fibrosis	Lungs
Cystic fibrosis	Lungs
Idiopathic pulmonary fibrosis	Lungs
Cirrhosis (associated with viral infection or other cause)	Liver
Non-alcoholic steatohepatitis	Liver
Endomyocardial fibrosis	Heart
Mediastinal fibrosis	Soft tissue of the mediastinum
Myelofibrosis	Bone marrow
Retroperitoneal fibrosis	Soft tissue of the retroperitoneum
Progressive massive fibrosis (a complication of coal workers' pneumoconiosis)	Lungs
Bronchiolitis obliterans	Lungs
Airway Remodeling associated with chronic asthma	Lungs
Kidney or Lung transplant fibrosis	Kidney, Lungs
Focal & Segmental Glomerulosclerosis	Kidney
Nephrogenic systemic fibrosis	Skin
Crohn's disease	Intestine
Keloid	Skin
Old myocardial infarction	Heart
Muscular dystrophy	Muscle
Scleroderma, systemic sclerosis	Skin, lungs
Arthrofibrosis	Knee, shoulder, other joints
Corneal fibrosis	Eyes
Retinal fibrosis associated with macular degeneration	Eyes

[063] The term "fibrosis" also comprises complex disorders such as pulmonary fibrosis, for example, idiopathic pulmonary fibrosis, pulmonary hypertension, diabetic nephropathy, ischemic renal injury, renal fibrosis, hepatic fibrosis, tubulointerstitial fibrosis, nephrosclerosis and nephrotoxicity.

[064] The term "antibody", as used herein, is intended to refer to immunoglobulin molecules comprised of four polypeptide chains, two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds (*i.e.*, "full antibody molecules"), as well as multimers thereof (*e.g.* IgM) or antigen-binding fragments thereof. Each heavy chain is comprised of a heavy chain variable region ("HCVR" or "V_H") and a heavy chain constant region (comprised of domains C_H1, C_H2 and C_H3). Each light chain is comprised of a light chain variable region ("LCVR or "V_L") and a light chain constant region (C_L). The V_H and V_L regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each V_H and V_L is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. In certain embodiments of the invention, the

FRs of the antibody (or antigen binding fragment thereof) may be identical to the human germline sequences, or may be naturally or artificially modified. An amino acid consensus sequence may be defined based on a side-by-side analysis of two or more CDRs.

[065] Substitution of one or more CDR residues or omission of one or more CDRs is also possible. Antibodies have been described in the scientific literature in which one or two CDRs can be dispensed with for binding. Padlan *et al.* (FASEB J. 1995, 9:133-139) analyzed the contact regions between antibodies and their antigens, based on published crystal structures, and concluded that only about one fifth to one third of CDR residues actually contact the antigen. Padlan also found many antibodies in which one or two CDRs had no amino acids in contact with an antigen (see also, Vajdos *et al.* 2002 J Mol Biol 320:415-428).

[066] CDR residues not contacting antigen can be identified based on previous studies (for example residues H60-H65 in CDRH2 are often not required), from regions of Kabat CDRs lying outside Chothia CDRs, by molecular modeling and/or empirically. If a CDR or residue(s) thereof is omitted, it is usually substituted with an amino acid occupying the corresponding position in another human antibody sequence or a consensus of such sequences. Positions for substitution within CDRs and amino acids to substitute can also be selected empirically. Empirical substitutions can be conservative or non-conservative substitutions.

[067] The fully human anti-GREM1 monoclonal antibodies disclosed herein may comprise one or more amino acid substitutions, insertions and/or deletions in the framework and/or CDR regions of the heavy and light chain variable domains as compared to the corresponding germline sequences. Such mutations can be readily ascertained by comparing the amino acid sequences disclosed herein to germline sequences available from, for example, public antibody sequence databases. The present invention includes antibodies, and antigen-binding fragments thereof, which are derived from any of the amino acid sequences disclosed herein, wherein one or more amino acids within one or more framework and/or CDR regions are mutated to the corresponding residue(s) of the germline sequence from which the antibody was derived, or to the corresponding residue(s) of another human germline sequence, or to a conservative amino acid substitution of the corresponding germline residue(s) (such sequence changes are referred to herein collectively as "germline mutations"). A person of ordinary skill in the art, starting with the heavy and light chain variable region sequences disclosed herein, can easily produce numerous antibodies and antigen-binding fragments which comprise one or more individual germline mutations or combinations thereof. In certain embodiments, all of the framework and/or CDR residues within the V_H and/or V_L domains are mutated back to the residues found in the original germline sequence from which the antibody was derived. In other embodiments, only certain residues are mutated back to the original germline sequence, *e.g.*, only the mutated residues found within the first 8 amino acids of FR1 or within the last 8 amino acids of FR4, or only the mutated residues found within CDR1, CDR2 or CDR3. In other embodiments, one or more of the framework and/or CDR residue(s) are mutated to the corresponding residue(s) of a

different germline sequence (*i.e.*, a germline sequence that is different from the germline sequence from which the antibody was originally derived). Furthermore, the antibodies of the present invention may contain any combination of two or more germline mutations within the framework and/or CDR regions, *e.g.*, wherein certain individual residues are mutated to the corresponding residue of a particular germline sequence while certain other residues that differ from the original germline sequence are maintained or are mutated to the corresponding residue of a different germline sequence. Once obtained, antibodies and antigen-binding fragments that contain one or more germline mutations can be easily tested for one or more desired property such as, improved binding specificity, increased binding affinity, improved or enhanced antagonistic or agonistic biological properties (as the case may be), reduced immunogenicity, etc. Antibodies and antigen-binding fragments obtained in this general manner are encompassed within the present invention.

[068] The present invention also includes fully human anti-GREM1 monoclonal antibodies comprising variants of any of the HCVR, LCVR, and/or CDR amino acid sequences disclosed herein having one or more conservative substitutions. For example, the present invention includes anti-GREM1 antibodies having HCVR, LCVR, and/or CDR amino acid sequences with, *e.g.*, 10 or fewer, 8 or fewer, 6 or fewer, 4 or fewer, etc. conservative amino acid substitutions relative to any of the HCVR, LCVR, and/or CDR amino acid sequences disclosed herein.

[069] The term "human antibody", as used herein, is intended to include antibodies having variable and constant regions derived from human germline immunoglobulin sequences. The human mAbs of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (*e.g.,* mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*), for example in the CDRs and in particular CDR3. However, the term "human antibody", as used herein, is not intended to include mAbs in which CDR sequences derived from the germline of another mammalian species (e.g., mouse), have been grafted onto human FR sequences.

[070] The term "specifically binds," or "binds specifically to", or the like, means that an antibody or antigen-binding fragment thereof forms a complex with an antigen that is relatively stable under physiologic conditions. Specific binding can be characterized by an equilibrium dissociation constant of at least about $1x10^{-6}$ M or less (*e.g.*, a smaller K_D denotes a tighter binding). Methods for determining whether two molecules specifically bind are well known in the art and include, for example, equilibrium dialysis, surface plasmon resonance, and the like. As described herein, antibodies which bind specifically to human GREM1 have been identified by surface plasmon resonance, *e.g.*, BIACORE™. Moreover, multi-specific antibodies that bind to one domain in GREM1 and one or more additional antigens or a bi-specific that binds to two different regions of GREM1 are nonetheless considered antibodies that "specifically bind", as used herein.

[071] The term "high affinity" antibody refers to those mAbs having a binding affinity to GREM1,

expressed as K_D, of at least 10^{-7} M; preferably 10^{-8} M; more preferably 10^{-9}M, even more preferably 10^{-10} M, even more preferably 10^{-11} M, as measured by surface plasmon resonance, e.g., BIACORE™ or solution-affinity ELISA.

[072] By the term "slow off rate", "Koff" or "kd" is meant an antibody that dissociates from GREM1, with a rate constant of 1×10^{-3} s^{-1} or less, preferably 1×10^{-4} s^{-1} or less, as determined by surface plasmon resonance, *e.g.*, BIACORE™.

[073] The terms "antigen-binding portion" of an antibody, "antigen-binding fragment" of an antibody, and the like, as used herein, include any naturally occurring, enzymatically obtainable, synthetic, or genetically engineered polypeptide or glycoprotein that specifically binds an antigen to form a complex. The terms "antigen-binding fragment" of an antibody, or "antibody fragment", as used herein, refers to one or more fragments of an antibody that retain the ability to bind to GREM1.

[074] In specific embodiments, antibody or antibody fragments of the invention may be conjugated to a therapeutic moiety ("immunoconjugate"), such as an antibiotic, a second anti-GREM1 antibody, or an antibody to a cytokine such as IL-1, IL-6, or TGF-β, or any other therapeutic moiety useful for treating a disease or condition including pulmonary fibrosis, renal fibrosis, liver fibrosis, ischemic renal injury, tubulointerstitial fibrosis, diabetic nephropathy, nephrosclerosis, or nephrotoxicity.

[075] An "isolated antibody", as used herein, is intended to refer to an antibody that is substantially free of other antibodies (Abs) having different antigenic specificities (*e.g.,* an isolated antibody that specifically binds human GREM1, or a fragment thereof, is substantially free of Abs that specifically bind antigens other than GREM1.

[076] A "blocking antibody" or a "neutralizing antibody", as used herein (or an "antibody that neutralizes GREM1 activity"), is intended to refer to an antibody whose binding to GREM1 results in inhibition of at least one biological activity of GREM1. For example, an antibody of the invention may aid in inhibiting or preventing the spread of fibrosis. Alternatively, an antibody of the invention may demonstrate the ability to treat fibrosis or at least one symptom caused by fibrosis, including dry cough or breathlessness. This inhibition of the biological activity of GREM1 can be assessed by measuring one or more indicators of GREM1 biological activity by one or more of several standard *in vitro* assays (such as a neutralization assay, as described herein) or *in vivo* assays known in the art (for example, animal models to look at protection from GREM1 activity following administration of one or more of the antibodies described herein).

[077] The term "surface plasmon resonance", as used herein, refers to an optical phenomenon that allows for the analysis of real-time biomolecular interactions by detection of alterations in protein concentrations within a biosensor matrix, for example using the BIACORE™ system (Pharmacia Biosensor AB, Uppsala, Sweden and Piscataway, N.J.).

[078] The term "K_D ", as used herein, is intended to refer to the equilibrium dissociation constant of a particular antibody-antigen interaction.

[079] The term "epitope" refers to an antigenic determinant that interacts with a specific antigen binding site in the variable region of an antibody molecule known as a paratope. A single antigen may have more than one epitope. Thus, different antibodies may bind to different areas on an antigen and may have different biological effects. The term "epitope" also refers to a site on an antigen to which B and/or T cells respond. It also refers to a region of an antigen that is bound by an antibody. Epitopes may be defined as structural or functional. Functional epitopes are generally a subset of the structural epitopes and have those residues that directly contribute to the affinity of the interaction. Epitopes may also be conformational, that is, composed of non-linear amino acids. In certain embodiments, epitopes may include determinants that are chemically active surface groupings of molecules such as amino acids, sugar side chains, phosphoryl groups, or sulfonyl groups, and, in certain embodiments, may have specific three-dimensional structural characteristics, and/or specific charge characteristics.

[080] The term "substantial identity" or "substantially identical," when referring to a nucleic acid or fragment thereof, indicates that, when optimally aligned with appropriate nucleotide insertions or deletions with another nucleic acid (or its complementary strand), there is nucleotide sequence identity in at least about 90%, and more preferably at least about 95%, 96%, 97%, 98% or 99% of the nucleotide bases, as measured by any well-known algorithm of sequence identity, such as FASTA, BLAST or GAP, as discussed below. A nucleic acid molecule having substantial identity to a reference nucleic acid molecule may, in certain instances, encode a polypeptide having the same or substantially similar amino acid sequence as the polypeptide encoded by the reference nucleic acid molecule.

[081] As applied to polypeptides, the term "substantial similarity" or "substantially similar" means that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 90% sequence identity, even more preferably at least 95%, 98% or 99% sequence identity. Preferably, residue positions, which are not identical, differ by conservative amino acid substitutions. A "conservative amino acid substitution" is one in which an amino acid residue is substituted by another amino acid residue having a side chain (R group) with similar chemical properties (e.g., charge or hydrophobicity). In general, a conservative amino acid substitution will not substantially change the functional properties of a protein. In cases where two or more amino acid sequences differ from each other by conservative substitutions, the percent or degree of similarity may be adjusted upwards to correct for the conservative nature of the substitution. Means for making this adjustment are well known to those of skill in the art. See, *e.g.*, Pearson (1994) Methods Mol. Biol. 24: 307-331, which is herein incorporated by reference. Examples of groups of amino acids that have side chains with similar chemical properties include 1) aliphatic side chains: glycine, alanine, valine, leucine and isoleucine; 2) aliphatic-hydroxyl side chains: serine and threonine; 3) amide-containing side chains: asparagine and glutamine; 4) aromatic side chains: phenylalanine, tyrosine, and tryptophan; 5) basic side chains: lysine, arginine, and histidine; 6) acidic side

chains: aspartate and glutamate, and 7) sulfur-containing side chains: cysteine and methionine. Preferred conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, glutamate-aspartate, and asparagine-glutamine. Alternatively, a conservative replacement is any change having a positive value in the PAM250 log-likelihood matrix disclosed in Gonnet *et al.* (1992) Science 256: 1443 45, herein incorporated by reference. A "moderately conservative" replacement is any change having a nonnegative value in the PAM250 log-likelihood matrix.

[082] Sequence similarity for polypeptides is typically measured using sequence analysis software. Protein analysis software matches similar sequences using measures of similarity assigned to various substitutions, deletions and other modifications, including conservative amino acid substitutions. For instance, GCG software contains programs such as GAP and BESTFIT which can be used with default parameters to determine sequence homology or sequence identity between closely related polypeptides, such as homologous polypeptides from different species of organisms or between a wild type protein and a mutein thereof. See, *e.g.,* GCG Version 6.1. Polypeptide sequences also can be compared using FASTA with default or recommended parameters; a program in GCG Version 6.1. FASTA (*e.g.,* FASTA2 and FASTA3) provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences (Pearson (2000) *supra*). Another preferred algorithm when comparing a sequence of the invention to a database containing a large number of sequences from different organisms is the computer program BLAST, especially BLASTP or TBLASTN, using default parameters. See, *e.g.,* Altschul *et al.* (1990) J. Mol. Biol. 215: 403-410 and (1997) Nucleic Acids Res. 25: 3389-3402, each of which is herein incorporated by reference.

[083] In specific embodiments, the antibody or antibody fragment for use in the method of the invention may be mono-specific, bi-specific, or multi-specific. Multi-specific antibodies may be specific for different epitopes of one target polypeptide or may contain antigen-binding domains specific for epitopes of more than one target polypeptide. An exemplary bi-specific antibody format that can be used in the context of the present invention involves the use of a first immunoglobulin (Ig) C_H3 domain and a second Ig C_H3 domain, wherein the first and second Ig C_H3 domains differ from one another by at least one amino acid, and wherein at least one amino acid difference reduces binding of the bi-specific antibody to Protein A as compared to a bi-specific antibody lacking the amino acid difference. In one embodiment, the first Ig C_H3 domain binds Protein A and the second Ig C_H3 domain contains a mutation that reduces or abolishes Protein A binding such as an H95R modification (by IMGT exon numbering; H435R by EU numbering). The second C_H3 may further comprise an Y96F modification (by IMGT; Y436F by EU). Further modifications that may be found within the second C_H3 include: D16E, L18M, N44S, K52N, V57M, and V82I (by IMGT; D356E, L358M, N384S, K392N, V397M, and V422I by EU) in the case of IgG1 mAbs; N44S, K52N, and V82I (IMGT; N384S, K392N, and V422I by EU) in the case of IgG2 mAbs; and Q15R, N44S, K52N, V57M, R69K, E79Q, and V82I (by

IMGT; Q355R, N384S, K392N, V397M, R409K, E419Q, and V422I by EU) in the case of IgG4 mAbs. Variations on the bi-specific antibody format described above are contemplated within the scope of the present invention.

[084] By the phrase "therapeutically effective amount" is meant an amount that produces the desired effect for which it is administered. The exact amount will depend on the purpose of the treatment, and will be ascertainable by one skilled in the art using known techniques (see, for example, Lloyd (1999) The Art, Science and Technology of Pharmaceutical Compounding).

General Description

[085] As an antagonist of bone morphogenetic proteins (BMPs), GREM1 gene plays a role in regulating organogenesis, body patterning, and tissue differentiation. GREM1 has been found to play an important role in lung development. However, expression of GREM1 in a healthy adult lung is low. Upregulated levels of GREM1 have been correlated with pulmonary hypertension and pulmonary fibrosis (Costello, et al., 2010, Am. J. Respir. Cell. Mol. Biol. 42: 517-523). Pulmonary fibrosis, especially of the idiopathic type is a progressive, scar-forming and disabling disease of the lung parenchyma with a poor prognosis and no efficacious therapy. Elevated GREM1 expression correlates negatively with lung function tests in idiopathic pulmonary fibrosis, suggesting that GREM1 may be an important marker of advanced stage fibrosis (Costello, et al., 2010, Am. J. Respir. Cell. Mol. Biol. 42: 517-523).

[086] GREM1 expression is also essential in kidney organogenesis. However, GREM1 expression in a healthy adult kidney is almost undetectable. Elevated GREM1 levels are found in patients with hyperglycemia and diabetic nephropathy (Lappin, et al., 2002, Nephrol. Dial. Transplant. 17: 65-67). GREM1 is found to be upregulated in areas of tubulointerstitial fibrosis in patients with diabetic nephropathy. Diabetic nephropathy is a complex disorder characterized by sclerosis and development of tubulointerstitial fibrosis. It is the leading cause of end-stage renal diseases and 20-40% of patients with diabetes ultimately develop diabetic nephropathy. Specific therapies to reverse or inhibit the progression of diabetic nephropathy to advanced stages are not available and current treatment strategies are limited to management of blood glucose levels and control of hypertension (Zhang et al., 2009, BBRC 383: 1-3).

[087] GREM1 has also been found to be upregulated in liver fibrosis (Boers et al., 2006, J. Biol. Chem. 281: 16289-16295). Hepatic fibrosis is a common response to most chronic liver injuries like viral hepatitis, parasitic infection, metabolic or autoimmune diseases, congenital abnormalities and drug and alcohol abuse. Fibrosis may also contribute to progressive cirrhosis of liver. Detection of liver disease is often delayed and effective medical treatment is not readily available.

[088] The antibodies described herein demonstrate specific binding to human GREM1 and in some embodiments, may be useful for treating patients suffering from fibrosis. The use of such antibodies may be an effective means of treating patients suffering from fibrosis, or may be used

to lessen the severity of the dry cough or difficulty in breathing associated with fibrosis. They may be used alone or as adjunct therapy with other therapeutic moieties or modalities known in the art for treating fibrosis, such as, but not limited to, a non-steroidal anti-inflammatory drug (NSIAD), a corticosteroid such as prednisone, or any other palliative therapy. They may be used in conjunction with a second or third different antibody specific for GREM1, or against a cytokine such as IL-1, IL-6 or TGF-β.

[089] In some embodiments, the antibodies described herein may be useful in treating or managing a disease or condition of fibrosis including (idiopathic) pulmonary fibrosis, renal fibrosis, liver fibrosis, ischemic renal injury, tubulointerstitial fibrosis, diabetic nephropathy, nephrosclerosis, or nephrotoxicity.

[090] In certain embodiments, the antibodies described herein may be useful for treating or managing cancer such as sarcoma, and carcinomas of the lung, uterine cervix, colon, breast, and pancreas.

[091] In certain embodiments, the antibodies of the invention are obtained from mice immunized with a primary immunogen, such as a native, full length human GREM1 (See GenBank accession number NP_037504 (SEQ ID NO: 594)) or with a recombinant form of GREM1 (SEQ ID NO: 595) or GREM1 fragments, followed by immunization with a secondary immunogen, or with an immunogenically active fragment of GREM1.

[092] The immunogen may be an immunogenic fragment of human GREM1 or DNA encoding the fragment thereof. The immunogen may GREM1 coupled to a histidine tag and/or to a fragment of Fc region of an antibody.

[093] The amino acid sequence of full length human GREM1 (also known by Gen bank accession number NP-037504) is shown as SEQ ID NO: 594. The full length amino acid sequence of recombinant GREM1 (aa 25-184 GREM1 coupled to Fc region and a histidine tag) is shown as SEQ ID NO: 595.

[094] The full-length DNA sequence of GREM1 is shown as SEQ ID NO: 593.

[095] In certain embodiments, antibodies that bind specifically to human GREM1 may be prepared using fragments of the above-noted regions, or peptides that extend beyond the designated regions by about 5 to about 20 amino acid residues from either, or both, the N or C terminal ends of the regions described herein. In certain embodiments, any combination of the above-noted regions or fragments thereof may be used in the preparation of human GREM1 specific antibodies. In certain embodiments, any one or more of the above-noted regions of human GREM1, or fragments thereof may be used for preparing monospecific, bispecific, or multispecific antibodies.

Antigen-Binding Fragments of Antibodies

[096] Unless specifically indicated otherwise, the term "antibody," as used herein, shall be understood to encompass antibody molecules comprising two immunoglobulin heavy chains

and two immunoglobulin light chains (*i.e.*, "full antibody molecules") as well as antigen-binding fragments thereof. The terms "antigen-binding portion" of an antibody, "antigen-binding fragment" of an antibody, and the like, as used herein, include any naturally occurring, enzymatically obtainable, synthetic, or genetically engineered polypeptide or glycoprotein that specifically binds an antigen to form a complex. The terms "antigen-binding fragment" of an antibody, or "antibody fragment", as used herein, refers to one or more fragments of an antibody that retain the ability to specifically bind to human GREM1. An antibody fragment may include a Fab fragment, a $F(ab')_2$ fragment, a Fv fragment, a dAb fragment, a fragment containing a CDR, or an isolated CDR. Antigen-binding fragments of an antibody may be derived, *e.g.*, from full antibody molecules using any suitable standard techniques such as proteolytic digestion or recombinant genetic engineering techniques involving the manipulation and expression of DNA encoding antibody variable and (optionally) constant domains. Such DNA is known and/or is readily available from, *e.g.*, commercial sources, DNA libraries (including, *e.g.*, phage-antibody libraries), or can be synthesized. The DNA may be sequenced and manipulated chemically or by using molecular biology techniques, for example, to arrange one or more variable and/or constant domains into a suitable configuration, or to introduce codons, create cysteine residues, modify, add or delete amino acids, etc.

[097] Non-limiting examples of antigen-binding fragments include: (i) Fab fragments; (ii) F(ab')2 fragments; (iii) Fd fragments; (iv) Fv fragments; (v) single-chain Fv (scFv) molecules; (vi) dAb fragments; and (vii) minimal recognition units consisting of the amino acid residues that mimic the hypervariable region of an antibody (*e.g.*, an isolated complementarity determining region (CDR) such as a CDR3 peptide), or a constrained FR3-CDR3-FR4 peptide. Other engineered molecules, such as domain-specific antibodies, single domain antibodies, domain-deleted antibodies, chimeric antibodies, CDR-grafted antibodies, diabodies, triabodies, tetrabodies, minibodies, nanobodies (*e.g.* monovalent nanobodies, bivalent nanobodies, etc.), small modular immunopharmaceuticals (SMIPs), and shark variable IgNAR domains, are also encompassed within the expression "antigen-binding fragment," as used herein.

[098] An antigen-binding fragment of an antibody will typically comprise at least one variable domain. The variable domain may be of any size or amino acid composition and will generally comprise at least one CDR, which is adjacent to or in frame with one or more framework sequences. In antigen-binding fragments having a V_H domain associated with a V_L domain, the V_H and V_L domains may be situated relative to one another in any suitable arrangement. For example, the variable region may be dimeric and contain V_H - V_H, V_H - V_L or V_L - V_L dimers. Alternatively, the antigen-binding fragment of an antibody may contain a monomeric V_H or V_L domain.

[099] In certain embodiments, an antigen-binding fragment of an antibody may contain at least one variable domain covalently linked to at least one constant domain. Non-limiting, exemplary configurations of variable and constant domains that may be found within an antigen-binding

fragment of an antibody of the present invention include: (i) V_H -C_H1; (ii) V_H -C_H2; (iii) V_H -C_H3; (iv) V_H -C_H1-C_H2; (v) V_H -C_H1-C_H2-C_H3; (vi) V_H -C_H2-C_H3; (vii) V_H -C_L; (viii) V_L -C_H1; (ix) V_L -C_H2; (x) V_L -C_H3; (xi) V_L -C_H1-C_H2; (xii) V_L -C_H1-C_H2-C_H3; (xiii) V_L -C_H2-C_H3; and (xiv) V_L -C_L. In any configuration of variable and constant domains, including any of the exemplary configurations listed above, the variable and constant domains may be either directly linked to one another or may be linked by a full or partial hinge or linker region. A hinge region may consist of at least 2 (*e.g.*, 5, 10, 15, 20, 40, 60 or more) amino acids, which result in a flexible or semi-flexible linkage between adjacent variable and/or constant domains in a single polypeptide molecule. Moreover, an antigen-binding fragment of an antibody of the present invention may comprise a homo-dimer or hetero-dimer (or other multimer) of any of the variable and constant domain configurations listed above in non-covalent association with one another and/or with one or more monomeric V_H or V_L domain (*e.g.*, by disulfide bond(s)).

[0100] As with full antibody molecules, antigen-binding fragments may be mono-specific or multi-specific (*e.g.*, bi-specific). A multi-specific antigen-binding fragment of an antibody will typically comprise at least two different variable domains, wherein each variable domain is capable of specifically binding to a separate antigen or to a different epitope on the same antigen. Any multi-specific antibody format, including the exemplary bi-specific antibody formats disclosed herein, may be adapted for use in the context of an antigen-binding fragment of an antibody of the present invention using routine techniques available in the art.

Preparation of Human Antibodies

[0101] Methods for generating human antibodies in transgenic mice are known in the art. Any such known methods can be used in the context of the present invention to make human antibodies that specifically bind to human GREM1.

[0102] Using VELOCIMMUNE™ technology (see, for example, US 6,596,541, Regeneron Pharmaceuticals, VELOCIMMUNE®) or any other known method for generating monoclonal antibodies, high affinity chimeric antibodies to human GREM1 are initially isolated having a human variable region and a mouse constant region. The VELOCIMMUNE® technology involves generation of a transgenic mouse having a genome comprising human heavy and light chain variable regions operably linked to endogenous mouse constant region loci such that the mouse produces an antibody comprising a human variable region and a mouse constant region in response to antigenic stimulation. The DNA encoding the variable regions of the heavy and light chains of the antibody are isolated and operably linked to DNA encoding the human heavy and light chain constant regions. The DNA is then expressed in a cell capable of expressing the fully human antibody.

[0103] Generally, a VELOCIMMUNE® mouse is challenged with the antigen of interest, and lymphatic cells (such as B-cells) are recovered from the mice that express antibodies. The lymphatic cells may be fused with a myeloma cell line to prepare immortal hybridoma cell lines,

and such hybridoma cell lines are screened and selected to identify hybridoma cell lines that produce antibodies specific to the antigen of interest. DNA encoding the variable regions of the heavy chain and light chain may be isolated and linked to desirable isotypic constant regions of the heavy chain and light chain. Such an antibody protein may be produced in a cell, such as a CHO cell. Alternatively, DNA encoding the antigen-specific chimeric antibodies or the variable domains of the light and heavy chains may be isolated directly from antigen-specific lymphocytes.

[0104] Initially, high affinity chimeric antibodies are isolated having a human variable region and a mouse constant region. As in the experimental section below, the antibodies are characterized and selected for desirable characteristics, including affinity, selectivity, epitope, etc. The mouse constant regions are replaced with a desired human constant region to generate the fully human antibody of the invention, for example wild-type or modified IgG1 or IgG4. While the constant region selected may vary according to specific use, high affinity antigen-binding and target specificity characteristics reside in the variable region.

[0105] In general, the antibodies of the instant invention possess very high affinities, typically possessing K_D of from about 10^{-12} through about 10^{-7} M, when measured by binding to antigen either immobilized on solid phase or in solution phase. The mouse constant regions are replaced with desired human constant regions to generate the fully human antibodies of the invention. While the constant region selected may vary according to specific use, high affinity antigen-binding and target specificity characteristics reside in the variable region.

Bioequivalents

[0106] The anti-human GREM1 antibodies and antibody fragments of the present invention encompass proteins having amino acid sequences that vary from those of the described antibodies, but that retain the ability to bind human GREM1. Such variant antibodies and antibody fragments comprise one or more additions, deletions, or substitutions of amino acids when compared to parent sequence, but exhibit biological activity that is essentially equivalent to that of the described antibodies. Likewise, the antibody-encoding DNA sequences of the present invention encompass sequences that comprise one or more additions, deletions, or substitutions of nucleotides when compared to the disclosed sequence, but that encode an antibody or antibody fragment that is essentially bioequivalent to an antibody or antibody fragment of the invention.

[0107] Two antigen-binding proteins, or antibodies, are considered bioequivalent if, for example, they are pharmaceutical equivalents or pharmaceutical alternatives whose rate and extent of absorption do not show a significant difference when administered at the same molar dose under similar experimental conditions, either single dose or multiple doses. Some antibodies will be considered equivalents or pharmaceutical alternatives if they are equivalent in the extent of their absorption but not in their rate of absorption and yet may be considered

bioequivalent because such differences in the rate of absorption are intentional and are reflected in the labeling, are not essential to the attainment of effective body drug concentrations on, *e.g.*, chronic use, and are considered medically insignificant for the particular drug product studied.

[0108] In one embodiment, two antigen-binding proteins are bioequivalent if there are no clinically meaningful differences in their safety, purity, and potency.

[0109] In one embodiment, two antigen-binding proteins are bioequivalent if a patient can be switched one or more times between the reference product and the biological product without an expected increase in the risk of adverse effects, including a clinically significant change in immunogenicity, or diminished effectiveness, as compared to continued therapy without such switching.

[0110] In one embodiment, two antigen-binding proteins are bioequivalent if they both act by a common mechanism or mechanisms of action for the condition or conditions of use, to the extent that such mechanisms are known.

[0111] Bioequivalence may be demonstrated by *in vivo* and/or *in vitro* methods. Bioequivalence measures include, *e.g.*, (a) an *in vivo* test in humans or other mammals, in which the concentration of the antibody or its metabolites is measured in blood, plasma, serum, or other biological fluid as a function of time; (b) an *in vitro* test that has been correlated with and is reasonably predictive of human *in vivo* bioavailability data; (c) an *in vivo* test in humans or other mammals in which the appropriate acute pharmacological effect of the antibody (or its target) is measured as a function of time; and (d) in a well-controlled clinical trial that establishes safety, efficacy, or bioavailability or bioequivalence of an antibody.

[0112] Bioequivalent variants of the antibodies of the invention may be constructed by, for example, making various substitutions of residues or sequences or deleting terminal or internal residues or sequences not needed for biological activity. For example, cysteine residues not essential for biological activity can be deleted or replaced with other amino acids to prevent formation of unnecessary or incorrect intramolecular disulfide bridges upon renaturation. In other contexts, bioequivalent antibodies may include antibody variants comprising amino acid changes, which modify the glycosylation characteristics of the antibodies, *e.g.*, mutations that eliminate or remove glycosylation.

Anti-GREM1 Antibodies Comprising Fc Variants

[0113] According to certain embodiments of the present invention, anti-GREM1 antibodies are provided comprising an Fc domain comprising one or more mutations which enhance or diminish antibody binding to the FcRn receptor, *e.g.*, at acidic pH as compared to neutral pH. For example, the present invention includes anti-GREM1 antibodies comprising a mutation in the C_H2 or a C_H3 region of the Fc domain, wherein the mutation(s) increases the affinity of the Fc domain to FcRn in an acidic environment (*e.g.,* in an endosome where pH ranges from about

5.5 to about 6.0). Such mutations may result in an increase in serum half-life of the antibody when administered to an animal. Non-limiting examples of such Fc modifications include, *e.g.*, a modification at position 250 (e.g., E or Q); 250 and 428 (e.g., L or F); 252 (e.g., L/Y/F/W or T), 254 (e.g., S or T), and 256 (e.g., S/R/Q/E/D or T); or a modification at position 428 and/or 433 (e.g., H/L/R/S/P/Q or K) and/or 434 (*e.g.*, A, W, H, F or Y [N434A, N434W, N434H, N434F or N434Y]); or a modification at position 250 and/or 428; or a modification at position 307 or 308 (*e.g.*, 308F, V308F), and 434. In one embodiment, the modification comprises a 428L (*e.g.*, M428L) and 434S (*e.g.*, N434S) modification; a 428L, 259I (*e.g.*, V259I), and 308F (*e.g.*, V308F) modification; a 433K (*e.g.*, H433K) and a 434 (*e.g.*, 434Y) modification; a 252, 254, and 256 (*e.g.*, 252Y, 254T, and 256E) modification; a 250Q and 428L modification (*e.g.*, T250Q and M428L); and a 307 and/or 308 modification (*e.g.*, 308F or 308P). In yet another embodiment, the modification comprises a 265A (*e.g.*, D265A) and/or a 297A (*e.g.*, N297A) modification.

[0114] For example, the present invention includes anti-GREM1 antibodies comprising an Fc domain comprising one or more pairs or groups of mutations selected from the group consisting of: 250Q and 248L (*e.g.*, T250Q and M248L); 252Y, 254T and 256E (*e.g.*, M252Y, S254T and T256E); 428L and 434S (*e.g.*, M428L and N434S); 257I and 311I (*e.g.*, P257I and Q311I); 257I and 434H (*e.g.*, P257I and N434H); 376V and 434H (*e.g.*, D376V and N434H); 307A, 380A and 434A (*e.g.*, T307A, E380A and N434A); and 433K and 434F (*e.g.*, H433K and N434F). All possible combinations of the foregoing Fc domain mutations, and other mutations within the antibody variable domains disclosed herein, are contemplated within the scope of the present invention.

[0115] The present invention also includes anti-GREM1 antibodies comprising a chimeric heavy chain constant (C_H) region, wherein the chimeric C_H region comprises segments derived from the C_H regions of more than one immunoglobulin isotype. For example, the antibodies of the invention may comprise a chimeric C_H region comprising part or all of a C_H2 domain derived from a human IgG1, human IgG2 or human IgG4 molecule, combined with part or all of a C_H3 domain derived from a human IgG1, human IgG2 or human IgG4 molecule. According to certain embodiments, the antibodies of the invention comprise a chimeric C_H region having a chimeric hinge region. For example, a chimeric hinge may comprise an "upper hinge" amino acid sequence (amino acid residues from positions 216 to 227 according to EU numbering) derived from a human IgG1, a human IgG2 or a human IgG4 hinge region, combined with a "lower hinge" sequence (amino acid residues from positions 228 to 236 according to EU numbering) derived from a human IgG1, a human IgG2 or a human IgG4 hinge region. According to certain embodiments, the chimeric hinge region comprises amino acid residues derived from a human IgG1 or a human IgG4 upper hinge and amino acid residues derived from a human IgG2 lower hinge. An antibody comprising a chimeric C_H region as described herein may, in certain embodiments, exhibit modified Fc effector functions without adversely affecting the therapeutic or pharmacokinetic properties of the antibody. (*See, e.g.*, U.S. Provisional Appl.

No. 61/759,578, filed February 1, 2013, the disclosure of which is hereby incorporated by reference in its entirety).

Biological Characteristics of the Antibodies

[0116] In general, the antibodies of the present invention may function by binding to human GREM1. In some embodiments, the antibodies of the present invention may bind to the catalytic domain of human GREM1, or to a fragment thereof. In some embodiments, the antibodies of the invention may bind to the secreted form of human GREM1 or to the membrane-associated form of human GREM1. In some embodiments, the antibodies of the present invention may bind to more than one domain (cross-reactive antibodies).

[0117] In certain embodiments of the invention, the antibodies may bind to an epitope located in the region between amino acid residues 25-184 of SEQ ID NO: 594 or SEQ ID NO: 595.

[0118] In certain embodiments, the antibodies of the present invention may function by blocking or inhibiting BMP signaling by binding to any other region or fragment of the full length native protein, the amino acid sequence of which is shown in SEQ ID NO: 594, which is encoded by the nucleic acid sequence shown in SEQ ID NO: 593. In one embodiment, the antibodies of the present invention may function by reversing the inhibition of BMP2, BMP4 or BMP7 by binding to full-length GREM1 or a fragment thereof. In some embodiments, the antibodies of the present invention may function by promoting BMP signaling or may block the binding between GREM1 and BMPs including BMP2, BMP4 or BMP7.

[0119] In certain embodiments, the antibodies of the present invention may function by blocking GREM1 binding to heparin and/or by inhibiting heparin-mediated VEGFR-2 activation.

[0120] In certain embodiments, the antibodies of the present invention may be bi-specific antibodies. The bi-specific antibodies of the invention may bind one epitope in one domain and may also bind one epitope in a second domain of human GREM1. In certain embodiments, the bi-specific antibodies of the invention may bind two different epitopes in the same domain.

[0121] In one embodiment, the invention provides a fully human monoclonal antibody or antigen-binding fragment thereof that binds to human GREM1, wherein the antibody or fragment thereof exhibits one or more of the following characteristics: (i) comprises a HCVR having an amino acid sequence selected from the group consisting of SEQ ID NO: 2, 18, 34, 50, 66, 82, 98, 114, 130, 146, 162, 178, 194, 210, 226, 242, 258, 274, 290, 306, 322, 338, 354, 370, 386, 402, 418, 434, 450, 466, 482, 498, 514, 530, 546, 562, and 578, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; (ii) comprises a LCVR having an amino acid sequence selected from the group consisting of SEQ ID NO: 10, 26, 42, 58, 74, 90, 106, 122, 138, 154, 170, 186, 202, 218, 234, 250, 266, 282, 298, 314, 330, 346, 362, 378, 394, 410, 426, 442, 458, 474, 490, 506, 522, 538, 554, 570, and 586, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; (iii) comprises a HCDR3 domain having an

amino acid sequence selected from the group consisting of SEQ ID NO: 8, 24, 40, 56, 72, 88, 104, 120, 136, 152, 168, 184, 200, 216, 232, 248, 264, 280, 296, 312, 328, 344, 360, 376, 392, 408, 424, 440, 456, 472, 488, 504, 520, 536, 552, 568, and 584, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and a LCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 16, 32, 48, 64, 80, 96, 112, 128, 144, 160, 176, 192, 208, 224, 240, 256, 272, 288, 304, 320, 336, 352, 368, 384, 400, 416, 432, 448, 464, 480, 496, 512, 528, 544, 560, 576, and 592, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; (iv) comprises a HCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 4, 20, 36, 52, 68, 84, 100, 116, 132, 148, 164, 180, 196, 212, 228, 244, 260, 276, 292, 308, 324, 340, 356, 372, 388, 404, 420, 436, 452, 468, 484, 500, 516, 532, 548, 564, and 580, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a HCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 6, 22, 38, 54, 70, 86, 102, 118, 134, 150, 166, 182, 198, 214, 230, 246, 262, 278, 294, 310, 326, 342, 358, 374, 390, 406, 422, 438, 454, 470, 486, 502, 518, 534, 550, 566, and 582, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a LCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 12, 28, 44, 60, 76, 92, 108, 124, 140, 156, 172, 188, 204, 220, 236, 252, 268, 284, 300, 316, 332, 348, 364, 380, 396, 412, 428, 444, 460, 476, 492, 508, 524, 540, 556, 572, and 588, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and a LCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 14, 30, 46, 62, 78, 94, 110, 126, 142, 158, 174, 190, 206, 222, 238, 254, 270, 286, 302, 318, 334, 350, 366, 382, 398, 414, 430, 446, 462, 478, 494, 510, 526, 542, 558, 574, and 590, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; (v) binds to GREM1 with a K_D equal to or less than 10^{-7}; (vi) blocks GREM1 binding to one of BMP2, BMP4 or BMP7; (vii) blocks GREM1 inhibition of BMP signaling and promotes cell differentiation; and (viii) blocks GREM1 binding to heparin.

[0122] Certain anti-GREM1 antibodies of the present invention are able to bind to and neutralize the activity of GREM1, as determined by *in vitro* or *in vivo* assays. The ability of the antibodies of the invention to bind to and neutralize the activity of GREM1 may be measured using any standard method known to those skilled in the art, including binding assays, or activity assays, as described herein.

[0123] Non-limiting, exemplary *in vitro* assays for measuring binding activity are illustrated in Example 4, herein. In Example 4, the binding affinities and kinetic constants of human anti-GREM1 antibodies were determined by surface plasmon resonance and the measurements were conducted on a T200 Biacore instrument. In Example 5, blocking assays were used to

determine the ability of the anti-GREM1 antibodies to block the BMP4 binding ability of GREM1 in vitro. Examples 6 and 7 describe the activity of the anti-GREM1 antibodies in promoting BMP4 signaling and cell differentiation. In Example 6, the anti-GREM1 antibodies blocked the GREM1 inhibition of BMP4 signaling. In Example 7, the anti-GREM1 antibodies promoted BMP4 signaling and cell differentiation of osteoblast progenitor cells. Example 9 describes inhibition of the GREM1-heparin binding interaction using GREM1-specific antibodies.

[0124] The present invention also includes anti-GREM1 antibodies and antigen binding fragments thereof which bind to at least one biologically active fragment of any of the following proteins, or peptides: SEQ ID NO: 594 (full length native human GREM1), or SEQ ID NO: 595 (recombinant form of human GREM1). Any of the GREM1 peptides described herein, or fragments thereof, may be used to generate anti-GREM1 antibodies.

[0125] The peptides may be modified to include addition or substitution of certain residues for tagging or for purposes of conjugation to carrier molecules, such as, KLH. For example, a cysteine may be added at either the N terminal or C terminal end of a peptide, or a linker sequence may be added to prepare the peptide for conjugation to, for example, KLH for immunization.

[0126] The antibodies specific for GREM1 may contain no additional labels or moieties, or they may contain an N-terminal or C-terminal label or moiety. In one embodiment, the label or moiety is biotin. In a binding assay, the location of a label (if any) may determine the orientation of the peptide relative to the surface upon which the peptide is bound. For example, if a surface is coated with avidin, a peptide containing an N-terminal biotin will be oriented such that the C-terminal portion of the peptide will be distal to the surface. In one embodiment, the label may be a radionuclide, a fluorescent dye or a MRI-detectable label. In certain embodiments, such labeled antibodies may be used in diagnostic assays including imaging assays.

Epitope Mapping and Related Technologies

[0127] The present invention includes anti-GREM1 antibodies which interact with one or more amino acids found within one or more regions of GREM1. The epitope to which the antibodies bind may consist of a single contiguous sequence of 3 or more (*e.g.*, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more) amino acids located within any of the aforementioned regions of the GREM1 molecule (*e.g.* a linear epitope in a domain). Alternatively, the epitope may consist of a plurality of non-contiguous amino acids (or amino acid sequences) located within either or both of the aforementioned regions of the GREM1 molecule (*e.g.* a conformational epitope).

[0128] Various techniques known to persons of ordinary skill in the art can be used to determine whether an antibody "interacts with one or more amino acids" within a polypeptide or protein. Exemplary techniques include, for example, routine cross-blocking assays, such as that described in Antibodies, Harlow and Lane (Cold Spring Harbor Press, Cold Spring Harbor, NY).

Other methods include alanine scanning mutational analysis, peptide blot analysis (Reineke (2004) Methods Mol Biol 248:443-63), peptide cleavage analysis crystallographic studies and NMR analysis. In addition, methods such as epitope excision, epitope extraction and chemical modification of antigens can be employed (Tomer (2000) Protein Science 9: 487-496). Another method that can be used to identify the amino acids within a polypeptide with which an antibody interacts is hydrogen/deuterium exchange detected by mass spectrometry. In general terms, the hydrogen/deuterium exchange method involves deuterium-labeling the protein of interest, followed by binding the antibody to the deuterium-labeled protein. Next, the protein/antibody complex is transferred to water and exchangeable protons within amino acids that are protected by the antibody complex undergo deuterium-to-hydrogen back-exchange at a slower rate than exchangeable protons within amino acids that are not part of the interface. As a result, amino acids that form part of the protein/antibody interface may retain deuterium and therefore exhibit relatively higher mass compared to amino acids not included in the interface. After dissociation of the antibody, the target protein is subjected to protease cleavage and mass spectrometry analysis, thereby revealing the deuterium-labeled residues which correspond to the specific amino acids with which the antibody interacts. *See, e.g.*, Ehring (1999) *Analytical Biochemistry* 267(2):252-259; Engen and Smith (2001) *Anal. Chem. 73*: 256A-265A.

[0129] The term "epitope" refers to a site on an antigen to which B and/or T cells respond. B-cell epitopes can be formed both from contiguous amino acids or noncontiguous amino acids juxtaposed by tertiary folding of a protein. Epitopes formed from contiguous amino acids are typically retained on exposure to denaturing solvents, whereas epitopes formed by tertiary folding are typically lost on treatment with denaturing solvents. An epitope typically includes at least 3, and more usually, at least 5 or 8-10 amino acids in a unique spatial conformation.

[0130] Modification-Assisted Profiling (MAP), also known as Antigen Structure-based Antibody Profiling (ASAP) is a method that categorizes large numbers of monoclonal antibodies (mAbs) directed against the same antigen according to the similarities of the binding profile of each antibody to chemically or enzymatically modified antigen surfaces (see US 2004/0101920, herein specifically incorporated by reference in its entirety). Each category may reflect a unique epitope either distinctly different from or partially overlapping with epitope represented by another category. This technology allows rapid filtering of genetically identical antibodies, such that characterization can be focused on genetically distinct antibodies. When applied to hybridoma screening, MAP may facilitate identification of rare hybridoma clones that produce mAbs having the desired characteristics. MAP may be used to sort the antibodies of the invention into groups of antibodies binding different epitopes.

[0131] In certain embodiments, the anti-GREM1 antibodies or antigen-binding fragments thereof bind an epitope within any one or more of the regions exemplified in GREM1, either in natural form, as exemplified in SEQ ID NO: 594, or recombinantly produced, as exemplified in SEQ ID NO: 595, or to a fragment thereof. In certain embodiments, the antibodies of the

invention, as shown in Table 1, interact with at least one amino acid sequence selected from the group consisting of amino acid residues ranging from about position 1 to about position 24 of SEQ ID NO: 594; or amino acid residues ranging from about position 25 to about position 184 of SEQ ID NO: 594. These regions are further exemplified in SEQ ID NO: 595.

[0132] The present invention includes anti-human GREM1 antibodies that bind to the same epitope, or a portion of the epitope, as any of the specific exemplary antibodies described herein in Table 1, or an antibody having the CDR sequences of any of the exemplary antibodies described in Table 1. Likewise, the present invention also includes anti-human GREM1 antibodies that compete for binding to GREM1 or a GREM1 fragment with any of the specific exemplary antibodies described herein in Table 1, or an antibody having the CDR sequences of any of the exemplary antibodies described in Table 1.

[0133] One can easily determine whether an antibody binds to the same epitope as, or competes for binding with, a reference anti-GREM1 antibody by using routine methods known in the art. For example, to determine if a test antibody binds to the same epitope as a reference anti-GREM1 antibody of the invention, the reference antibody is allowed to bind to a GREM1 protein or peptide under saturating conditions. Next, the ability of a test antibody to bind to the GREM1 molecule is assessed. If the test antibody is able to bind to GREM1 following saturation binding with the reference anti-GREM1 antibody, it can be concluded that the test antibody binds to a different epitope than the reference anti-GREM1 antibody. On the other hand, if the test antibody is not able to bind to the GREM1 protein following saturation binding with the reference anti-GREM1 antibody, then the test antibody may bind to the same epitope as the epitope bound by the reference anti-GREM1 antibody of the invention.

[0134] To determine if an antibody competes for binding with a reference anti-GREM1 antibody, the above-described binding methodology is performed in two orientations: In a first orientation, the reference antibody is allowed to bind to a GREM1 protein under saturating conditions followed by assessment of binding of the test antibody to the GREM1 molecule. In a second orientation, the test antibody is allowed to bind to a GREM1 molecule under saturating conditions followed by assessment of binding of the reference antibody to the GREM1 molecule. If, in both orientations, only the first (saturating) antibody is capable of binding to the GREM1 molecule, then it is concluded that the test antibody and the reference antibody compete for binding to GREM1. As will be appreciated by a person of ordinary skill in the art, an antibody that competes for binding with a reference antibody may not necessarily bind to the identical epitope as the reference antibody, but may sterically block binding of the reference antibody by binding an overlapping or adjacent epitope.

[0135] Two antibodies bind to the same or overlapping epitope if each competitively inhibits (blocks) binding of the other to the antigen. That is, a 1-, 5-, 10-, 20- or 100-fold excess of one antibody inhibits binding of the other by at least 50% but preferably 75%, 90% or even 99% as measured in a competitive binding assay (see, *e.g.,* Junghans *et al.*, Cancer Res. 1990

50:1495-1502). Alternatively, two antibodies have the same epitope if essentially all amino acid mutations in the antigen that reduce or eliminate binding of one antibody reduce or eliminate binding of the other. Two antibodies have overlapping epitopes if some amino acid mutations that reduce or eliminate binding of one antibody reduce or eliminate binding of the other.

[0136] Additional routine experimentation (*e.g.*, peptide mutation and binding analyses) can then be carried out to confirm whether the observed lack of binding of the test antibody is in fact due to binding to the same epitope as the reference antibody or if steric blocking (or another phenomenon) is responsible for the lack of observed binding. Experiments of this sort can be performed using ELISA, RIA, surface plasmon resonance, flow cytometry or any other quantitative or qualitative antibody-binding assay available in the art.

Immunoconjugates

[0137] The invention encompasses a human anti-GREM1 monoclonal antibody conjugated to a therapeutic moiety ("immunoconjugate"), such as an agent that is capable of reducing the severity of fibrosis, or to ameliorate at least one symptom associated with fibrosis, including dry persistent cough and/or difficulty in breathing, or the severity thereof. As used herein, the term "immunoconjugate" refers to an antibody which is chemically or biologically linked to a radioactive agent, a cytokine, an interferon, a target or reporter moiety, an enzyme, a toxin, or a therapeutic agent. The antibody may be linked to the radioactive agent, cytokine, interferon, target or reporter moiety, enzyme, toxin, or therapeutic agent at any location along the molecule so long as it is able to bind its target. An example of immunoconjugate is antibody drug conjugate. In some embodiments, the agent may be a second different antibody to human GREM1, or to a cytokine such as IL-1, IL-6, or a chemokine such as TGF-β. The type of therapeutic moiety that may be conjugated to the anti-GREM1 antibody and will take into account the condition to be treated and the desired therapeutic effect to be achieved. For example, if the desired therapeutic effect is to treat the sequelae or symptoms associated with fibrosis, or any other condition resulting from fibrosis, such as, but not limited to, inflammation or weight loss, it may be advantageous to conjugate an agent appropriate to treat the sequelae or symptoms of the condition, or to alleviate any side effects of the antibodies of the invention. Examples of suitable agents for forming immunoconjugates are known in the art; see for example, WO 05/103081. The preparation of immunoconjugates and immunotoxins is generally well known in the art (see, e.g., U.S. Pat. No. 4340535). Immunoconjugates are described in detail, for example, in US 7250492, US 7420040 and US 7411046, each of which is incorporated herein in their entirety.

Multi-specific Antibodies

[0138] The antibodies of the present invention may be mono-specific, bi-specific, or multi-specific. Multi-specific antibodies may be specific for different epitopes of one target

polypeptide or may contain antigen-binding domains specific for more than one target polypeptide. See, *e.g.,* Tutt et al., 1991, J. Immunol. 147:60-69; Kufer et al., 2004, Trends Biotechnol. 22:238-244.The antibodies of the present invention can be linked to or co-expressed with another functional molecule, *e.g.*, another peptide or protein. For example, an antibody or fragment thereof can be functionally linked (*e.g.,* by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other molecular entities, such as another antibody or antibody fragment to produce a bi-specific or a multi-specific antibody with a second binding specificity. For example, the present invention includes bi-specific antibodies wherein one arm of an immunoglobulin is specific for the N-terminal region of GREM1, or a fragment thereof, and the other arm of the immunoglobulin is specific for the C-terminal region of GREM1, or a second therapeutic target, or is conjugated to a therapeutic moiety. An exemplary bi-specific antibody format that can be used in the context of the present invention involves the use of a first immunoglobulin (Ig) C_{H3} domain and a second Ig C_{H3} domain, wherein the first and second Ig C_{H3} domains differ from one another by at least one amino acid, and wherein at least one amino acid difference reduces binding of the bi-specific antibody to Protein A as compared to a bi-specific antibody lacking the amino acid difference. In one embodiment, the first Ig C_{H3} domain binds Protein A and the second Ig C_{H3} domain contains a mutation that reduces or abolishes Protein A binding such as an H95R modification (by IMGT exon numbering; H435R by EU numbering). The second C_{H3} may further comprise a Y96F modification (by IMGT; Y436F by EU). Further modifications that may be found within the second C_{H3} include: D16E, L18M, N44S, K52N, V57M, and V82I (by IMGT; D356E, L358M, N384S, K392N, V397M, and V422I by EU) in the case of IgG1 antibodies; N44S, K52N, and V82I (IMGT; N384S, K392N, and V422I by EU) in the case of IgG2 antibodies; and Q15R, N44S, K52N, V57M, R69K, E79Q, and V82I (by IMGT; Q355R, N384S, K392N, V397M, R409K, E419Q, and V422I by EU) in the case of IgG4 antibodies. Variations on the bi-specific antibody format described above are contemplated within the scope of the present invention.

[0139] Other exemplary bispecific formats that can be used in the context of the present invention include, without limitation, *e.g.*, scFv-based or diabody bispecific formats, IgG-scFv fusions, dual variable domain (DVD)-Ig, Quadroma, knobs-into-holes, common light chain (*e.g.*, common light chain with knobs-into-holes, etc.), CrossMab, CrossFab, (SEED)body, leucine zipper, Duobody, IgG1/IgG2, dual acting Fab (DAF)-IgG, and Mab^2 bispecific formats (*see, e.g.*, Klein *et al.* 2012, mAbs 4:6, 1-11, and references cited therein, for a review of the foregoing formats). Bispecific antibodies can also be constructed using peptide/nucleic acid conjugation, *e.g.*, wherein unnatural amino acids with orthogonal chemical reactivity are used to generate site-specific antibody-oligonucleotide conjugates which then self-assemble into multimeric complexes with defined composition, valency and geometry. (*See, e.g.*, Kazane *et al., J. Am. Chem. Soc.* [*Epub: Dec. 4, 2012*]).

Therapeutic Administration and Formulations

[0140] The invention provides therapeutic compositions comprising the anti-GREM1 antibodies or antigen-binding fragments thereof of the present invention. The administration of therapeutic compositions in accordance with the invention will be administered with suitable carriers, excipients, and other agents that are incorporated into formulations to provide improved transfer, delivery, tolerance, and the like. A multitude of appropriate formulations can be found in the formulary known to all pharmaceutical chemists: Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, PA. These formulations include, for example, powders, pastes, ointments, jellies, waxes, oils, lipids, lipid (cationic or anionic) containing vesicles (such as LIPOFECTIN™), DNA conjugates, anhydrous absorption pastes, oil-in-water and water-in-oil emulsions, emulsions carbowax (polyethylene glycols of various molecular weights), semi-solid gels, and semi-solid mixtures containing carbowax. See also Powell *et al.* "Compendium of excipients for parenteral formulations" PDA (1998) J Pharm Sci Technol 52:238-311.

[0141] The dose of antibody may vary depending upon the age and the size of a subject to be administered, target disease, conditions, route of administration, and the like. When the antibody of the present invention is used for treating fibrosis in an adult patient, or for treating pulmonary hypertension, or for lessening the severity of the disease, it is advantageous to intravenously administer the antibody of the present invention normally at a single dose of about 0.1 to about 100 mg/kg body weight, more preferably about 5 to about 100, about 10 to about 90, or about 20 to about 70 mg/kg body weight. Depending on the severity of the condition, the frequency and the duration of the treatment can be adjusted. In certain embodiments, the antibody or antigen-binding fragment thereof of the invention can be administered as an initial dose of at least about 0.1 mg to about 800 mg, about 1 to about 500 mg, about 5 to about 300 mg, or about 10 to about 200 mg, to about 100 mg, or to about 50 mg. In certain embodiments, the initial dose may be followed by administration of a second or a plurality of subsequent doses of the antibody or antigen-binding fragment thereof in an amount that can be approximately the same or less than that of the initial dose, wherein the subsequent doses are separated by at least 1 day to 3 days; at least one week, at least 2 weeks; at least 3 weeks; at least 4 weeks; at least 5 weeks; at least 6 weeks; at least 7 weeks; at least 8 weeks; at least 9 weeks; at least 10 weeks; at least 12 weeks; or at least 14 weeks.

[0142] Various delivery systems are known and can be used to administer the pharmaceutical composition of the invention, *e.g.,* encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the mutant viruses, receptor mediated endocytosis (see, *e.g.*, Wu et al. (1987) J. Biol. Chem. 262:4429-4432). Methods of introduction include, but are not limited to, intradermal, transdermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural and oral routes. The composition may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.,* oral mucosa, rectal and intestinal mucosa, etc.) and may be

administered together with other biologically active agents. Administration can be systemic or local. The pharmaceutical composition can be also delivered in a vesicle, in particular a liposome (see, for example, Langer (1990) Science 249:1527-1533).

[0143] The use of nanoparticles to deliver the antibodies of the present invention is also contemplated herein. Antibody-conjugated nanoparticles may be used both for therapeutic and diagnostic applications. Antibody-conjugated nanoparticles and methods of preparation and use are described in detail by Arruebo, M., et al. 2009 ("Antibody-conjugated nanoparticles for biomedical applications" in J. Nanomat. Volume 2009, Article ID 439389, 24 pages, doi: 10.1155/2009/439389), incorporated herein by reference. Nanoparticles for drug delivery have also been described in, for example, US 8277812, US 8258256, US 8257740, US 8246995, US 8236330, each incorporated herein in its entirety.

[0144] In certain situations, the pharmaceutical composition can be delivered in a controlled release system. In one embodiment, a pump may be used. In another embodiment, polymeric materials can be used. In yet another embodiment, a controlled release system can be placed in proximity of the composition's target, thus requiring only a fraction of the systemic dose.

[0145] The injectable preparations may include dosage forms for intravenous, subcutaneous, intracutaneous and intramuscular injections, drip infusions, etc. These injectable preparations may be prepared by methods publicly known. For example, the injectable preparations may be prepared, *e.g.*, by dissolving, suspending or emulsifying the antibody or its salt described above in a sterile aqueous medium or an oily medium conventionally used for injections. As the aqueous medium for injections, there are, for example, physiological saline, an isotonic solution containing glucose and other auxiliary agents, etc., which may be used in combination with an appropriate solubilizing agent such as an alcohol (*e.g.*, ethanol), a polyalcohol (e.g., propylene glycol, polyethylene glycol), a nonionic surfactant [e.g., polysorbate 80, HCO-50 (polyoxyethylene (50 mol) adduct of hydrogenated castor oil)], etc. As the oily medium, there are employed, *e.g.*, sesame oil, soybean oil, etc., which may be used in combination with a solubilizing agent such as benzyl benzoate, benzyl alcohol, etc. The injection thus prepared is preferably filled in an appropriate ampoule.

[0146] A pharmaceutical composition of the present invention can be delivered subcutaneously or intravenously with a standard needle and syringe. In addition, with respect to subcutaneous delivery, a pen delivery device readily has applications in delivering a pharmaceutical composition of the present invention. Such a pen delivery device can be reusable or disposable. A reusable pen delivery device generally utilizes a replaceable cartridge that contains a pharmaceutical composition. Once all of the pharmaceutical composition within the cartridge has been administered and the cartridge is empty, the empty cartridge can readily be discarded and replaced with a new cartridge that contains the pharmaceutical composition. The pen delivery device can then be reused. In a disposable pen delivery device, there is no replaceable cartridge. Rather, the disposable pen delivery device comes prefilled with the pharmaceutical

composition held in a reservoir within the device. Once the reservoir is emptied of the pharmaceutical composition, the entire device is discarded.

[0147] Numerous reusable pen and autoinjector delivery devices have applications in the subcutaneous delivery of a pharmaceutical composition of the present invention. Examples include, but certainly are not limited to AUTOPEN™ (Owen Mumford, Inc., Woodstock, UK), DISETRONIC™ pen (Disetronic Medical Systems, Burghdorf, Switzerland), HUMALOG MIX 75/25™ pen, HUMALOG™ pen, HUMALIN 70/30™ pen (Eli Lilly and Co., Indianapolis, IN), NOVOPEN™ I, II and III (Novo Nordisk, Copenhagen, Denmark), NOVOPEN JUNIOR™ (Novo Nordisk, Copenhagen, Denmark), BD™ pen (Becton Dickinson, Franklin Lakes, NJ), OPTIPEN™, OPTIPEN PRO™, OPTIPEN STARLET™, and OPTICLIK™ (Sanofi-aventis, Frankfurt, Germany), to name only a few. Examples of disposable pen delivery devices having applications in subcutaneous delivery of a pharmaceutical composition of the present invention include, but certainly are not limited to the SOLOSTAR™ pen (Sanofi-aventis), the FLEXPEN™ (Novo Nordisk), and the KWIKPEN™ (Eli Lilly), the SURECLICK ™ Autoinjector (Amgen, Thousand Oaks, CA), the PENLET ™ (Haselmeier, Stuttgart, Germany), the EPIPEN (Dey, L.P.) and the HUMIRA ™ Pen (Abbott Labs, Abbott Park, IL), to name only a few.

[0148] Advantageously, the pharmaceutical compositions for oral or parenteral use described above are prepared into dosage forms in a unit dose suited to fit a dose of the active ingredients. Such dosage forms in a unit dose include, for example, tablets, pills, capsules, injections (ampoules), suppositories, etc. The amount of the aforesaid antibody contained is generally about 5 to about 500 mg per dosage form in a unit dose; especially in the form of injection, it is preferred that the aforesaid antibody is contained in about 5 to about 100 mg and in about 10 to about 250 mg for the other dosage forms.

Therapeutic Uses of the Antibodies

[0149] In certain embodiments of the invention, the present antibodies are useful for treating a disease or condition associated with fibrosis, or at least one symptom associated with the disease or condition, such as persistent cough, breathlessness, weight loss or loss of appetite, or for lessening the severity of the disease. In some embodiments, the antibodies may be useful for treating a condition or symptom of fibrosis at a later stage in the disease. The antibodies of the invention are also contemplated for prophylactic use in patients at risk for developing fibrosis. These patients include the elderly, or patients with a family history, or patients immunocompromised due to illness or treatment with immunosuppressive therapeutics, or patients who may have an underlying medical condition such as diabetes that predisposes them to fibrosis, or patients who may be predisposed to fibrosis due to lifestyle choices such as smoking or alcohol abuse. It is contemplated that the antibodies of the invention may be used alone, or in conjunction with a second agent, or third agent for treating fibrosis, or for alleviating at least one symptom or complication associated with fibrosis, such loss of kidney function or

liver function associated with, or resulting from fibrosis. The second or third agents may be delivered concurrently with the antibodies of the invention, or they may be administered separately, either before or after the antibodies of the invention.

[0150] Symptoms for fibrosis disorders include, but are not limited to, dry cough, difficulty in breathing, loss of appetite, weight loss, fatigue, nausea, swelling and fluid accumulation, liver damage, liver failure, hypertension, and loss of renal function. Other signs or symptoms include, but are not limited to, malaise, poor sleep, or complications such as pneumonia or urinary tract infection, hyperglycemia, and proteinuria. The antibodies of the present invention may be used to relieve or to prevent or to decrease the severity of one or more of the symptoms or conditions listed above.

[0151] In certain embodiments, the present antibodies are useful for treating a condition or indication associated with cancer including, but not limited to sarcoma, or carcinoma of lung, ovary, kidney, breast, colon, pancreas and uterine cervix.

[0152] In certain embodiments, one or more antibodies of the present invention may be used alone or in combination to block GREM1 binding to heparin and/or heparin-mediated angiogenesis.

[0153] In a further embodiment of the invention the present antibodies are used for the preparation of a pharmaceutical composition for treating patients suffering from fibrosis or cancer, or a symptom associated with fibrosis or cancer. In yet another embodiment of the invention the present antibodies are used for the preparation of a pharmaceutical composition for reducing the tissue damage or for preventing progressive degeneration or for protecting kidney function or liver function in fibrosis. In one embodiment of the invention the present antibodies are used as adjunct therapy with any other agent useful for treating fibrosis or cancer, including an analgesic, a NSAID, an anti-tumor drug, chemotherapy, radiotherapy, a glucocorticoid, a vascular endothelial growth factor (VEGF) antagonist [*e.g.*, a "VEGF-Trap" such as aflibercept or other VEGF-inhibiting fusion protein as set forth in US 7,087,411, or an anti-VEGF antibody or antigen binding fragment thereof (*e.g.*, bevacizumab, or ranibizumab)], a second antibody to GREM1, an antibody to GREM2 or to an inflammatory cytokine such as IL-1, IL-6, or TGF-β, or any other palliative therapy known to those skilled in the art.

Combination Therapies

[0154] Combination therapies may include an anti-GREM1 antibody of the invention and any additional therapeutic agent that may be advantageously combined with an antibody of the invention, or with a biologically active fragment of an antibody of the invention.

[0155] For example, a second or third therapeutic agent such as a non-steroidal anti-inflammatory agent (NSAID) or an analgesic may be employed to aid in alleviating the symptoms of fibrosis such as dry cough or difficulty in breathing. An example of a common analgesic is acetaminophen. Exemplary NSAIDs include aspirin, ibuprofen, and naproxen. The

additional therapeutic agent may be an antibiotic to treat a complication such as urinary tract infection. The antibodies of the present invention may be combined with an antihypertensive agent to slow down the development of fibrosis. For example, for patients suffering from diabetic nephropathy, the antibodies may be combined with treatment such as angiotensin-converting enzyme inhibitors to reduce blood pressure and protect kidney function.

[0156] The antibodies may be used in conjunction with other therapies, such as corticosteroids, or nutritional supplements in fibrosis treatment. Anti-fibrotic drugs such as pirfenidone have been found both to produce durable symptomatic remissions and to delay or halt progression of fibrosis. This is important as such damage is usually irreversible. Anti-inflammatories and analgesics improve pain but do not prevent tissue damage or slow the disease progression. In some embodiments, second or third therapeutic agents may be used to minimize clinical symptoms such as nausea and swelling, as well as prevent fibrotic tissue damage. An additional therapeutic agent may comprise cortisone therapy, e.g., a low dosage of prednisone or prednisolone may be used in conjunction with one or more antibodies of the present invention in a long term treatment plan for fibrosis. The use of one or more antibodies directed to a cytokine such as IL-1, IL-6, or TGF-β in fibrosis treatments are also envisaged within the scope of the present invention. The antibodies of the present invention may be combined with additional therapeutic agents to minimize or prevent complications such as urinary tract infection, hyperglycemia or blood pressure.

[0157] The antibodies of the present invention may also be administered in combination with other treatment options for fibrosis including physical therapy, lifestyle changes (including exercise and weight control), pulmonary rehabilitation, oxygen therapy, and dietary changes. Transplant surgery of liver, lungs or kidney may be required in advanced forms of fibrosis.

[0158] The antibodies of the present invention may be combined synergistically with one or more anti-cancer drugs or therapy used to treat cancer. Examples of anti-cancer drugs and therapy that may be used include, but are not limited to, cytotoxins, chemotherapeutic agents, radiation and surgery. In some embodiments, one or more antibodies of the present invention may be used in combination with an anti-inflammatory drug (*e.g.*, corticosteroids, and non-steroidal anti-inflammatory drugs), an antibody to a tumor-specific antigen (*e.g.,* CA9, CA125, melanoma-associated antigen (MAGE), carcinoembryonic antigen (CEA), vimentin, tumor-M2-PK, prostate-specific antigen (PSA), MART-1, and CA19-9), a vascular endothelial growth factor (VEGF) antagonist [*e.g.,* a "VEGF-Trap" such as aflibercept or other VEGF-inhibiting fusion protein as set forth in US 7,087,411, or an anti-VEGF antibody or antigen binding fragment thereof (*e.g.*, bevacizumab, or ranibizumab)], a dietary supplement such as anti-oxidants or any palliative care to treat cancer.

[0159] The additional therapeutically active component(s) may be administered prior to, concurrent with, or after the administration of the anti-GREM1 antibody of the present invention. For purposes of the present disclosure, such administration regimens are considered the

administration of an anti-GREM1 antibody "in combination with" a second therapeutically active component.

Diagnostic Uses of the Antibodies

[0160] The anti-GREM1 antibodies of the present invention may also be used to detect and/or measure GREM1 in a sample, *e.g.*, for diagnostic purposes. It is envisioned that any one or more of the antibodies of the invention may be used to detect severity of tissue damage in fibrosis. Exemplary diagnostic assays for GREM1 may comprise, *e.g.*, contacting a sample, obtained from a patient, with an anti-GREM1 antibody of the invention, wherein the anti-GREM1 antibody is labeled with a detectable label or reporter molecule or used as a capture ligand to selectively isolate GREM1 from patient samples. Alternatively, an unlabeled anti-GREM1 antibody can be used in diagnostic applications in combination with a secondary antibody which is itself detectably labeled. The detectable label or reporter molecule can be a radioisotope, such as ^{3}H, ^{14}C, ^{32}P, ^{35}S, or ^{125}I; a fluorescent or chemiluminescent moiety such as fluorescein isothiocyanate, or rhodamine; or an enzyme such as alkaline phosphatase, β-galactosidase, horseradish peroxidase, or luciferase. Specific exemplary assays that can be used to detect or measure GREM1 in a sample include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluorescence-activated cell sorting (FACS).

[0161] Samples that can be used in GREM1 diagnostic assays according to the present invention include any tissue or fluid sample obtainable from a patient, which contains detectable quantities of either GREM1 protein, or fragments thereof, under normal or pathological conditions. Generally, levels of GREM1 in a particular sample obtained from a healthy patient (*e.g.*, a patient not afflicted with fibrosis) will be measured to initially establish a baseline, or standard, level of GREM1. This baseline level of GREM1 can then be compared against the levels of GREM1 measured in samples obtained from individuals suspected of having fibrosis related condition, or symptoms associated with such condition.

[0162] The antibodies specific for GREM1 may contain no additional labels or moieties, or they may contain an N-terminal or C-terminal label or moiety. In one embodiment, the label or moiety is biotin. In a binding assay, the location of a label (if any) may determine the orientation of the peptide relative to the surface upon which the peptide is bound. For example, if a surface is coated with avidin, a peptide containing an N-terminal biotin will be oriented such that the C-terminal portion of the peptide will be distal to the surface. In some embodiments, the label may be detectable label such as a radionuclide, a fluorescent dye or a MRI-detectable label. Detectable labels may be linked to the antibodies wherein such antibodies may be used in imaging assays. Methods using imaging assays may be useful for fibrosis diagnosis and prognosis, or monitoring fibrotic activity.

[0163] Aspects of the invention relate to use of the disclosed antibodies as markers for predicting prognosis of fibrosis in patients. GREM1 has been found to be upregulated in fibrotic

tissues in, for example, lung or liver or kidneys. Elevated levels of GREM1 have been correlated to diabetic nephropathy or pulmonary hypertension and could be used for evaluation of patient's prognosis. Antibodies of the present invention may be used in diagnostic assays to evaluate prognosis of fibrotic disease in a patient and to predict survival.

EXAMPLES

[0164] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the methods and compositions of the invention, and are not intended to limit the scope of what the inventors regard as their invention. Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric.

Example 1. Generation of Human Antibodies to human GREM1

[0165] In certain embodiments, the immunogen may be a peptide from the N terminal or C terminal end of human GREM1. In certain embodiments of the invention, the immunogen is the mature protein of human GREM1 that ranges from about amino acid residues 25-184 of SEQ ID NO: 594. In one embodiment, the antibodies of the invention were obtained from mice immunized with full length recombinant human GREM1.

[0166] In certain embodiments, antibodies that bind specifically to human GREM1 may be prepared using fragments of the above-noted regions, or peptides that extend beyond the designated regions by about 5 to about 20 amino acid residues from either, or both, the N or C terminal ends of the regions described herein. In certain embodiments, any combination of the above-noted regions or fragments thereof may be used in the preparation of GREM1 specific antibodies. In certain embodiments, any one or more of the above-noted domains of hGREM1, or fragments thereof may be used for preparing monospecific, bispecific, or multispecific antibodies (see Example 8 below for details).

[0167] The full length proteins, or fragments thereof, that were used as immunogens, as noted above, were administered directly, with an adjuvant to stimulate the immune response, to a VELOCIMMUNE® mouse comprising DNA encoding human Immunoglobulin heavy and kappa light chain variable regions. The antibody immune response was monitored by a GREM1-specific immunoassay. When a desired immune response was achieved splenocytes were harvested and fused with mouse myeloma cells to preserve their viability and form hybridoma cell lines. The hybridoma cell lines were screened and selected to identify cell lines that produce GREM1-specific antibodies. Using this technique, and the various immunogens described above, several anti-GREM1, as well as cross-reactive, chimeric antibodies (*i.e.*,

antibodies possessing human variable domains and mouse constant domains) were obtained; exemplary antibodies generated in this manner were designated as H1M2907N, H2M2780N, H2M2782N, H2M2783N, H4H2783N2, H2M2784N, H2M2785N, H2M2786N, H2M2889N, H2M2890N, H2M2891N, H2M2892N, H2M2895N, H2M2897N, H2M2898N, H2M2899N, H2M2901N, H2M2906N, H2M2926N, H3M2788N, and H3M2929N.

[0168] Anti-GREM1 antibodies were also isolated directly from antigen-positive B cells without fusion to myeloma cells, as described in U.S. 2007/0280945A1, herein specifically incorporated by reference in its entirety. Using this method, several fully human anti-GREM1 antibodies (*i.e.*, antibodies possessing human variable domains and human constant domains) were obtained; exemplary antibodies generated in this manner were designated as follows: H4H6232P, H4H6233P, H4H6236P, H4H6238P, H4H6240P, H4H6243P, H4H6245P, H4H6246P, H4H6248P, H4H6250P, H4H6251P, H4H6252S, H4H6256P, H4H6260P, H4H6269P, and H4H6270P.

[0169] The biological properties of the exemplary antibodies generated in accordance with the methods of this Example are described in detail in the Examples set forth below.

Example 2. Heavy and Light Chain Variable Region Amino Acid Sequences

[0170] Table 1 sets forth the heavy and light chain variable region amino acid sequence pairs of selected antibodies specific for human GREM1 and their corresponding antibody identifiers. Antibodies are typically referred to herein according to the following nomenclature: Fc prefix (e.g. "H4H", "H1M, "H2M"), followed by a numerical identifier (e.g. "2907" as shown in Table 1), followed by a "P" or "N" suffix. Thus, according to this nomenclature, an antibody may be referred to as, e.g. "H1H2907". The H4H, H1M, and H2M prefixes on the antibody designations used herein indicate the particular Fc region of the antibody. For example, an "H2M" antibody has a mouse IgG2 Fc, whereas an "H4H" antibody has a human IgG4 Fc. As will be appreciated by a person of ordinary skill in the art, an H1M or H2M antibody can be converted to an H4H antibody, and vice versa, but in any event, the variable domains (including the CDRs), which are indicated by the numerical identifiers shown in Table 1, will remain the same. Antibodies having the same numerical antibody designation, but differing by a letter suffix of N, B or P refer to antibodies having heavy and light chains with identical CDR sequences but with sequence variations in regions that fall outside of the CDR sequences (*i.e.,* in the framework regions). Thus, N, B and P variants of a particular antibody have identical CDR sequences within their heavy and light chain variable regions but differ from one another within their framework regions.

Table 1

	SEQ ID NOs:							
Antibody Designation	**HCVR**	**HCDR1**	**HCDR2**	**HCDR3**	**LCVR**	**LCDR1**	**LCDR2**	**LCDR3**

2907N	2	4	6	8	10	12	14	16
2780N	18	20	22	24	26	28	30	32
2782N	34	36	38	40	42	44	46	48
2783N	50	52	54	56	58	60	62	64
2783N2	66	68	70	72	74	76	78	80
2784N	82	84	86	88	90	92	94	96
2785N	98	100	102	104	106	108	110	112
2786N	114	116	118	120	122	124	126	128
2889N	130	132	134	136	138	140	142	144
2890N	146	148	150	152	154	156	158	160
2891N	162	164	166	168	170	172	174	176
2892N	178	180	182	184	186	188	190	192
2895N	194	196	198	200	202	204	206	208
2897N	210	212	214	216	218	220	222	224
2898N	226	228	230	232	234	236	238	240
2899N	242	244	246	248	250	252	254	256
2901N	258	260	262	264	266	268	270	272
2906N	274	276	278	280	282	284	286	288
2926N	290	292	294	296	298	300	302	304
2788N	306	308	310	312	314	316	318	320
2929N	322	324	326	328	330	332	334	336
6232P	338	340	342	344	346	348	350	352
6233P	354	356	358	360	362	364	366	368
6236P	370	372	374	376	378	380	382	384
6238P	386	388	390	392	394	396	398	400
6240P	402	404	406	408	410	412	414	416
6243P	418	420	422	424	426	428	430	432
6245P	434	436	438	440	442	444	446	448
6246P	450	452	454	456	458	460	462	464
6248P	466	468	470	472	474	476	478	480
6250P	482	484	486	488	490	492	494	496
6251P	498	500	502	504	506	508	510	512
6252P	514	516	518	520	522	524	526	528
6256P	530	532	534	536	538	540	542	544
6260P	546	548	550	552	554	556	558	560
6269P	562	564	566	568	570	572	574	576
6270P	578	580	582	584	586	588	590	592

Example 3. Variable Gene Utilization Analysis

[0171] To analyze the structure of antibodies produced, the nucleic acids encoding antibody variable regions were cloned and sequenced. From the nucleic acid sequence and predicted amino acid sequence of the antibodies, gene usage was identified for each Heavy Chain Variable Region (HCVR) and Light Chain Variable Region (LCVR). Table 2 sets forth the gene usage for selected antibodies in accordance with the invention.

Table 2

Antibody	HCVR			LCVR	
	V_H	D_H	J_H	V_K	J_K
H4H2780N	3-21	1-26	6	1-27	3
H2bM2782N	3-21	1-7	4	1-27	3
H4H2784N	3-33	3-10	4	3-15	2
H2bM2785N	3-23	6-13	3	1-33	2
H2bM2786N	3-23	2-8	3	1-33	2
H2M2783N	3-23	6-13	3	1-33	2
H4H2783N2	3-23	6-13	3	1-33	2
H3M2788N	3-33	4-23	4	1-17	1
H4H2897N	3-30	2-21	4	2-28	3
H2bM2891N	3-7	3-3	4	1-12	2
H2AM2898N	3-13	2-2	6	1-6	1
H2BM2906N	3-7	3-3	4	1-12	2
H4H2892N	3-33	1-1	2	1-9	1
H1M2907N	3-33	1-1	3	3-11	2
H2BM2890N	3-33	1-14	4	3-11	4
H2AM2899N	1-8	6-6	4	1-33	3
H4H2895N	3-53	3-9	4	6-21	1
H4H2926N	3-9	6-13	4	1-33	4
H3M2929N	3-33	3-10	4	3-15	1
H2AM2901N	3-21	2-12	4	1-27	3
H4H2889N	4-59	4-4	6	1-6	1
H4H6232P	1-24	3-9	6	1-39	3
H4H6233P	3-33	1-7	4	3-11	2
H4H6236P	1-24	3-9	6 or 3	1-39	3
H4H6238P	3-11	1-1	4	3-15	2
H4H6240P	3-33	1-7	4	3D-15	3
H4H6243P	1-24	3-9	6 or 4	1-39	3
H4H6245P	3-7	2-15	4	1-33	2
H4H6246P	3-33	4-17	4	1-17	3
H4H6248P	3-33	4-17	4	3-15	1
H4H6250P	3-33	4-17	4	1-17	3
H4H6251P	3-33	4-17	4	3-15	1
H4H6252P	3-33	4-17	4	1-17	3
H4H6256P	3-33	4-17	5 or 4	3-15	1

H4H6260P	3-33	1-1	3	3-11	5
H4H6269P	4-31	1-1	4	1-6	4
H4H6270P	3-33	3-10	4	3-15	2

Example 4. Antibody Binding to human GREM1 as Determined by Surface Plasmon Resonance

[0172] Binding associative and dissociative rate constants (k_a and k_d, respectively) and calculated equilibrium dissociation constants and dissociative half-lives (K_D and $t_{1/2}$, respectively) for antigen binding to purified anti-Gremlin1 (GREM1) antibodies were determined using a real-time surface plasmon resonance biosensor (Biacore T200) assay at 25°C and at 37°C.

[0173] Anti-GREM1 antibodies were captured on either a goat anti-mouse IgG polyclonal antibody (GE Healthcare, # BR-1008-38) or a mouse anti-human IgG monoclonal antibody (GE Healthcare, # BR-1008-39) surface created through direct amine coupling to a Biacore CM5 sensor chip. Kinetic experiments were carried out using HBS-EP + heparin [10mM HEPES, 150mM NaCl, 3mM EDTA, 0.05% (v/v) surfactant P20, 10µg/ml heparin sodium salt, pH 7.4] as both the running buffer and the sample buffer. Antigen-antibody association rates were measured by injecting various concentrations (ranging from 11 to 100 nM, 3-fold dilutions) of human GREM1 (hGREM1-His; SEQ ID NO: 595) over the captured anti-GREM1 antibody surface. Antigen-antibody association was monitored for 150 seconds while dissociation in buffer was monitored for 420 seconds. Kinetic analysis was performed using Scrubber software version 2.0a or Biacore T200 evaluation software v1.0 to determine k_a and k_d values. K_D and $t_{1/2}$ were then calculated from the experimentally determined k_a and k_d values as $K_D = k_d / k_a$ and $t_{1/2} = \ln(2)/k_d$.

[0174] As shown in Table 3, thirty-five anti-GREM1 antibodies when captured on the Biacore sensor exhibited binding to hGREM1-His protein injected over the surface at 25°C, with K_D values ranging from 625pM to 270nM. Two of the antibodies tested, H2aM2898N and H2bM2785N, did not bind hGREM1-His under these experimental conditions. A subset of the 37 anti-GREM1 antibodies was tested again at 37°C. As shown in Table 4, twenty-four anti-GREM1 antibodies when captured on the Biacore sensor exhibited binding to hGREM1-His protein injected over the surface at 37°C, with K_D values ranging from 1.23nM to 275nM.

Table 3: Biacore affinities at 25°C for hGREM1-His binding to captured anti-GREM1 monoclonal antibodies

mAb captured	k_a(1/Ms)	k_d(1/s)	K_D (M)	$t_{1/2}$ (min)
H4H2895N	1.70E+05	1.32E-04	7.78E-10	88
H4H2780N	7.12E+04	8.80E-04	1.24E-08	13
H4H2783N2	3.04E+04	4.76E-04	1.57E-08	24
H4H2784N	6.18E+04	1.05E-03	1.70E-08	11
H4H2897N	2.77E+03	4.10E-04	1.48E-07	28
H4H2889N	6.27E+04	1.18E-04	1.89E-09	98
H4H2892N	1.42E+05	1.73E-04	1.22E-09	67

H4H2926N	9.10E+04	5.02E-04	5.52E-09	23
H4H6232P	7.68E+04	9.60E-05	1.25E-09	120
H4H6233P	9.13E+04	1.72E-04	1.88E-09	67
H4H6236P	4.20E+04	1.25E-04	2.99E-09	92
H4H6238P	2.41E+04	2.25E-04	9.32E-09	51
H4H6240P	7.97E+04	3.19E-04	4.00E-09	36
H4H6243P	1.58E+04	1.78E-04	1.13E-08	65
H4H6245P	7.79E+04	1.17E-04	1.51E-09	98
H4H6246P	7.39E+04	1.92E-04	2.59E-09	60
H4H6248P	5.03E+04	7.73E-05	1.54E-09	149
H4H6250P	9.01E+04	2.97E-04	3.30E-09	39
H4H6251P	2.82E+04	6.52E-04	2.31E-08	18
H4H6252P	7.46E+04	7.58E-05	1.02E-09	152
H4H6256P	9.00E+04	1.07E-04	1.19E-09	108
H4H6260P	9.74E+04	6.51E-05	6.69E-10	177
H4H6269P	1.01E+05	6.32E-05	6.25E-10	183
H4H6270P	4.29E+04	1.75E-04	4.08E-09	66
H1M2907N	6.20E+04	2.81E-03	4.53E-08	4.1
H2aM2898N	NB	NB	NB	NB
H2aM2899N	8.00E+03	2.00E-03	2.70E-07	5.8
H2aM2901N	1.64E+05	1.45E-03	8.80E-09	8.0
H2bM2782N	1.23E+05	1.89E-03	1.53E-08	6.1
H2bM2785N	NB	NB	NB	NB
H2bM2786N	1.80E+05	6.00E-03	3.30E-08	1.9
H2bM2890N	1.00E+04	1.00E-03	1.30E-07	11.6
H2bM2891N	2.00E+04	1.20E-03	6.00E-08	9.6
H2bM2906N	8.00E+04	1.30E-03	1.50E-08	8.9
H3M2788N	8.00E+04	3.50E-04	4.20E-09	33.0
H2bM2783N	2.02E+05	1.50E-03	7.40E-09	7.7
H3M2929N	1.25E+05	3.62E-03	2.90E-08	3

NB = no binding under the conditions tested

Table 4: Biacore affinities at 37°C for hGREM1-His binding to captured anti-GREM1 monoclonal antibodies

mAb captured	k_a(1/Ms)	k_d(1/s)	K_D (M)	$t_{1/2}$ (min)
H4H2895N	1.75E+05	2.15E-04	1.23E-09	54
H4H2780N	5.74E+04	1.61E-03	2.80E-08	7
H4H2783N2	1.39E+04	8.49E-04	6.11E-08	14
H4H2784N	1.42E+05	1.97E-03	1.38E-08	6
H4H2897N	7.03E+04	2.02E-03	2.88E-08	6
H4H2889N	1.04E+05	4.22E-04	4.06E-09	27
H4H2892N	1.71E+05	4.00E-04	2.35E-09	29
H4H2926N	1.26E+05	1.40E-03	1.11E-08	8
H4H6232P	1.13E+05	1.95E-04	1.73E-09	59

H4H6233P	1.57E+05	5.12E-04	3.26E-09	23
H4H6236P	9.30E+04	2.45E-04	2.63E-09	47
H4H6238P	5.69E+04	9.31E-04	1.64E-08	12
H4H6240P	1.21E+05	1.18E-03	9.75E-09	10
H4H6243P	7.05E+03	4.84E-04	6.87E-08	24
H4H6245P	1.03E+05	2.62E-04	2.53E-09	44
H4H6246P	1.27E+05	4.44E-04	3.50E-09	26
H4H6248P	8.55E+04	4.87E-04	5.69E-09	24
H4H6250P	1.49E+05	8.82E-04	5.93E-09	13
H4H6251P	2.53E+04	6.94E-03	2.75E-07	2
H4H6252P	1.11E+05	4.59E-04	4.14E-09	25
H4H6256P	1.14E+05	4.66E-04	4.08E-09	25
H4H6260P	1.66E+05	2.85E-04	1.71E-09	41
H4H6269P	1.28E+05	2.54E-04	1.98E-09	46
H4H6270P	9.58E+04	6.50E-04	6.79E-09	18

Example 5. Determination of the GREM1 inhibitory activity of the anti-hGREM1 antibodies

[0175] To further characterize the anti-human Gremlin 1 (GREM1) antibodies, their ability to block GREM1 binding to human bone morphogenetic protein 4 (BMP4) was examined via ELISA. Plates were coated with recombinant human BMP4 (2ug/mL) (hBMP4; R&D, # 314-BP/CF, residues S293-R408 of accession # Q53XC5, expressed in NS0 cells) overnight and then serial dilutions of antibodies were incubated with a constant amount (100pM) of recombinant human GREM1 protein (hGREM1-His; SEQ ID NO: 595) modified with a biotin tag for 1 hour at 25°C before this complex was added to coated plates and allowed to incubate for an additional hour at 25°C. The plates were then washed and plate bound biotin-hGREM1-His was detected with streptavidin conjugated with horseradish peroxidase (Pierce, # N200). Plates were then developed with a TMB solution (BD Biosciences, # 555214) to produce a colorimetric reaction and the reaction was quenched by acidification with sulfuric acid before reading absorbance at 450nm on a PerkinElmer Victor X5 plate reader. Data were analyzed using a sigmoidal dose-response model within Prism™ software. The calculated IC_{50} value, defined as the antibody concentration required to achieve 50% of maximum blocking, was used as an indicator of blocking potency. The IC_{50} value for several samples was reported at a fixed, lower-bound value of 2.5E-11M, which represents the theoretical lower-limit of this assay, given the fixed concentration of biotin-hGREM1-His used in the assay. Percent blockade was calculated as the ratio of the reduction in signal observed in the presence of antibody relative to the difference between the signal with GREM1 alone and background (signal from HRP-conjugated secondary antibody alone). The absorbance measured for the constant concentration of 100 pM biotin-hGREM1-His alone is defined as 0% blocking and the absorbance measured for no added GREM1 is defined as 100% blocking. The absorbance values of the wells containing the

highest concentration for each antibody were used to determine the percent maximum blocking. All 24 anti-GREM1 antibodies tested in this assay blocked biotin-hGREM1-His with IC_{50} values ranging from <25pM to 1.9nM. At a concentration of 20nM of antibody, the 24 antibodies exhibited from 42 to 96-percent blockade of biotin-hGREM1-His binding to hBMP4.

Table 5: Anti-GREM1 antibodies blocking bone morphogenic protein 4 (hBMP4) binding to biotin-hGREM1-His

Ab PID	IC_{50} (M)	% blocked at maximum Ab tested (20nM)
H4H2780N	1.0E-10	69
H4H2783N2	< 2.5E-11	76
H4H2784N	4.5E-10	59
H4H2889N	< 2.5E-11	86
H4H2892N	1.7E-10	82
H4H2895N	3.6E-11	94
H4H2897N	4.2E-11	73
H4H2926N	5.2E-11	93
H4H6232P	< 2.5E-11	93
H4H6233P	6.9E-10	66
H4H6236P	< 2.5E-11	95
H4H6238P	9.7E-10	64
H4H6240P	6.5E-10	60
H4H6243P	7.2E-12	92
H4H6245P	3.6E-11	96
H4H6246P	1.5E-09	62
H4H6248P	2.1E-10	62
H4H6250P	1.1E-10	42
H4H6251P	1.1E-10	62
H4H6252P	1.9E-10	53
H4H6256P	8.7E-11	75
H4H6260P	< 2.5E-11	87
H4H6269P	2.6E-11	73
H4H6270P	1.9E-09	62
H4H121N isotype control	>2.00E-08	5.9

Calculated IC50 values <2.50E-11 below theoretical assay bottom and reported as <2.50E-11

Example 6. Effect of GREM1 on BMP4 signaling

[0176] Gremlin 1 (GREM1) is a negative regulator of bone morphogenetic protein (BMP) signaling (Walsh et al. 2010). BMPs belong to the TGF-β superfamily and are involved in regulation of many physiological processes including proliferation, differentiation, and cell-fate determination during embryonic and postnatal development (Hogan, 1996). Activation of BMP receptors leads to phosphorylation of SMAD proteins and transcriptional activation of BMP-responsive genes. GREM1 binds to BMP2, BMP4, and BMP7 and blocks binding to their

receptors. A bioassay was developed to detect the regulation of BMP4 signaling by GREM1 in a mammalian cell line, W-20-17, a mouse bone marrow stromal cell line previously shown to be responsive to BMP2 (Thies et al. 1992). This cell line was modified to stably express a BMP-responsive luciferase reporter. The resulting stable cell line (W-20-17/BRE-luc cells) was isolated and maintained in 10% fetal bovine serum, DMEM, NEAA, penicillin/streptomycin, and 200µg/ml G418.

[0177] For the bioassay, the W-20-17/BRE-luc cells were seeded onto 96-well assay plates at 10,000 cells/well and incubated at 37°C and 5% CO_2 overnight. The next day, recombinant human BMP4 (hBMP4; R&D, # 314-BP/CF, residues S293-R408 of accession # Q53XC5, expressed in NS0 cells) was serially diluted at 1:3 and added to cells starting from 100nM to 0.002nM including no hBMP4 control for dose response. For inhibition of hBMP4 by GREM1, recombinant human GREM1 (hGREM1-His; C-terminal 10His tagged, R&D, # 5190-GR, residues K25-D184 of accession # O60565, expressed in NS0 cells) was serially diluted at 1:2 starting from 400nM to 0.4nM including no hGREM1-His control and added to cells along with 200pM or 100pM hBMP4. For inhibition of hGREM1-His, antibodies were serially diluted at 1:3 starting from 100nM to 0.002nM including no antibody control and added to cells along with hBMP4 and hGREM1-His at final concentrations of either 200pM and 20nM or 100pM and 10nM, respectively. Luciferase activity was detected after 5.5 hours of incubation in 37°C and 5% CO_2.

[0178] Thirty-four of the 36 anti-GREM1 antibodies tested in the W-20-17/BRE-luc bioassay fully blocked hGREM1-His inhibition of hBMP signaling at 10nM hGREM1-His and 100pM hBMP4 or 20nM hGREM1-His and 200pM hBMP4. One antibody, H4H2780N, showed partial blocking of hGREM1-His and another antibody, H4H6269P, did not inhibit hGREM1-His. Isotype control antibodies (Control mAb1 and Control mAb2) were also included. IC_{50} values are shown in Tables 6 and 7. hBMP4 activated the W-20-17/BRE-luc cells with EC_{50} values of 39 to 116pM. hGREM1-His inhibited 200pM hBMP4 with an IC_{50} value of 10.3nM and 100pM hBMP4 with an IC_{50} value of 2.9 - 6.0nM.

Table 6: Inhibition of hGREM-His by anti-human GREM1-antibodies in a cell based assay

hBMP4 EC_{50} (pM)	116	56	75	73	39
hGrem1-His IC_{50} (nM)	10.3	6.0	4.3	4.2	5.4
hBMP4 Constant	200pM	100pM			
hGrem1-His Constant	20nM	10nM			
AbPID	***IC_{50} [M]***	***IC_{50} [M]***	***IC_{50} [M]***	***IC_{50} [M]***	***IC_{50} [M]***
H2bM2782N	3.5E-09				
H2bM2785N	2.7E-08				
H2bM2786N	1.9E-08				
H3M2788N	2.5E-08				
H2bM2890N		2.1E-09			
H2bM2891N		1.1E-09			

H2aM2898N		1.4E-09			
H2aM2899N		2.1E-09			
H2aM2901N				1.7E-09	
H2bM2906N			1.6E-09		
H1M2907N		1.8E-09			
H3M2929N					2.2E-09
Control mAb1	Not Block	Not Block	Not Block	Not Block	Not Block

Table 7: Inhibition of hGREM-His by anti-human GREM1-antibodies in a cell based assay

hBMP4 EC_{50} (pM)	60
hGrem1-His IC_{50} (nM)	2.9
hBMP4 Constant	100pM
hGrem1-His Constant	10nM
AbPID	***IC_{50} [M]***
H4H2780N	49% Inhibition
H4H2783N2	1.3E-09
H4H2784N	9.5E-10
H4H2889N	1.2E-09
H4H2892N	9.5E-10
H4H2895N	5.8E-10
H4H2897N	4.5E-10
H4H2926N	1.2E-09
H4H6232P	7.8E-10
H4H6233P	4.1E-10
H4H6236P	5.4E-10
H4H6238P	1.1E-09
H4H6240P	8.1E-10
H4H6243P	5.4E-10
H4H6245P	5.3E-10
H4H6246P	5.6E-10
H4H6248P	5.3E-10
H4H6250P	4.4E-10
H4H6251P	6.3E-10
H4H6252P	8.2E-10
H4H6256P	5.4E-10
H4H6260P	4.6E-10
H4H6269P	Not Block
H4H6270P	5.4E-10
Control mAb2	Not Block

Example 7. Effect of anti-GREM1 on BMP signaling and cell differentiation

[0179] In order to determine the potency of anti-human Gremlin 1 (GREM1) antibodies, their ability to block GREM1 induced inhibition of bone morphogenetic protein 4 (BMP4) signaling was investigated. W-20-17 cells are an osteoblast progenitor cell line and can differentiate in response to BMP4 signaling. GREM1, a known BMP inhibitor, blocks this differentiation. Blocking of GREM1 results in a reversal of BMP4 inhibition in this assay. Differentiation can be measured colorimetrically by using a substrate to detect endogenous expression of alkaline

phosphatase, an early marker of osteoblast differentiation. A total of 24 anti-GREM1 antibodies were tested.

[0180] W-20-17 cells were grown in DMEM/10% fetal bovine serum/glutamine/penicillin/streptomycin (complete media) to 100% confluency at 37°C in 5% CO_2. Cells were washed in 1X PBS, trypsinized (trypsin containing EDTA), plated at 3000 cells/well in clear plastic 96 well plates, an\\d grown overnight in complete media at a volume of 100uL/well. The next day recombinant human GREM1 protein (hGREM1-His; C-terminal 10His tagged, R&D, # 5190-GR, residues K25-D184 of accession # O60565, expressed in NS0 cells) was mixed with anti-GREM1 antibodies in complete media and incubated at room temperature (RT) for 40 minutes. Recombinant human BMP4 (hBMP4; R&D, # 314-BP/CF, residues S293-R408 of accession # Q53XC5, expressed in NS0 cells), also diluted in complete media, was added to the hGREM1-His/GREM1 antibody mixtures and then incubated at RT for an additional 30 minutes. After incubation, 50uL of these mixtures was added to W-20-17 cells plated in 100uL of complete media. The final concentration of hBMP4 and hGREM1-His on W-20-17 cells in each well was 1.5nM and 6nM, respectively, and the antibody concentration varied over an 11-point, 2-fold dilution series (maximum antibody concentration of 200 nM). After 3 days of growth at 37°C in 5% CO_2, media was aspirated and 50uL of water was added to each well. Ninety-six well plates were frozen at -80°C for 2 hours and then thawed on ice. Alkaline phosphatase was measured using p-nitrophenyl phosphate substrate prepared as directed (Sigma, #N2770-50SET). Absorbance at 405 nm was measured on a Victor plate reader seven minutes after addition of 50 uL of substrate. Graphs were plotted in Prism as mean +/- SEM (4 replicates for each condition).

[0181] Twenty-two of the 24 anti-GREM1 antibodies tested blocked hGREM1-His inhibition of hBMP4 in this assay with IC_{50} values as shown in Table 8. Two antibodies, H4H6269P and H4H2780N, did not exhibit any measurable blocking of hGREM1-His activity in this assay.

Table 8: Gremlin 1 blocking antibodies in W-20-17 cell differentiation assay

AbPID	hGREM1-His Inhibition IC_{50}, M
H4H2895N	1.9E-09
H4H2889N	5.4E-09
H4H2892N	4.4E-09
H4H2783N2	5.6E-09
H4H2784N	2.4E-08
H4H2897N	1.8E-08
H4H2926N	1.3E-08
H4H6232P	4.5E-09
H4H6233P	2.3E-08
H4H6236P	3.9E-09
H4H6238P	2.2E-08

H4H6240P	7.8E-09
H4H6243P	5.9E-09
H4H6245P	6.1E-09
H4H6246P	6.2E-09
H4H6248P	5.6E-09
H4H6250P	1.2E-08
H4H6251P	1.1E-08
H4H6252P	6.1E-09
H4H6256P	5.7E-09
H4H6260P	3.3E-09
H4H6270P	3.1E-09

hBMP4 and hGREM1-His activity in W-20-17 cell differentiation assay:

hBMP4 EC_{50} (M)	5.6E-10
hGREM1-His, IC_{50} (M)	4.4E-09

Example 8. Generation of a Bi-specific Antibody

[0182] Various bi-specific antibodies are generated for use in practicing the methods of the invention. For example, hGREM1-specific antibodies are generated in a bi-specific format (a "bi-specific") in which variable regions binding to distinct regions of hGREM1 are linked together to confer dual-domain specificity within a single binding molecule. Appropriately designed bi-specifics may enhance overall GREM1 inhibitory efficacy through increasing both specificity and binding avidity. Variable regions with specificity for individual regions or epitopes of GREM1 are paired on a structural scaffold that allows each region to bind simultaneously to the separate epitopes. In one example for a bi-specific, heavy chain variable regions (V_H) from a binder with specificity for one region are recombined with light chain variable regions (V_L) from a series of binders with specificity for a second region to identify non-cognate V_L partners that can be paired with an original V_H without disrupting the original specificity for that V_H. In this way, a single V_L segment (*e.g.*, V_L1) can be combined with two different V_H domains (*e.g.*, V_H1 and V_H2) to generate a bi-specific comprised of two binding "arms" (V_H1- V_L1 and V_H2- V_L1). Use of a single V_L segment reduces the complexity of the system and thereby simplifies and increases efficiency in cloning, expression, and purification processes used to generate the bi-specific (See, for example, USSN13/022759 and US2010/0331527).

[0183] Alternatively, antibodies that bind to GREM1 and a second target, such as, but not limited to, for example, a second different anti-GREM1 antibody, or a drug specific for fibrosis, may be prepared in a bi-specific format using techniques described herein, or other techniques known to those skilled in the art. Antibody variable regions binding to distinct catalytic domain regions may be linked together with variable regions that bind to relevant sites on, for example, the signal peptide domain, to confer dual-antigen specificity within a single binding molecule.

Appropriately designed bi-specifics of this nature serve a dual function. For example, in the case of a bi-specific antibody that binds both the domains, one may be able to better neutralize both the domains concurrently, without the need for administration of a composition containing two separate antibodies. Variable regions with specificity for the catalytic domain are combined with a variable region with specificity for the signal peptide domain and are paired on a structural scaffold that allows each variable region to bind to the separate antigens.

Example 9. Inhibition of the GREM1-heparin binding interaction using GREM1-specific antibodies

[0184] In the present study, Bio-Layer Interferometry was used to confirm previous results showing GREM1 binding to heparin and to evaluate the ability of GREM1 specific monoclonal antibodies to interfere with this binding interaction. GREM1 and other structurally related cysteine knot-containing proteins including GREM2 and cerberus were also tested for their ability to bind to heparin. Using these observations, amino acid residues involved in the binding of GREM1 to heparin were hypothesized by comparing the sequences and known heparin-binding properties of other cysteine-knot containing DAN family proteins. The binding of GREM1 to heparin was competed by different GAGs including heparin, HS, and dermatan sulfate (DS) to varying degrees, demonstrating the specificity of the interaction. Finally, individual antibodies were tested for their ability to interfere with the binding of GREM1 to heparin. Of the twenty-four antibodies tested, four were demonstrated to partially affect aspects of this binding interaction. In an attempt to completely block the interaction, the four antibodies that promoted partial blockade of the GREM1 and heparin binding interaction when tested alone were tested in combination. Some of the combinations including three of the antibodies and the mixture containing all four antibodies were able to completely inhibit the GREM1 and heparin binding interaction. These results give more insight into the binding mechanics of GREM1 and heparin and demonstrate a possible method using combinations of antibodies for inhibiting the angiogenic interaction of GREM1 with HS for therapeutic treatment.

Reagents and Instrumentation

[0185] Carrier free recombinant human GREM1 with a C-terminal decahistidine tag was obtained from R&D Systems (Minneapolis, MN). Heparin-biotin sodium salt from porcine intestinal mucosa and heparin sodium salt from porcine intestinal mucosa were obtained from Millipore (Billerica, MA). Heparan sulfate (HS) sodium salt from porcine intestinal mucosa and dermatan sulfate (DS) sodium salt from porcine intestinal mucosa were obtained from Celsus (Cincinnati, Ohio).

[0186] Binding measurements were conducted using an Octet Red96 label-free biomolecular interaction instrument (ForteBio). All experiments were performed at 25°C using plate agitation at 1000 rpm. Solutions were made in an aqueous buffer containing 10 mM HEPES, 150 mM NaCl, 3mM EDTA 0.05% P20, 0.1 mg/mL BSA and adjusted to pH of 7.4 (HBST buffer).

Binding kinetics of GREM1 interacting with captured heparin

[0187] Super Streptavidin biosensors were loaded with 10 µg/mL heparin-biotin solutions for 90 seconds. Following a 120 second wash, the heparin-captured biosensors were submerged in wells containing 11.1 nM, 33.3 nM, 100 nM, and 300 nM GREM1 solutions to measure association for 240 seconds. The biosensors were then submerged in HBST buffer to measure dissociation for 240 seconds. The measured association and dissociation rate constants (k_{on} = 1.17×10^6 $M^{-1}s^{-1}$ and k_{off} = 3.27×10^{-3} s^{-1}) demonstrate that GREM1 binds to heparin with high affinity ($K_D = k_{off} / k_{on} = 2.8 \times 10^{-9}$ M).

Inhibition of GREM1 binding to captured heparin by glycosaminoglycans

[0188] Super Streptavidin biosensors were loaded with 10 µg/mL heparin-biotin solutions for 90 seconds. Following a 120 second wash, the heparin-captured biosensors were submerged in wells containing 100 nM GREM1 solution pre-mixed with increasing concentrations (0 nM, 20 nM, 100 nM, 2 µM) of heparin, heparan sulfate (HS), or dermatan sulfate (DS) to measure association for 240 seconds.

[0189] GREM1 exhibited reduced binding to captured heparin-biotin in the presence of increasing concentrations of heparin, HS, and DS, as reflected in the binding signals observed after 240 seconds of association (Table 9). Exogenously added heparin was most effective in blocking the binding 100nM GREM1 to captured heparin-biotin, exhibiting complete inhibition at 100 nM. Nearly complete inhibition of GREM1 binding to captured heparin-biotin was observed when GREM1 was mixed with 2 µM of HS and DS. The complete inhibition of 100 nM GREM1 binding to captured heparin-biotin with soluble heparin at 100nM supports the specificity of this binding. Partial inhibition with the other glycosaminoglycans, which are all negatively charged, suggests that electrostatic interactions may be important in the GREM1-heparin binding interaction.

Table 9: The effect of soluble glycosaminoglycans on the GREM1-heparin binding interaction

	Binding Response (nm) for 100 nM GREM1 Binding to Captured Biotin-Heparin in the Presence of Increasing Concentrations of Glycosaminoglycans		
Concentration of Glycosaminoglycan	**Heparin**	**Heparan Sulfate**	**Dermatan Sulfate**
0 nM	0.12	0.11	0.09
20 nM	0.07	0.11	0.08
100 nM	0	0.07	0.05
2 µM	0	0.04	0.02

Inhibition of GREM1 binding to GREM1 specific monoclonal antibodies by heparin

[0190] Anti-Human IgG Fc Capture biosensors (ForteBio) were separately loaded with 50 µg/mL solutions of 24 different anti-GREM1 antibodies for 60 seconds. Following a 30 second wash, duplicate biosensors for each antibody were submerged into wells containing either 100

nM GREM1 solutions or 100 nM GREM1 solutions containing 5 μM of heparin, measuring association for 300 seconds. The presence or absence of heparin for some of the antibodies influenced the association rate, and this effect is summarized in Table 10 where observed signals at both 30 seconds and 300 seconds are provided. The binding of antibodies H4H2895N, H4H2780N, H4H6269P, and H4H2892N to GREM1 was minimally affected by the presence of heparin (the binding signals at 30 seconds were reduced by 0.05 nm or less). The binding of eight antibodies (H4H2897N, H4H6252P, H4H6245P, H4H6251P, H4H6232P, H4H2783N2, H4H6236P, and H4H6243P) exhibited the greatest reduction in binding to GREM1 in the presence of heparin (binding signals reduced by 0.19 nm or greater at 30 seconds). The other antibodies tested showed intermediate levels of binding inhibition to GREM1 in the presence of heparin.

Table 10: The inhibitory effect of soluble glycosaminoglycans on GREM1-antibody binding interactions

	100nM GREM1		100nM GREM1 + 5uM Heparin			
mAb Captured	Binding Signal with 30sec Association (nm)	Binding Signal with 300sec Association (nm)	Binding Signal with 30sec Association (nm)	Binding Signal with 300sec Association (nm)	Reduction in Signal from Heparin at 30sec	Reduction in Signal from Heparin at 300sec
H4H2895N	0.15	0.5	0.22	0.54	-0.07	-0.04
H4H2780N	0.13	0.58	0.13	0.58	0.00	0.00
H4H6269P	0.15	0.6	0.14	0.52	0.01	0.08
H4H2892N	0.2	0.49	0.15	0.49	0.05	0.00
H4H6233P	0.19	0.47	0.08	0.41	0.11	0.06
H4H6238P	0.13	0.41	0.02	0.33	0.11	0.08
H4H6246P	0.19	0.38	0.06	0.34	0.13	0.04
H4H6256P	0.2	0.4	0.07	0.38	0.13	0.02
H4H6250P	0.2	0.37	0.06	0.32	0.14	0.05
H4H2889N	0.2	0.46	0.05	0.44	0.15	0.02
H4H6270P	0.2	0.39	0.05	0.33	0.15	0.06
H4H2926N	0.23	0.49	0.07	0.42	0.16	0.07
H4H6248P	0.21	0.42	0.04	0.37	0.17	0.05
H4H6260P	0.24	0.42	0.07	0.37	0.17	0.05
H4H2784N	0.24	0.52	0.06	0.46	0.18	0.06
H4H6240P	0.23	0.53	0.05	0.37	0.18	0.16
H4H2897N	0.2	0.68	0.01	0.26	0.19	0.42
H4H6252P	0.23	0.44	0.04	0.35	0.19	0.09
H4H6245P	0.23	0.41	0.02	0.22	0.21	0.19
H4H6251P	0.27	0.45	0.03	0.36	0.24	0.09
H4H6232P	0.33	0.48	0.06	0.46	0.27	0.02
H4H2783N2	0.29	0.47	0.01	0.29	0.28	0.18
H4H6236P	0.33	0.45	0.05	0.43	0.28	0.02
H4H6243P	0.3	0.47	0	0.21	0.30	0.26

Inhibition of GREM1 binding to heparin by combinations of GREM1 antibodies

[0191] Super Streptavidin biosensors were loaded with 10 µg/mL solutions of heparin-biotin for 60 seconds. Following a 30 second wash, the biosensors were submerged into separate GREM1 solutions, each containing all 15 possible combinations of four antibodies (H4H2783N2, H4H2897N, H4H6243P, H4H6245P) chosen from the group in the previous experiment that exhibited the strongest inhibition of GREM1-heparin binding when tested alone. The mixtures included single antibodies and combinations of two, three, or four antibodies. Each antibody had a concentration of 600 nM in the mixtures. Also included were a negative isotype control antibody known not to bind to GREM1, two antibodies (H4H2892N and H4H2780N) whose binding to GREM1 was minimally affected by the presence of heparin, and the reference condition of GREM1 alone. As shown in Table 11, the individual antibodies only partially reduced binding of GREM1 to captured heparin in this binding format. Combinations of antibodies decreased binding of GREM1 to captured heparin more effectively. The solution containing H4H2897N, H4H6243P, and H4H6245P and the solution containing all four antibodies completely inhibited GREM1 from binding to captured heparin.

[0192] The GREM1 and heparin binding interaction was completely inhibited using combinations of antibodies that were each individually identified to be effective at partially interfering with this binding interaction. Given the highly negatively charged nature of heparin, the results suggest that heparin binds to GREM1 at multiple positively charged surface residues on GREM1. With this insight, it is proposed that in order to completely inhibit the angiogenesis-inducing HS and GREM1 binding interaction, multiple antibodies are required that have diverse epitopes overlapping with multiple heparin-binding sites in the structure of GREM1.

Table 11: The inhibitory effect of GREM1-specific antibodies on the GREM1-heparin binding interaction

PID	Binding Response (nm) at 300sec of 100 nM GREM1 binding to captured heparin in the presence of 600nM of each antibody alone or as a combination
H4H2783N2	0.08
H4H2897N	0.20
H4H6243P	0.13
H4H6245P	0.19
H4H2783N2 + H4H2897N	0.06
H4H2783N2 + H4H6243P	0.06
H4H2783N2 + H4H6245P	0.04
H4H2897N + H4H6243P	0.07
H4H2897N + H4H6245P	0.19
H4H6243P + H4H6245P	0.05
H4H2783N2 + H4H2897N + H4H6243P	0.04
H4H2897N + H4H6243P + H4H6245P	-0.01
H4H2783N2 + H4H6243P +	0.02

H4H6245P	
H4H2783N2 + H4H2897N + H4H6245P	0.10
H4H2783N2 + H4H2897N + H4H6243P + H4H6245P	-0.01
H4H2892N	0.40
H4H2780N	0.29
no antibody	0.13
negative control antibody	0.12

[0193] The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and the accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

What is claimed is:

1. An isolated human monoclonal antibody or antigen-binding fragment thereof that binds specifically to human gremlin-1 (GREM1).

2. The isolated human monoclonal antibody or antigen-binding fragment thereof of claim 1, wherein the antibody or antigen-binding fragment thereof blocks GREM1 binding to one of bone morphogenetic protein – 2 (BMP2), BMP4, BMP7 or heparin.

3. An isolated human antibody or antigen-binding fragment thereof that binds specifically to GREM1, wherein the antibody or antigen-binding fragment thereof exhibits one or more properties selected from the group consisting of:

(a) binds GREM1 at 37°C with a binding dissociation equilibrium constant (K_D) of less than about 275nM as measured by surface plasmon resonance;

(b) binds to GREM1 at 37°C with a dissociative half-life (t½) of greater than about 3 minutes as measured by surface plasmon resonance;

(c) binds GREM1 at 25°C with a K_D of less than about 280nM as measured by surface plasmon resonance;

(d) binds to GREM1 at 25°C with a t½ of greater than about 2 minutes as measured by surface plasmon resonance;

(e) blocks GREM1 binding to BMP4 with an IC_{50} of less than about 1.9nM as measured in a competition ELISA assay at 25°C;

(f) blocks GREM1-mediated inhibition of BMP signaling and promotes cell differentiation; and

(g) blocks GREM1 binding to heparin.

4. The isolated antibody or antigen-binding fragment thereof of any one of claims 1 – 3, wherein the antibody competes for specific binding to GREM1 with an antibody or antigen-binding fragment comprising the complementarity determining regions (CDRs) of a heavy chain variable region (HCVR), wherein the HCVR has an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 18, 34, 50, 66, 82, 98, 114, 130, 146, 162, 178, 194, 210, 226, 242, 258, 274, 290, 306, 322, 338, 354, 370, 386, 402, 418, 434, 450, 466, 482, 498, 514, 530, 546, 562, and 578; and the

5. The isolated antibody or antigen-binding fragment thereof of any one of claims 1 – 4, wherein the antibody competes for specific binding to GREM1 with an antibody or antigen-binding fragment thereof comprising the CDRs of a light chain variable region (LCVR), wherein the LCVR has an amino acid sequence selected from the group consisting of SEQ ID NOs: 10,

26, 42, 58, 74, 90, 106, 122, 138, 154, 170, 186, 202, 218, 234, 250, 266, 282, 298, 314, 330, 346, 362, 378, 394, 410, 426, 442, 458, 474, 490, 506, 522, 538, 554, 570, and 586.

6. An isolated human antibody or antigen-binding fragment thereof that specifically binds to GREM1, wherein the antibody comprises three heavy chain complementarity determining regions (CDRs) (HCDR1, HCDR2 and HCDR3) contained within any one of the heavy chain variable region (HCVR) sequences selected from the group consisting of SEQ ID NOs: 2, 18, 34, 50, 66, 82, 98, 114, 130, 146, 162, 178, 194, 210, 226, 242, 258, 274, 290, 306, 322, 338, 354, 370, 386, 402, 418, 434, 450, 466, 482, 498, 514, 530, 546, 562, and 578; and three light chain CDRs (LCDR1, LCDR2 and LCDR3) contained within any one of the light chain variable region (LCVR) sequences selected from the group consisting of SEQ ID NOs: 10, 26, 42, 58, 74, 90, 106, 122, 138, 154, 170, 186, 202, 218, 234, 250, 266, 282, 298, 314, 330, 346, 362, 378, 394, 410, 426, 442, 458, 474, 490, 506, 522, 538, 554, 570, and 586.

7. The isolated human antibody or antigen-binding fragment thereof of claim 6, comprising a HCVR having an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 18, 34, 50, 66, 82, 98, 114, 130, 146, 162, 178, 194, 210, 226, 242, 258, 274, 290, 306, 322, 338, 354, 370, 386, 402, 418, 434, 450, 466, 482, 498, 514, 530, 546, 562, and 578.

8. The isolated human antibody or antigen-binding fragment thereof of either claim 6 or 7, comprising a LCVR having an amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 26, 42, 58, 74, 90, 106, 122, 138, 154, 170, 186, 202, 218, 234, 250, 266, 282, 298, 314, 330, 346, 362, 378, 394, 410, 426, 442, 458, 474, 490, 506, 522, 538, 554, 570, and 586.

9. The isolated human antibody or antigen-binding fragment thereof of any one of claims 6 – 8, comprising: (a) a HCVR having an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 18, 34, 50, 66, 82, 98, 114, 130, 146, 162, 178, 194, 210, 226, 242, 258, 274, 290, 306, 322, 338, 354, 370, 386, 402, 418, 434, 450, 466, 482, 498, 514, 530, 546, 562, and 578; and (b) a LCVR having an amino acid sequence selected from the group consisting of SEQ ID NO: 10, 26, 42, 58, 74, 90, 106, 122, 138, 154, 170, 186, 202, 218, 234, 250, 266, 282, 298, 314, 330, 346, 362, 378, 394, 410, 426, 442, 458, 474, 490, 506, 522, 538, 554, 570, and 586.

10. The isolated human antibody or antigen-binding fragment thereof of any one of claims 6 – 9, comprising:

(a) a HCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 4, 20, 36, 52, 68, 84, 100, 116, 132, 148, 164, 180, 196, 212, 228, 244, 260, 276, 292, 308, 324, 340, 356, 372, 388, 404, 420, 436, 452, 468, 484, 500, 516, 532, 548, 564, and 580;

(b) a HCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 6, 22, 38, 54, 70, 86, 102, 118, 134, 150, 166, 182, 198, 214, 230, 246, 262, 278, 294, 310, 326, 342, 358, 374, 390, 406, 422, 438, 454, 470, 486, 502, 518, 534, 550, 566, and 582;

(c) a HCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 8, 24, 40, 56, 72, 88, 104, 120, 136, 152, 168, 184, 200, 216, 232, 248, 264, 280, 296, 312, 328, 344, 360, 376, 392, 408, 424, 440, 456, 472, 488, 504, 520, 536, 552, 568, and 584;

(d) a LCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 12, 28, 44, 60, 76, 92, 108, 124, 140, 156, 172, 188, 204, 220, 236, 252, 268, 284, 300, 316, 332, 348, 364, 380, 396, 412, 428, 444, 460, 476, 492, 508, 524, 540, 556, 572, and 588;

(e) a LCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 14, 30, 46, 62, 78, 94, 110, 126, 142, 158, 174, 190, 206, 222, 238, 254, 270, 286, 302, 318, 334, 350, 366, 382, 398, 414, 430, 446, 462, 478, 494, 510, 526, 542, 558, 574, and 590; and

(f) a LCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 16, 32, 48, 64, 80, 96, 112, 128, 144, 160, 176, 192, 208, 224, 240, 256, 272, 288, 304, 320, 336, 352, 368, 384, 400, 416, 432, 448, 464, 480, 496, 512, 528, 544, 560, 576, and 592.

11. The isolated human antibody or antigen-binding fragment of any one of claims 6 – 10, comprising a HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NOs: 2/10, 18/26, 34/42, 50/58, 66/74, 82/90, 98/106, 114/122, 130/138, 146/154, 162/170, 178/186, 194/202, 210/218, 226/234, 242/250, 258/266, 274/282, 290/298, 306/314, 322/330, 338/346, 354/362, 370/378, 386/394, 402/410, 418/426, 434/442, 450/458, 466/474, 482/490, 498/506, 514/522, 530/538, 546/554, 562/570, and 578/586.

12. An isolated antibody or antigen-binding fragment thereof that binds the same epitope on GREM1 as an antibody or antigen-binding fragment comprising the complementarity determining regions (CDRs) of a heavy chain variable region (HCVR), wherein the HCVR has an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 18, 34, 50, 66, 82, 98, 114, 130, 146, 162, 178, 194, 210, 226, 242, 258, 274, 290, 306, 322, 338, 354, 370, 386, 402, 418, 434, 450, 466, 482, 498, 514, 530, 546, 562, and 578; and the CDRs of a light chain variable region (LCVR), wherein the LCVR has an amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 26, 42, 58, 74, 90, 106, 122, 138, 154, 170, 186, 202, 218, 234, 250, 266, 282, 298, 314, 330, 346, 362, 378, 394, 410, 426, 442, 458, 474, 490, 506, 522, 538, 554, 570, and 586.

13. A pharmaceutical composition comprising an isolated human antibody or antigen-binding fragment thereof that binds to GREM1 according to any one of claims 1 – 12, and a pharmaceutically acceptable carrier or diluent.

14. A method for treating fibrosis comprising administering an effective amount of an antibody or an antigen-binding fragment thereof according to any of claims 1 – 12; or a pharmaceutical composition according to claim 13 to a patient in need thereof.

15. The method of claim 14, wherein the antibody or antigen-binding fragment thereof, or the pharmaceutical composition comprising the antibody or antigen-binding fragment thereof, is administered to the patient in combination with a second therapeutic agent.

16. The method of claim 15, wherein the second therapeutic agent is selected from the group consisting of an anti-fibrotic agent, an anti-inflammatory drug, a corticosteroid, a nutritional supplement, an anti-hypertensive agent, an antibiotic, another antibody to GREM1, an antibody to a cytokine such as IL-1, IL-6, or TGF-β, and any other palliative therapy.

17. The method of any of claims 14 – 16, wherein the antibody or antigen-binding fragment thereof is administered subcutaneously, intravenously, intradermally, orally, or intramuscularly.

18. The method of any of claims 14 – 17, wherein the antibody or antigen-binding fragment is administered at a dose of about 0.1 mg/kg of body weight to about 100 mg/kg of body weight of the patient.

19. The method of any of claims 14 – 18, wherein the fibrosis is present in liver, lungs or kidney.

20. The method of any of claims 14 – 19, wherein the fibrosis is selected from the group comprising pulmonary fibrosis, pulmonary hypertension, idiopathic pulmonary fibrosis, liver fibrosis, renal fibrosis, diabetic nephropathy, ischemic renal injury, nephrosclerosis and nephrotoxicity.

21. A method for treating cancer comprising administering an effective amount of an antibody or an antigen-binding fragment thereof according to any of claims 1 – 12; or a pharmaceutical composition according to claim 13 to a patient in need thereof.

22. The method of claim 21, wherein the antibody or antigen-binding fragment thereof, or the pharmaceutical composition comprising the antibody or antigen-binding fragment thereof, is administered to the patient in combination with a second therapeutic agent.

23. The method of claim 22, wherein the second therapeutic agent is selected from the group consisting of another antibody to GREM1, a VEGF antagonist, a cytotoxic agent, a chemotherapeutic agent, radiation, surgery, an anti-inflammatory drug, a corticosteroid, a nutritional supplement, and any other palliative therapy.

24. The method of claim 23, wherein the VEGF antagonist is an anti-VEGF antibody or a VEGF-inhibiting fusion protein.

25. The method of claim 23, wherein the VEGF antagonist is aflibercept.

26. A method for inhibiting angiogenesis comprising administering an effective amount of an antibody or an antigen-binding fragment thereof according to any of claims 1 – 12; or a pharmaceutical composition according to claim 13 to a patient in need thereof.

27. The method of claim 26, wherein the antibody is administered to a patient with cancer.

28. The method of claim 26, wherein the antibody is administered in combination with a second therapeutic agent.

29. The method of claim 28, wherein the second therapeutic agent is another antibody to GREM1 or a VEGF antagonist.

30. An antibody or antigen-binding fragment thereof of any one of claims 1 – 12 for use in promoting BMP signaling and cell differentiation.

31. A composition comprising one or more antibodies or antigen-binding fragments thereof of any one of claims 1 – 12 for use in inhibiting heparin-mediated angiogenesis.

32. The composition of claim 31, wherein the one or more antibodies or antigen-binding fragments thereof comprise a HCVR selected from the group consisting of SEQ ID NOs: 66, 210, 418 and 434; and a LCVR selected from the group consisting of SEQ ID NOs: 74, 218, 426 and 442.

33. An antibody or antigen-binding fragment thereof of any one of claims 1 – 12 for use in treating a patient with fibrosis or cancer.

34. Use of the isolated antibody or antigen-binding fragment thereof of any one of claims 1 – 12 in the manufacture of a medicament for treating a patient with fibrosis or cancer.

35. A method of blocking interaction of (a) a polypeptide comprising the amino acid sequence of SEQ ID NO: 594 or SEQ ID NO: 595 with (b) heparin, the method comprising exposing a mixture comprising (a) and (b) to the antibody of any one of claims 1 – 12, or to the composition of claim 32.

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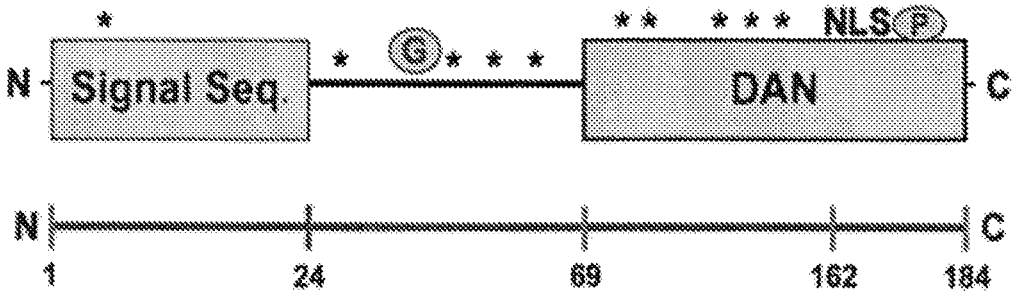

(From: Wordinger, R.J., et al 2008, Exp. Eye Res. 87: 78-79)

Figure 1

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2014/021471

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/18 A61K39/395 A61P35/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MICHAEL S. MULVIHILL ET AL: "Gremlin is Overexpressed in Lung Adenocarcinoma and Increases Cell Growth and Proliferation in Normal Lung Cells", PLOS ONE, vol. 7, no. 8, 1 August 2012 (2012-08-01), pages 1-8, XP055121719, ISSN: 1932-6203, DOI: 10.1371/journal.pone.0042264 abstract figure 1 page 6, right-hand column, paragraph 1 ----- -/--	1-13, 21-25, 33,34

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
5 June 2014	17/06/2014

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Malamoussi, A

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/021471

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:

   a. (means)

   - [ ] on paper
   - [X] in electronic form

   b. (time)

   - [X] in the international application as filed
   - [ ] together with the international application in electronic form
   - [ ] subsequently to this Authority for the purpose of search

2. [ ] In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2014/021471

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MINSOO KIM ET AL: "Gremlin-1 Induces BMP-Independent Tumor Cell Proliferation, Migration, and Invasion", PLOS ONE, vol. 7, no. 4, 13 April 2012 (2012-04-13), page e35100, XP055121712, DOI: 10.1371/journal.pone.0035100 cited in the application abstract page 2, left-hand column, paragraph 3	21-25, 33,34
X	KATRI KOLI ET AL: "Bone Morphogenetic Protein-4 Inhibitor Gremlin Is Overexpressed in Idiopathic Pulmonary Fibrosis", THE AMERICAN JOURNAL OF PATHOLOGY, vol. 169, no. 1, 1 July 2006 (2006-07-01), pages 61-71, XP055121792, ISSN: 0002-9440, DOI: 10.2353/ajpath.2006.051263 abstract page 70, left-hand column, paragraph 3	14-20, 33,34
X	US 2009/041757 A1 (ZHEN HANSON [US] ET AL) 12 February 2009 (2009-02-12) page 1, paragraph 0005 - paragraph 0006 claims 5,6 page 5, paragraph 0050	21-25, 30,33,34
X	CHANG-LI WANG: "The bone morphogenetic protein antagonist Gremlin is overexpressed in human malignant mesothelioma", ONCOLOGY REPORTS, 20 September 2011 (2011-09-20), pages 58-64, XP055121632, ISSN: 1021-335X, DOI: 10.3892/or.2011.1463 abstract page 63, right-hand column, paragraph 1	21-25, 33,34
X	S. MITOLA ET AL: "Gremlin is a novel agonist of the major proangiogenic receptor VEGFR2", BLOOD, vol. 116, no. 18, 4 November 2010 (2010-11-04), pages 3677-3680, XP055121628, ISSN: 0006-4971, DOI: 10.1182/blood-2010-06-291930 "Introduction"; page 3677	26-29

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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2014/021471

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	P. CHIODELLI ET AL: "Heparan Sulfate Proteoglycans Mediate the Angiogenic Activity of the Vascular Endothelial Growth Factor Receptor-2 Agonist Gremlin", ARTERIOSCLEROSIS, THROMBOSIS, AND VASCULAR BIOLOGY, vol. 31, no. 12, 15 September 2011 (2011-09-15), pages e116-e127, XP055121690, ISSN: 1079-5642, DOI: 10.1161/ATVBAHA.111.235184 abstract ----	31,32,35
A	S. A. ROXBURGH ET AL: "Allelic Depletion of grem1 Attenuates Diabetic Kidney Disease", DIABETES, vol. 58, no. 7, 28 April 2009 (2009-04-28) , pages 1641-1650, XP055121724, ISSN: 0012-1797, DOI: 10.2337/db08-1365 abstract ----	1-35
A	QINGXIAN ZHANG ET AL: "In Vivo Delivery of Gremlin siRNA Plasmid Reveals Therapeutic Potential against Diabetic Nephropathy by Recovering Bone Morphogenetic Protein-7", PLOS ONE, vol. 5, no. 7, 22 July 2010 (2010-07-22), page e11709, XP055121728, DOI: 10.1371/journal.pone.0011709 page 8, left-hand column, paragraph 1 ----	1-35
A	LONBERG ET AL: "Fully human antibodies from transgenic mouse and phage display platforms", CURRENT OPINION IN IMMUNOLOGY, ELSEVIER, OXFORD, GB, vol. 20, no. 4, 1 August 2008 (2008-08-01) , pages 450-459, XP025771204, ISSN: 0952-7915, DOI: 10.1016/J.COI.2008.06.004 [retrieved on 2008-07-21] the whole document ----	1-13

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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2014/021471

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MING-HUANG CHEN ET AL: "Expression of gremlin 1 correlates with increased angiogenesis and progression-free survival in patients with pancreatic neuroendocrine tumors", JOURNAL OF GASTROENTEROLOGY, SPRINGER-VERLAG, TO, vol. 48, no. 1, 16 June 2012 (2012-06-16), pages 101-108, XP035162725, ISSN: 1435-5922, DOI: 10.1007/S00535-012-0614-Z abstract --- Helena Stabile ET AL: "Bone morphogenic protein antagonist Drm/gremlin is a novel proangiogenic factor", , 1 March 2007 (2007-03-01), pages 1834-1840, XP055121826, DOI: 10.1182/blood-2006- Retrieved from the Internet: URL:http://bloodjournal.hematologylibrary.org/content/bloodjournal/109/5/1834.full.pdf#zoom=75 [retrieved on 2014-06-05] abstract page 1836, left-hand column, paragraph 6 --- J. B. SNEDDON ET AL: "Bone morphogenetic protein antagonist gremlin 1 is widely expressed by cancer-associated stromal cells and can promote tumor cell proliferation", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, vol. 103, no. 40, 3 October 2006 (2006-10-03), pages 14842-14847, XP055121828, ISSN: 0027-8424, DOI: 10.1073/pnas.0606857103 abstract page 14846, right-hand column, paragraph 5 figure 2 ---	1-35 1-35 1-35

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2014/021471

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2009041757 A1	12-02-2009	NONE	

说 明 书 摘 要

本发明提供与人 gremlin-1 (GREM1)结合的抗体，和使用方法。根据本发明的某些实施方案，抗体是与 GREM1 结合的全人抗体。本发明的抗体适用于抑制或中和 GREM1 活性，因此提供治疗 GREM1 相关疾病或病症(如纤维化和癌症)的手段。在一些实施方案中，本发明的抗体用于治疗肝、肺或肾的纤维化的至少一种症状或并发症。