(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
27 November 2014 (27.11.2014)

WIPO | PCT

(10) International Publication Number
WO 2014/190316 A1

(51) International Patent Classification:
A61K 39/00 (2006.01) *C07K 16/40* (2006.01)
C07K 16/24 (2006.01) *C07K 16/46* (2006.01)

(21) International Application Number:
PCT/US2014/039437

(22) International Filing Date:
23 May 2014 (23.05.2014)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/826,692 23 May 2013 (23.05.2013) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

[Continued on next page]

(54) Title: ANTI-CCL2 AND ANTI-LOXL2 COMBINATION THERAPY FOR TREATMENT OF SCLERODERMA

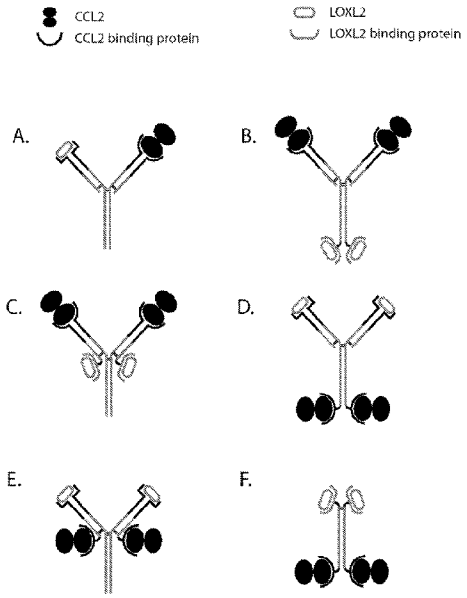

FIG. 1

(57) Abstract: The present invention provides, among other things, bi-specific molecules including, but not limited to, antibodies, fynomers, aptamers, fusion proteins, and protein binding domains that bind both CCL2 and LOXL2 and uses thereof, in particular, for treatment of scleroderma and related fibrotic and/or inflammatory diseases, disorders and conditions. In some embodiments, the present invention further provides methods and compositions for treatment of scleroderma and related fibrotic and/or inflammatory diseases, disorders and conditions based on the combination of mono-specific anti-CCL2 and anti-LOXL2 molecules.

WO 2014/190316 A1

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

— *with sequence listing part of description (Rule 5.2(a))*

ANTI-CCL2 AND ANTI-LOXL2 COMBINATION THERAPY FOR TREATMENT OF SCLERODERMA

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims benefit under 35 USC § 119(e) of U.S. Provisional Patent Application Serial No. 61/826,692 filed May 23, 2013, which application is hereby incorporated by reference in its entirety.

SEQUENCE LISTING

[0002] The present specification makes reference to a Sequence Listing submitted in electronic form as an ASCII.txt file named "2006685-0569_ST25" on May 23, 2014. The .txt file was generated on May 12, 2014 and is 7 KB in size.

BACKGROUND

[0003] Systemic sclerosis (scleroderma) is a clinically heterogeneous disorder of the connective tissue, resulting in hardening and tightening of the skin. It is an autoimmune-type of disease characterized by immune activation, vascular damage, and fibrosis. Major organ-based complications involving the lungs, heart, kidneys, and gastrointestinal tract can contribute to mortality and morbidity. The pathogenesis is unknown.

[0004] The feature most commonly associated with scleroderma is fibrosis—a buildup of collagen in the skin and organs. The buildup of collagen contributes to symptoms of the disorder, including hair loss, skin hardening and tightening, skin discoloration, joint pain, stiffness of fingers and joints, digestive tract problems and breathing complications (dry cough, shortness of breath, wheezing). Scleroderma may be classified into two major subgroups: limited cutaneous scleroderma and diffuse cutaneous scleroderma. In limited cutaneous scleroderma, fibrosis is mainly restricted to the hands, arms, and face. Diffuse cutaneous scleroderma is a rapidly progressing disorder that affects large areas of the skin and compromises one or more internal organs. Patients with limited cutaneous scleroderma have a relatively better long term prognosis than patients with diffuse cutaneous scleroderma. Widespread systemic scleroderma can damage the

heart, kidney, lungs, or GI tract, which may cause death. Pulmonary fibrosis is the most common cause of death in patients with scleroderma.

[0005] Thus, scleroderma is an extremely debilitating disease with potentially fatal repercussions. There are about 50,000 patients in the US. The ratio of female patients to male patients is about 4:1. Current treatment methods are based only on symptomatic treatment and management of complications that arise through the course of the disease (e.g., corticosteroids, NSAIDs, and immune-suppressing medications such as Metotrexate and Cytoxan). There is no treatment shown to reverse or halt progression of disease. Therefore, there is a high unmet medical need for an effective treatment of scleroderma.

SUMMARY OF THE INVENTION

[0006] The present invention provides, among other things, improved methods and compositions for effective treatment of scleroderma, in particular, based on bi-specific binding molecules, including, but not limited to, antibodies, fynomers, aptamers, fusion proteins, protein binding domains (e.g., those derived from receptors) that can specifically bind to lysyl oxidase-like-2 ("LOXL2") and C-C chemokine ligand-2 ("CCL2"), and/or combination therapy based on such molecules that specifically bind to LOXL2 and CCL2. CCL2 is known to be a validated target for scleroderma. Several studies have shown that scleroderma fibroblasts display increased constitutive expression of CCL2 mRNA and protein. In scleroderma skin sections, expression of CCL2 was detected in fibroblasts, keratinocytes, and mononuclear cells, whereas it was undetectable in normal skin (Galindo et al., *Arthritis Rheum.* 2001 Jun; 44(6):1382-6; Distler et al., *Arthritis Rheum.* 2001 Nov; 44(11):2665-78; Lioyd et al., *Exp Med.* 1997 Apr 7;185(7):1371-80; Yamamoto et al., *J Dermatol Sci.* 2001 Jun; 26(2):133-9; Denton et al.; *Trends Immunol.* 2005 Nov; 26(11):596-602. Epub 2005 Sep 15.). However, prior to the present invention, no effective treatment for scleroderma has been developed based on anti-CCL2 antibodies. The present inventors observe that high levels of CCL2 in plasma sequester anti-CCL2 antibodies injected intravenously, resulting in wasted anti-CCL2 antibodies and ineffective targeting of CCL2 in diseased tissues. To solve this problem, the present inventors contemplate the use of bi-specific molecules that allow sequestering anti-CCL2 activity in diseased tissues with free anti-CCL2 arms that bind to tissue CCL2, which provides tissue specific targeting of CCL2. Thus, the present invention provides methods and

compositions that preferentially inhibit tissue CCL2 as opposed to plasma CCL2, resulting in highly effective treatment of scleroderma.

[0007] Thus, in one aspect, the present invention provides bi-specific binding molecules (e.g., bi-specific antibodies, fynomers, aptamers, fusion proteins, or protein binding domains) comprising a first antigen-binding site that specifically binds to LOXL2 and a second antigen-binding site that specifically binds to CCL2.

[0008] In some embodiments, the first antigen-binding site specifically binds to LOXL2 with a binding affinity of 100 nM or greater (e.g., 10nM or greater, 1nM or greater, 500pM or greater, 100pM or greater, 50pM or greater, 10pM or greater, 1pM or greater, 500fM or greater, 400fM or greater, 300fM or greater, 200fM or greater, 100fM or greater, 50fM or greater, 10fM or greater, or 1fM or greater).

[0009] In some embodiments, the second antigen-binding site specifically binds to CCL2 with a binding affinity of between about 500nM and 1fM (e.g., between 500nM and 10fM, between 500nM and 100fM, between 500nM and 1pM, between 10nM and 1fM, between 10nM and 100fM, between 10nM and 1pM, between 1nM and 1fM, between 1nM and 100fM, between 1nM and 500fM, between 1nM and 1pM, between 1nM and 10pM, between 1nM and 50pM, between 1nM and 100pM, between 1nM and 500pM). In some embodiments, the second antigen-binding site specifically binds to CCL2 with a binding affinity of greater than about 500nM (e.g., greater than about 500nM, 100nM, 10nM, 1nM, 500pM, 100pM, 50pM, 10pM, 1pM, 500fM, 400fM, 300fM, 200fM, 100fM, 50fM, 10fM, 1fM).

[0010] In some embodiments, the first antigen-binding site comprises a first full length heavy chain and a first full length light chain. In some embodiments, the first antigen-binding site comprises a first Fab fragment. In some embodiments, the first antigen-binding site comprises a first single-chain variable fragments (scFvs).

[0011] In some embodiments, the second antigen-binding site comprises a second full length heavy chain and a second full length light chain. In some embodiments, the second antigen-binding site comprises a second Fab fragment. In some embodiments, the second antigen-binding site comprises a second single-chain variable fragments (scFvs).

[0012] In some embodiments, the first and second antigen-binding sites are linked by a peptide linker. In some embodiments, the peptide linker is ≥ 5 (e.g., 6, 7, 8, 9, 10, 11,

12, 13, 14, 15, 20, 25 or more) amino acids long. In some embodiments, the first and second antigen binding sites are configured such that they form a single polypeptide chain.

[0013] In some embodiments, the first and second antigen-binding sites are associated via chemical cross-linking.

[0014] In some embodiments, a bi-specific binding molecule according to the invention is a bi-specific antibody. In some embodiments, the bi-specific antibody comprises an Fc region.

[0015] In some embodiments, the bi-specific antibody is human. In some embodiments, the bi-specific antibody is humanized.

[0016] In another aspect, the present invention provides pharmaceutical compositions comprising the bi-specific binding molecule (e.g., a bi-specific antibody, fynomer, aptamer, fusion protein, protein binding domain) as described herein and a pharmaceutically acceptable carrier.

[0017] In further aspect, the present invention provides methods of treating scleroderma comprising administering to an individual who is suffering from or susceptible to scleroderma a bi-specific binding molecule (e.g., a bi-specific antibody, fynomer, aptamer, fusion protein, protein binding domain) as described herein. In some embodiments, the bi-specific antibody is administered at a therapeutically effective dose and an administration interval such that at least one symptom or feature of scleroderma on a target tissue is reduced in intensity, severity, or frequency, or has delayed onset.

[0018] In some embodiments, the at least one pathological feature of scleroderma is ameliorated, including but not limited to, endothelial-cell damage, proliferation of basal-lamina layers, perivascular mononuclear-cell infiltration, fibrosis, derangement of visceral-organ architecture, rarefaction of blood vessels, hypoxia, and combination thereof.

[0019] In some embodiments, the target tissue is selected from the group consisting of skin, blood vessels, lung, heart, kidney, gastrointestinal tract (including liver), musculoskeletal system and combinations thereof. In some embodiments, the target tissue is lung. In some embodiments, the target tissue is heart.

[0020] In some embodiments, the individual is suffering from or susceptible to limited cutaneous scleroderma. In some embodiments, the individual is suffering from or susceptible to diffuse cutaneous scleroderma.

[0021] In some embodiments, the bi-specific antibody is administered parenterally. In some embodiments, the parenteral administration is selected from intravenous, intradermal, inhalation, transdermal (topical), subcutaneous, and/or transmucosal administration. In some embodiments, the parenteral administration is intravenous administration.

[0022] In some embodiments, the bi-specific antibody is administered orally.

[0023] In certain embodiments, the bi-specific antibody is administered bimonthly, monthly, triweekly, biweekly, weekly, daily, or at variable intervals.

[0024] In some embodiments, the bi-specific antibody is co-administered with one or more anti-fibrotic or anti-inflammatory agents.

[0025] In another aspect, the present invention provides use of a bi-specific binding molecule as described herein in the manufacture of a medicament for treatment of scleroderma, wherein the treatment comprises administering to an individual who is suffering from or susceptible to scleroderma an effective amount of the bi-specific molecule, wherein the bi-specific binding molecule comprises a first antigen-binding site that specifically binds to LOXL2 and a second antigen-binding site that specifically binds to CCL2

[0026] In some embodiments, the first antigen-binding site specifically binds to LOXL2 with a binding affinity of 100 nM or greater (e.g., 10nM or greater, 1nM or greater, 500pM or greater, 100pM or greater, 50pM or greater, 10pM or greater, 1pM or greater, 500fM or greater, 400fM or greater, 300fM or greater, 200fM or greater, 100fM or greater, 50fM or greater, 10fM or greater, or 1fM or greater).

[0027] In some embodiments, the second antigen-binding site specifically binds to CCL2 with a binding affinity of between about 500nM and 1fM (e.g., between 500nM and 10fM, between 500nM and 100fM, between 500nM and 1pM, between 10nM and 1fM, between 10nM and 100fM, between 10nM and 1pM, between 1nM and 1fM, between 1nM and 100fM, between 1nM and 500fM, between 1nM and 1pM, between 1nM and 10pM, between 1nM and 50pM, between 1nM and 100pM, between 1nM and 500pM). In some embodiments, the second antigen-binding site specifically binds to CCL2 with a

binding affinity of greater than about 500nM (e.g., greater than about 500nM, 100nM, 10nM, 1nM, 500pM, 100pM, 50pM, 10pM, 1pM, 500fM, 400fM, 300fM, 200fM, 100fM, 50fM, 10fM, 1fM).

[0028] In some embodiments, the first antigen-binding site comprises a first full length heavy chain and a first full length light chain. In some embodiments, the first antigen-binding site comprises a first Fab fragment. In some embodiments, the first antigen-binding site comprises a first single-chain variable fragments (scFvs).

[0029] In some embodiments, the second antigen-binding site comprises a second full length heavy chain and a second full length light chain. In some embodiments, the second antigen-binding site comprises a second Fab fragment. In some embodiments, the second antigen-binding site comprises a second single-chain variable fragments (scFvs).

[0030] In some embodiments, the first and second antigen-binding sites are linked by a peptide linker. In some embodiments, the peptide linker is ≥ 5 (e.g., 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 25 or more) amino acids long. In some embodiments, the first and second antigen binding sites are configured such that they form a single polypeptide chain.

[0031] In some embodiments, the first and second antigen-binding sites are associated via chemical cross-linking.

[0032] In some embodiments, a bi-specific binding molecule according to the invention is a bi-specific antibody. In some embodiments, the bi-specific antibody comprises an Fc region.

[0033] In some embodiments, the bi-specific antibody is humanized.

[0034] In another aspect, the present invention provides a bi-specific binding molecule as described herein for use in a method of treating scleroderma comprising a step of administering an effective amount of the bi-specific binding molecule to a subject who is suffering from or susceptible to scleroderma, wherein the bi-specific binding molecule comprises a first antigen-binding site that specifically binds to LOXL2 and a second antigen-binding site that specifically binds to CCL2.

[0035] In some embodiments, the first antigen-binding site specifically binds to LOXL2 with a binding affinity of 100 nM or greater (e.g., 10nM or greater, 1nM or greater, 500pM or greater, 100pM or greater, 50pM or greater, 10pM or greater, 1pM or

greater, 500fM or greater, 400fM or greater, 300fM or greater, 200fM or greater, 100fM or greater, 50fM or greater, 10fM or greater, or 1fM or greater).

[0036] In some embodiments, the second antigen-binding site specifically binds to CCL2 with a binding affinity of between about 500nM and 1fM (e.g., between 500nM and 10fM, between 500nM and 100fM, between 500nM and 1pM, between 10nM and 1fM, between 10nM and 100fM, between 10nM and 1pM, between 1nM and 1fM, between 1nM and 100fM, between 1nM and 500fM, between 1nM and 1pM, between 1nM and 10pM, between 1nM and 50pM, between 1nM and 100pM, between 1nM and 500pM). In some embodiments, the second antigen-binding site specifically binds to CCL2 with a binding affinity of greater than about 500nM (e.g., greater than about 500nM, 100nM, 10nM, 1nM, 500pM, 100pM, 50pM, 10pM, 1pM, 500fM, 400fM, 300fM, 200fM, 100fM, 50fM, 10fM, 1fM).

[0037] In some embodiments, the first antigen-binding site comprises a first full length heavy chain and a first full length light chain. In some embodiments, the first antigen-binding site comprises a first Fab fragment. In some embodiments, the first antigen-binding site comprises a first single-chain variable fragments (scFvs).

[0038] In some embodiments, the second antigen-binding site comprises a second full length heavy chain and a second full length light chain. In some embodiments, the second antigen-binding site comprises a second Fab fragment. In some embodiments, the second antigen-binding site comprises a second single-chain variable fragments (scFvs).

[0039] In some embodiments, the first and second antigen-binding sites are linked by a peptide linker. In some embodiments, the peptide linker is ≥ 5 (e.g., 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 25 or more) amino acids long. In some embodiments, the first and second antigen binding sites are configured such that they form a single polypeptide chain.

[0040] In some embodiments, the first and second antigen-binding sites are associated via chemical cross-linking.

[0041] In some embodiments, a bi-specific binding molecule according to the invention is a bi-specific antibody. In some embodiments, the bi-specific antibody comprises an Fc region.

[0042] In some embodiments, the bi-specific antibody is human. In some embodiments, the bi-specific antibody is humanized.

[0043] In yet another aspect, the present invention provides methods of treating fibrotic diseases, disorders or conditions comprising administering to an individual who is suffering from or susceptible to a fibrotic disease, disorder or condition a bi-specific binding molecule (e.g., a bi-specific antibody, fynomer, aptamer, fusion protein, protein binding domain) as described herein.

[0044] In another aspect, the present invention provides use of a bi-specific binding molecule as described herein in the manufacture of a medicament for treatment of fibrotic diseases, disorders or conditions, wherein the treatment comprises administering to an individual who is suffering from or susceptible to a fibrotic disease, disorder or condition the bi-specific binding molecule, wherein the bi-specific molecule comprises a first antigen-binding site that specifically binds to LOXL2 and a second antigen-binding site that specifically binds to CCL2.

[0045] In another aspect, the present invention provides a bi-specific molecule for use in a method of treating fibrotic diseases, disorders or conditions comprising a step of administering to an individual who is suffering from or susceptible to a fibrotic disease, disorder or condition the bi-specific binding molecule, wherein the bi-specific molecule comprises a first antigen-binding site that specifically binds to LOXL2 and a second antigen-binding site that specifically binds to CCL2.

[0046] In various embodiments, the fibrotic disease, disorder or condition is selected from the group consisting of skin fibrosis, kidney fibrosis, liver fibrosis, lung fibrosis, heart fibrosis, muscle fibrosis, and combination thereof.

[0047] In another aspect, the present invention provides methods of treating inflammatory diseases, disorders or conditions comprising administering to an individual who is suffering from or susceptible to an inflammatory disease, disorder or condition a bi-specific binding molecule as described herein.

[0048] In another aspect, the present invention provides use of a bi-specific binding molecule as described herein in the manufacture of a medicament for treatment of inflammatory diseases, disorders or conditions, wherein the treatment comprises administering to an individual who is suffering from or susceptible to an inflammatory diseases, disorders or condition the bi-specific binding molecule, wherein the bi-specific molecule comprises a first antigen-binding site that specifically binds to LOXL2 and a second antigen-binding site that specifically binds to CCL2.

[0049] In another aspect, the present invention provides a bi-specific molecule for use in a method of treating fibrotic diseases, disorders or conditions comprising a step of administering to an individual who is suffering from or susceptible to a fibrotic disease, disorder or condition the bi-specific binding molecule, wherein the bi-specific molecule comprises a first antigen-binding site that specifically binds to LOXL2 and a second antigen-binding site that specifically binds to CCL2.

[0050] In various embodiments, the inflammatory disease, disorder or condition is selected from the group consisting of psoriasis, rheumatoid arthritis, atherosclerosis, epilepsy, Alzheimer's disease, obesity, lupus nephritis, general kidney inflammation, multiple sclerosis, Crohn's disease, asthma, discoid lupus erythematosus, inflammatory bowel disease, or systemic lupus erythematosus.

[0051] In another aspect, the present invention provides methods of treating scleroderma comprising administering to an individual who is suffering from or susceptible to scleroderma an anti-CCL2 antibody, or fragment thereof, and an anti-LOXL2 antibody, or fragment thereof.

[0052] In another aspect, the present invention provides use of an anti-CCL2 antibody, or fragment thereof, and an anti-LOXL2 antibody, or fragment thereof, in the manufacture of a medicament for treatment of scleroderma, wherein the treatment comprises a step of administering the anti-CCL2 antibody, or fragment thereof, and the anti-LOXL2 antibody, or fragment thereof, to an individual who is suffering from or susceptible to scleroderma.

[0053] In another aspect, the present invention provides an anti-CCL2 antibody, or fragment thereof, and an anti-LOXL2 antibody, or fragment thereof, for use in a method of treating scleroderma comprising a step of administering the anti-CCL2 antibody, or fragment thereof, and the anti-LOXL2 antibody, or fragment thereof, to an individual who is suffering from or susceptible to scleroderma.

[0054] In some embodiments, the anti-CCL2 antibody, or fragment thereof, and the anti-LOXL2 antibody, or fragment thereof, are administered simultaneously. In some embodiments, the anti-CCL2 antibody, or fragment thereof, and the anti-LOXL2 antibody, or fragment thereof, are administered sequentially.

[0055] In some embodiments, the anti-CCL2 antibody, or fragment thereof, has a binding affinity of 1nM or greater (e.g., 500pM or greater, 100pM or greater, 50pM or

greater, 10pM or greater, 1pM or greater, 500fM or greater, 400fM or greater, 300fM or greater, 200fM or greater, 100fM or greater, 50fM or greater, 10fM or greater, 1fM or greater).

[0056] In some embodiments, the anti-LOXL2 antibody, or fragment thereof, has a binding affinity of 1pM or greater (e.g., 500fM or greater, 400fM or greater, 300fM or greater, 200fM or greater, 100fM or greater, 50fM or greater, 10fM or greater, 1fM or greater).

[0057] In some embodiments, the anti-CCL2 antibody, or fragment thereof, is selected from the group consisting of intact IgG, F(ab')2, F(ab)2, Fab', Fab, ScFvs, diabodies, triabodies and tetrabodies.

[0058] In some embodiments, the anti-LOXL2 antibody, or fragment thereof, is selected from the group consisting of intact IgG, F(ab')2, F(ab)2, Fab', Fab, ScFvs, diabodies, triabodies and tetrabodies.

[0059] In some embodiments, one or both of the anti-CCL2 antibody, or fragment thereof, and the anti-LOXL2 antibody, or fragment thereof, are humanized.

[0060] In some embodiments, the anti-CCL2 antibody, or fragment thereof, and the anti-LOXL2 antibody, or fragment thereof, are administered via same administration route. In some embodiments, the anti-CCL2 antibody, or fragment thereof, and the anti-LOXL2 antibody, or fragment thereof, are administered via different administration route.

[0061] In some embodiments, the anti-CCL2 antibody, or fragment, is administered intravenously, intradermally, by inhalation, transdermally (topically), subcutaneously, transmucosally, and/or orally.

[0062] In some embodiments, the anti-CCL2 antibody, or fragment thereof, is administered bimonthly, monthly, triweekly, biweekly, weekly, daily, or at variable intervals.

[0063] In some embodiments, the anti-LOXL2 antibody, or fragment, is administered intravenously, intradermally, by inhalation, transdermally (topically), subcutaneously, transmucosally, and/or orally.

[0064] In some embodiments, the anti-LOXL2 antibody, or fragment thereof, is administered bimonthly, monthly, triweekly, biweekly, weekly, daily, or at variable intervals.

[0065] In another aspect, the present invention provides methods of treating fibrotic diseases, disorders or conditions comprising administering to an individual who is suffering from or susceptible to a fibrotic disease, disorder or condition an anti-CCL2 antibody, or fragment thereof, and an anti-LOXL2 antibody, or fragment thereof.

[0066] In another aspect, the present invention provides use of an anti-CCL2 antibody, or fragment thereof, and an anti-LOXL2 antibody, or fragment thereof, in the manufacture of a medicament for treatment of fibrotic diseases, disorders or conditions, wherein the treatment comprises a step of administering the anti-CCL2 antibody, or fragment thereof, and the anti-LOXL2 antibody, or fragment thereof, to an individual who is suffering from or susceptible to a fibrotic disease, disorder or condition.

[0067] In another aspect, the present invention provides an anti-CCL2 antibody, or fragment thereof, and an anti-LOXL2 antibody, or fragment thereof, for use in a method of treating fibrotic diseases, disorders or conditions comprising a step of administering the anti-CCL2 antibody, or fragment thereof, and the anti-LOXL2 antibody, or fragment thereof, to an individual who is suffering from or susceptible to a fibrotic disease, disorder or condition.

[0068] In another aspect, the present invention provides methods of treating inflammatory diseases, disorders or conditions comprising administering to an individual who is suffering from or susceptible to an inflammatory disease, disorder or condition an anti-CCL2 antibody, or fragment thereof, and an anti-LOXL2 antibody, or fragment thereof.

[0069] In another aspect, the present invention provides use of an anti-CCL2 antibody, or fragment thereof, and an anti-LOXL2 antibody, or fragment thereof, in the manufacture of a medicament for treatment of inflammatory diseases, disorders or conditions, wherein the treatment comprises a step of administering the anti-CCL2 antibody, or fragment thereof, and the anti-LOXL2 antibody, or fragment thereof, to an individual who is suffering from or susceptible to an inflammatory disease, disorder or condition.

[0070] In another aspect, the present invention provides an anti-CCL2 antibody, or fragment thereof, and an anti-LOXL2 antibody, or fragment thereof, for use in a method of treating inflammatory diseases, disorders or conditions comprising a step of administering the anti-CCL2 antibody, or fragment thereof, and the anti-LOXL2 antibody, or fragment

thereof, to an individual who is suffering from or susceptible to an inflammatory disease, disorder or condition.

[0071] In another aspect, the present disclosure provides kits comprising an anti-CCL2 antibody, or fragment thereof, and an anti-LOXL2 antibody, or fragment thereof.

[0072] Other features, objects, and advantages of the present invention are apparent in the detailed description, drawings and claims that follow. It should be understood, however, that the detailed description, the drawings, and the claims, while indicating embodiments of the present invention, are given by way of illustration only, not limitation. Various changes and modifications within the scope of the invention will become apparent to those skilled in the art.

BRIEF DESCRIPTION OF THE DRAWING

[0073] The Drawing included herein, which is comprised of the following Figures, is for illustration purposes only not for limitation.

[0074] **FIGs. 1A-1F** illustrate diagrams depicting exemplary anti-CCL2 and anti-LOXL2 bi-specific antibodies.

[0075] **FIG. 2** illustrates an exemplary diagram depicting the Modified Rodnan Skin Score. Locations on the body where skin fibrosis is assessed are indicated.

[0076] **FIG. 3** depicts an exemplary graph plotting serum and tissue concentration of CCL2 following equilibration.

[0077] **FIG. 4** illustrates an exemplary diagram depicting CCL2 targeting in plasma and in diseased tissue.

[0078] **FIG. 5** depicts an exemplary graph plotting concentration of CCL2 as a function of days post treatment with either anti-CCL2 (Mono) or anti-CCL2/LOXL2 (Bi), illustrating preliminary bi-specific modeling results.

[0079] **FIG. 6** shows the percentage of skin ulcers observed in C57BL/6 mice treated with IgG, anti-CCL2 antibody, anti-LOXL2 antibody, or anti-CCL2 and anti-LOXL2 antibodies. PBS: negative control. BOTH: combination treatment with anti-CCL2 antibody and anti-LOXL2 antibody.

[0080] **FIG. 7** shows the fold-change in skin thickness observed in C57BL/6 mice treated with IgG, anti-CCL2 antibody, anti-LOXL2 antibody, or anti-CCL2 and anti-

LOXL2 antibodies. PBS: negative control. BOTH: combination treatment with anti-CCL2 antibody and anti-LOXL2 antibody.

[0081] **FIG. 8** shows the Ashcroft score for lung tissue samples of C57BL/6 mice treated with IgG, anti-CCL2 antibody, anti-LOXL2 antibody, or anti-CCL2 and anti-LOXL2 antibodies. PBS: negative control. BOTH: combination treatment with anti-CCL2 antibody and anti-LOXL2 antibody.

[0082] **FIG. 9** shows Arginase 1 (Arg1)-staining in lung tissue samples of C57BL/6 mice treated with IgG, anti-CCL2 antibody, anti-LOXL2 antibody, or anti-CCL2 and anti-LOXL2 antibodies. PBS: negative control. BOTH: combination treatment with anti-CCL2 antibody and anti-LOXL2 antibody.

[0083] **FIG. 10** shows a correlation plot of Arginase 1 (Arg1)-staining as a function of Ashcroft score in lung tissue samples of C57BL/6 mice treated with IgG, anti-CCL2 antibody, anti-LOXL2 antibody, or anti-CCL2 and anti-LOXL2 antibodies. PBS: negative control. BOTH: combination treatment with anti-CCL2 antibody and anti-LOXL2 antibody.

[0084] **FIG. 11** shows representative histological sections of lung tissue samples stained with Trichrome for various treatment groups of mice at 4X power. Top row: PBS (left), IgG (right). Middle row: anti-CCL2 (left), anti-LOXL2 (right). Bottom row: anti-CCL2 and anti-LOXL2.

[0085] **FIG. 12** shows representative histological sections of lung tissue samples stained with Arginase 1 (Arg1) for various treatment groups of mice. Top row: PBS (left), IgG (right). Middle row: anti-CCL2 (left), anti-LOXL2 (right). Bottom row: anti-CCL2 and anti-LOXL2.

[0086] **FIG. 13** shows representative histological sections of lung tissue samples stained with Arginase 1 (Arg1) for IgG treatment groups at 20X (left) and 40X (right) power.

DEFINITIONS

[0087] In order for the present invention to be more readily understood, certain terms are first defined. Additional definitions for the following terms and other terms are set forth throughout the specification.

[0088] ***Affinity:*** As is known in the art, "affinity" is a measure of the tightness with which a particular ligand binds to (e.g., associates non-covalently with) and/or the rate or frequency with which it dissociates from, its partner. As is known in the art, any of a variety of technologies can be utilized to determine affinity. In many embodiments, affinity represents a measure of specific binding.

[0089] ***Affinity-matured* (or *affinity-matured antibody*):** As used herein, refers to an antibody with one or more alterations in one or more CDRs thereof which result an improvement in the affinity of the antibody for antigen, compared to a parent antibody which does not possess those alteration(s). In some embodiments, affinity matured antibodies will have nanomolar or even picomolar affinities for a target antigen. Affinity matured antibodies may be produced by any of a variety of procedures known in the art. Marks et al. BioTechnology 10:779-783 (1992) describes affinity maturation by V_H and V_L domain shuffling. Random mutagenesis of CDR and/or framework residues is described by: Barbas et al. Proc Nat. Acad. Sci, USA 91:3809-3813 (1994); Schier et al. Gene 169:147-155 (1995); Yelton et al. J. Immunol. 155:1994-2004 (1995); Jackson et al., J. Immunol. 154(7):3310-9 (1995); and Hawkins et al, J. Mol. Biol. 226:889-896 (1992).

[0090] ***Antibody*:** As used herein, the term "antibody" refers to a polypeptide consisting of one or more polypeptides substantially encoded by immunoglobulin genes or fragments of immunoglobulin genes. The recognized immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon and mu constant region genes, as well as myriad immunoglobulin variable region genes. Light chains are typically classified as either kappa or lambda. Heavy chains are typically classified as gamma, mu, alpha, delta, or epsilon, which in turn define the immunoglobulin classes, IgG, IgM, IgA, IgD and IgE, respectively. A typical immunoglobulin (antibody) structural unit is known to comprise a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kD) and one "heavy" chain (about 50-70 kD). The N-terminus of each chain defines a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The terms "variable light chain" (V_L) and "variable heavy chain" (V_H) refer to these light and heavy chains respectively. An antibody can be specific for a particular antigen. The antibody or its antigen can be either an analyte or a binding partner. Antibodies exist as intact immunoglobulins or as a number of well-characterized fragments produced by digestion with various peptidases. Thus, for example, pepsin digests an antibody below the disulfide linkages in the hinge

region to produce F(ab)'2, a dimer of Fab which itself is a light chain joined to V_H-C_H1 by a disulfide bond. The F(ab)'2 may be reduced under mild conditions to break the disulfide linkage in the hinge region thereby converting the (Fab')2 dimer into an Fab' monomer. The Fab' monomer is essentially an Fab with part of the hinge region (see, Fundamental Immunology, W. E. Paul, ed., Raven Press, N.Y. (1993), for a more detailed description of other antibody fragments). While various antibody fragments are defined in terms of the digestion of an intact antibody, one of ordinary skill in the art will appreciate that such Fab' fragments may be synthesized de novo either chemically or by utilizing recombinant DNA methodology. Thus, the term "antibody," as used herein also includes antibody fragments either produced by the modification of whole antibodies or synthesized de novo using recombinant DNA methodologies. In some embodiments, antibodies are single chain antibodies, such as single chain Fv (scFv) antibodies in which a variable heavy and a variable light chain are joined together (directly or through a peptide linker) to form a continuous polypeptide. A single chain Fv ("scFv") polypeptide is a covalently linked V_H::V_L heterodimer which may be expressed from a nucleic acid including V_H- and V_L-encoding sequences either joined directly or joined by a peptide-encoding linker. (See, e.g., Huston, et al. (1988) Proc. Nat. Acad. Sci. USA, 85:5879-5883, the entire contents of which are herein incorporated by reference.) A number of structures exist for converting the naturally aggregated, but chemically separated light and heavy polypeptide chains from an antibody V region into an scFv molecule which will fold into a three dimensional structure substantially similar to the structure of an antigen-binding site. See, e.g. U.S. Pat. Nos. 5,091,513 and 5,132,405 and 4,956,778.

[0091] ***Approximately:*** As used herein, the term "approximately" or "about," as applied to one or more values of interest, refers to a value that is similar to a stated reference value. In certain embodiments, the term "approximately" or "about" refers to a range of values that fall within 25%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less in either direction (greater than or less than) of the stated reference value unless otherwise stated or otherwise evident from the context (except where such number would exceed 100% of a possible value).

[0092] ***Binding agent:*** As used herein, the term "binding agent" includes any naturally occurring, synthetic or genetically engineered agent, such as protein, that binds an antigen or a target protein or peptide. "Binding agent" is also referred to as "binding protein." Binding agents can be derived from naturally occurring antibodies or

synthetically engineered. A binding protein or agent can function similarly to an antibody by binding to a specific antigen to form a complex and elicit a biological response (e.g., agonize or antagonize a particular biological activity). Binding agents or proteins can include isolated fragments, "Fv" fragments consisting of the variable regions of the heavy and light chains of an antibody, recombinant single chain polypeptide molecules in which light and heavy chain variable regions are connected by a peptide linker ("ScFv proteins"), and minimal recognition units consisting of the amino acid residues that mimic the hypervariable region. The term Binding Agent as used herein can also include antibody fragments either produced by the modification of whole antibodies or synthesized *de novo* using recombinant DNA methodologies. In some embodiments, antibodies are single chain antibodies, such as single chain Fv (scFv) antibodies in which a variable heavy and a variable light chain are joined together (directly or through a peptide linker) to form a continuous polypeptide. A single chain Fv ("scFv") polypeptide is a covalently linked VH::VL heterodimer which may be expressed from a nucleic acid including VH- and VL-encoding sequences either joined directly or joined by a peptide-encoding linker. (See, *e.g.*, Huston, *et al.* (1988) Proc. Nat. Acad. Sci. USA, 85:5879-5883, the entire contents of which are herein incorporated by reference.) A number of structures exist for converting the naturally aggregated, but chemically separated light and heavy polypeptide chains from an antibody V region into an scFv molecule which will fold into a three dimensional structure substantially similar to the structure of an antigen-binding site. See, e.g. U.S. Pat. Nos. 5,091,513 and 5,132,405 and 4,956,778. In some embodiments, the term Binding Agent as used herein can also include antibody. See the definition of Antibody.

[0093] ***Bi-specific*:** The term "bi-specific" as used herein refers to a molecule having two distinct binding specificities. Typically, a bi-specific binding molecule contains at least two antigen-binding sites, each of which specifically binds to a different antigen or epitope. A bi-specific molecule can be, for example, a bi-specific antibody, fynomer, aptamer, fusion protein, protein binding domain. As used herein, bi-specific molecules encompass molecules (e.g., antibodies, fynomers, aptamers, fusion proteins, protein binding domains or other binding agents) having higher valencies (i.e., the ability to bind more than two antigens, or epitopes), which are also referred to as multispecific molecules.

[0094] ***Bispecific antibody:*** The term "bispecific antibody" as used herein, refers to a bispecific binding molecule in which at least one, and typically both, of the binding

moieties is or comprises an antibody component or fragment. A variety of different bi-specific antibody structures is known in the art. In some embodiments, each binding moiety in a bispecific antibody that is or comprises an antibody component or fragment includes V_H and/or V_L regions; in some such embodiments, the V_H and/or V_L regions are those found in a particular monoclonal antibody. In some embodiments, where the bispecific antibody contains two antibody component binding moieties, each includes V_H and/or V_L regions from different monoclonal antibodies.

[0095] ***Bispecific binding molecule:*** The term "bispecific binding molecule" as used herein, refers to a polypeptide with two discrete binding moieties, each of which binds with a distinct target. In some embodiments, a bispecific binding molecule is a single polypeptide; in some embodiments, a bispecific binding molecule is or comprises a plurality of peptides which, in some such embodiments may be covalently associated with one another, for example by cross-linking. In some embodiments, the two binding moieties of a bispecific binding molecule recognize different sites (e.g., epitopes) the same target (e.g., antigen); in some embodiments, they recognize different targets. In some embodiments, a bispecific binding molecule is capable of binding simultaneously to two targets which are of different structure.

[0096] ***CDR***: The term "CDR" as used herein, refers to a complementarity determining region within an antibody variable region. There are three CDRs in each of the variable regions of the heavy chain and the light chain, which are designated CDR1, CDR2 and CDR3, for each of the variable regions. A "set of CDRs" or "CDR set" refers to a group of three or six CDRs that occur in either a single variable region capable of binding the antigen or the CDRs of cognate heavy and light chain variable regions capable of binding the antigen. Boundaries of CDRs have been defined differently depending on the system, of which several are known in the art (e.g., Kabat, Chothia, etc.).

[0097] ***Chimeric:*** A "chimeric" antibody as used herein, is a recombinant protein that contains the variable domains including the complementarity-determining regions (CDRs) of an antibody derived from one species, preferably a rodent antibody, while the constant domains of the antibody molecule is derived from those of a human antibody. For veterinary applications, the constant domains of the chimeric antibody may be derived from that of other species, such as a cat or dog.

[0098] ***Combination:*** The term "in combination" as used herein, refers to the use of more than one prophylactic and/or therapeutic agents (e.g., an anti-CCL2 antibody and an anti-LOXL2 antibody). The use of the term "in combination" does not restrict the order in which prophylactic and/or therapeutic agents are administered to a subject with a disorder. A first prophylactic or therapeutic agent (e.g., an anti-CCL2 antibody) can be administered prior to (e.g., 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks before), concomitantly with, or subsequent to (e.g., 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks after) the administration of a second prophylactic or therapeutic agent (e.g., an anti-LOXL2 antibody) to a subject with a disorder.

[0099] ***Compound and Agent*:** The terms “compound” and “agent” are used herein interchangeably. They refer to any naturally occurring or non-naturally occurring (i.e., synthetic or recombinant) molecule, such as a biological macromolecule (e.g., nucleic acid, polypeptide or protein), organic or inorganic molecule, or an extract made from biological materials such as bacteria, plants, fungi, or animal (particularly mammalian, including human) cells or tissues. The compound may be a single molecule or a mixture or complex of at least two molecules.

[0100] ***Comparable:*** The term “comparable” as used herein, refers to describe two (or more) sets of conditions or circumstances that are sufficiently similar to one another to permit comparison of results obtained or phenomena observed. In some embodiments, comparable sets of conditions or circumstances are characterized by a plurality of substantially identical features and one or a small number of varied features. Those of ordinary skill in the art will appreciate that sets of conditions are comparable to one another when characterized by a sufficient number and type of substantially identical features to warrant a reasonable conclusion that differences in results obtained or phenomena observed under the different sets of conditions or circumstances are caused by or indicative of the variation in those features that are varied.

[0101] ***Control:*** As used herein, the term “control” has its art-understood meaning of being a standard against which results are compared. Typically, controls are used to augment integrity in experiments by isolating variables in order to make a conclusion

about such variables. In some embodiments, a control is a reaction or assay that is performed simultaneously with a test reaction or assay to provide a comparator. In one experiment, the "test" (*i.e.*, the variable being tested) is applied. In the second experiment, the "control," the variable being tested is not applied. In some embodiments, a control is a historical control (*i.e.*, of a test or assay performed previously, or an amount or result that is previously known). In some embodiments, a control is or comprises a printed or otherwise saved record. A control may be a positive control or a negative control.

[0102] ***Dosing regimen:*** A "dosing regimen" (or "therapeutic regimen"), as that term is used herein, is a set of unit doses (typically more than one) that are administered individually to a subject, typically separated by periods of time. In some embodiments, a given therapeutic agent has a recommended dosing regimen, which may involve one or more doses. In some embodiments, a dosing regimen comprises a plurality of doses each of which are separated from one another by a time period of the same length; in some embodiments, a dosing regimen comprises a plurality of doses and at least two different time periods separating individual doses.

[0103] ***Diagnosis:*** As used herein, the term "*diagnosis*" refers to a process aimed at determining if an individual is afflicted with a disease or ailment. In the context of the present invention, "*diagnosis of scleroderma*" refers to a process aimed at one or more of: determining if an individual is afflicted with scleroderma, identifying a scleroderma subtype (*i.e.*, diffuse or limited cutaneous scleroderma), and determining the severity of the disease.

[0104] ***Effective amount*:** As used herein, the term "effective amount" refers to an amount of a compound or agent that is sufficient to fulfill its intended purpose(s). In the context of the present invention, the purpose(s) may be, for example: to modulate the cause or symptoms of scleroderma; and/or to delay or prevent the onset of scleroderma; and/or to slow down or stop the progression, aggravation, or deterioration of the symptoms of scleroderma; and/or to alleviate one or more symptoms associated with scleroderma; and/or to bring about amelioration of the symptoms of scleroderma, and/or to cure scleroderma.

[0105] ***Framework* or *framework region*:** As used herein, refers to the sequences of a variable region minus the CDRs. Because a CDR sequence can be determined by different systems, likewise a framework sequence is subject to correspondingly different

interpretations. The six CDRs divide the framework regions on the heavy and light chains into four sub-regions (FR1, FR2, FR3 and FR4) on each chain, in which CDR1 is positioned between FR1 and FR2, CDR2 between FR2 and FR3, and CDR3 between FR3 and FR4. Without specifying the particular sub-regions as FR1, FR2, FR3 or FR4, a framework region, as referred by others, represents the combined FRs within the variable region of a single, naturally occurring immunoglobulin chain. As used herein, a FR represents one of the four sub-regions, FR1, for example, represents the first framework region closest to the amino terminal end of the variable region and 5' with respect to CDR1, and FRs represents two or more of the sub-regions constituting a framework region.

[0106] ***Human antibody*:** As used herein, is intended to include antibodies having variable and constant regions generated (or assembled) from human immunoglobulin sequences. In some embodiments, antibodies (or antibody components) may be considered to be "human" even though their amino acid sequences include residues or elements not encoded by human germline immunoglobulin sequences (e.g., include sequence variations, for example that may (originally) have been introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*), for example in one or more CDRs and in particular CDR3.

[0107] ***Humanized*:** As is known in the art, the term "humanized" is commonly used to refer to antibodies (or antibody components) whose amino acid sequence includes V_H and V_L region sequences from a reference antibody raised in a non-human species (e.g., a mouse), but also includes modifications in those sequences relative to the reference antibody intended to render them more "human-like", i.e., more similar to human germline variable sequences. In some embodiments, a "*humanized*" antibody (or antibody component) is one that immunospecifically binds to an antigen of interest and that has a framework (FR) region having substantially the amino acid sequence as that of a human antibody, and a complementary determining region (CDR) having substantially the amino acid sequence as that of a non-human antibody. A humanized antibody comprises substantially all of at least one, and typically two, variable domains (Fab, Fab', F(ab')2, FabC, Fv) in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin (i.e., donor immunoglobulin) and all or substantially all of the framework regions are those of a human immunoglobulin consensus sequence. In some embodiments, a humanized antibody also comprises at least a portion of an

immunoglobulin constant region (Fc), typically that of a human immunoglobulin constant region. In some embodiments, a humanized antibody contains both the light chain as well as at least the variable domain of a heavy chain. The antibody also may include a CH_1, hinge, CH_2, CH_3, and, optionally, a CH_4 region of a heavy chain constant region. In some embodiments, a humanized antibody only contains a humanized V_L region. In some embodiments, a humanized antibody only contains a humanized V_H region. In some certain embodiments, a humanized antibody contains humanized V_H and V_L regions.

[0108] ***Fynomers*:** As used herein, the term "fynomers" refers to a class of binding proteins derived from the Src homology (SH3) domain of the human Fyn kinase, which is a human protein composed of 63 amino acid residues (D. Grabulovski et al. *J. Biol. Chem.* 282, 3196–3204 (2007)). Fynomers can bind to target molecules with the same affinity and specificity as antibodies. It can be produced in bacteria with high yields. Moreover, several Fynomers can be linked to yield a protein with multiple binding specificities

[0109] ***Improve, increase, or reduce*:** As used herein, the terms "improve," "increase" or "reduce," or grammatical equivalents, indicate values that are relative to a baseline measurement, such as a measurement in the same individual prior to initiation of the treatment described herein, or a measurement in a control individual (or multiple control individuals) in the absence of the treatment described herein. A "control individual" is an individual afflicted with the same type and approximately the same severity of scleroderma as the individual being treated, who is about the same age as the individual being treated (to ensure that the stages of the disease in the treated individual and the control individual(s) are comparable).

[0110] ***Kit*:** As used herein, the term "kit" refers to any delivery system for delivering materials. Such delivery systems may include systems that allow for the storage, transport, or delivery of various diagnostic or therapeutic reagents (e.g., oligonucleotides, enzymes, etc. in the appropriate containers) and/or supporting materials (e.g., buffers, written instructions for performing the assay etc.) from one location to another. For example, kits include one or more enclosures (e.g., boxes) containing the relevant reaction reagents and/or supporting materials. As used herein, the term "fragmented kit" refers to a delivery systems comprising two or more separate containers that each contain a subportion of the total kit components. The containers may be delivered to the intended recipient together or separately. For example, a first container

may contain an enzyme for use in an assay, while a second container contains oligonucleotides. The term "fragmented kit" is intended to encompass kits containing Analyte Specific Reagents (ASR's) regulated under section 520(e) of the Federal Food, Drug, and Cosmetic Act, but are not limited thereto. Indeed, any delivery system comprising two or more separate containers that each contain a subportion of the total kit components are included in the term "fragmented kit." In contrast, a "combined kit" refers to a delivery system containing all of the components in a single container (e.g., in a single box housing each of the desired components). The term "kit" includes both fragmented and combined kits.

[0111] ***Normal*:** As used herein, the term "normal," when used to modify the term "individual" or "subject" refers to an individual or group of individuals who does not have a particular disease or condition and is also not a carrier of the disease or condition. The term "normal" is also used herein to qualify a biological specimen or sample isolated from a normal or wild-type individual or subject, for example, a "normal biological sample."

[0112] ***Nucleic Acid*:** As used herein the term "nucleic acid" refers to an oligonucleotide, nucleotide or polynucleotide, and fragments or portions thereof, and to DNA or RNA of genomic or synthetic origin that may be single or double stranded, and represents the sense or antisense strand.

[0113] ***Nucleic Acid Molecule*:** The terms "nucleic acid molecule" and "polynucleotide" are used herein interchangeably. They refer to a deoxyribonucleotide or ribonucleotide polymer in either single- or double-stranded form, and unless otherwise stated, encompass known analogs of natural nucleotides that can function in a similar manner as naturally occurring nucleotides. The terms encompasses nucleic acid-like structures with synthetic backbones, as well as amplification products.

[0114] ***Protein*:** In general, a "protein" is a polypeptide (i.e., a string of at least two amino acids linked to one another by peptide bonds). Proteins may include moieties other than amino acids (e.g., may be glycoproteins) and/or may be otherwise processed or modified. Those of ordinary skill in the art will appreciate that a "protein" can be a complete polypeptide chain as produced by a cell (with or without a signal sequence), or can be a functional portion thereof. Those of ordinary skill will further appreciate that a protein can sometimes include more than one polypeptide chain, for example linked by one or more disulfide bonds or associated by other means.

[0115] ***Sample*:** As used herein, the term "sample" encompasses any sample obtained from a biological source. The terms "biological sample" and "sample" are used interchangeably. A biological sample can, by way of non-limiting example, include skin tissue, liver tissue, kidney tissue, lung tissue, cerebrospinal fluid (CSF), blood, amniotic fluid, sera, urine, feces, epidermal sample, skin sample, cheek swab, sperm, amniotic fluid, cultured cells, bone marrow sample and/or chorionic villi. Cell cultures of any biological samples can also be used as biological samples. A biological sample can also be, e.g., a sample obtained from any organ or tissue (including a biopsy or autopsy specimen), can comprise cells (whether primary cells or cultured cells), medium conditioned by any cell, tissue or organ, tissue culture. In some embodiments, biological samples suitable for the invention are samples which have been processed to release or otherwise make available a nucleic acid for detection as described herein. Fixed or frozen tissues also may be used.

[0116] ***Subject*:** As used herein, the term "subject" refers to a human or any non-human animal (e.g., mouse, rat, rabbit, dog, cat, cattle, swine, sheep, horse or primate). A human includes pre- and post-natal forms. In many embodiments, a subject is a human being. A subject can be a patient, which refers to a human presenting to a medical provider for diagnosis or treatment of a disease. The term "subject" is used herein interchangeably with "individual" or "patient." A subject can be afflicted with or is susceptible to a disease or disorder but may or may not display symptoms of the disease or disorder.

[0117] ***Suffering from*:** An individual who is "suffering from" a disease, disorder, and/or condition (e.g., scleroderma) has been diagnosed with or displays one or more symptoms of the disease, disorder, and/or condition.

[0118] ***Susceptible to:*** An individual who is "susceptible to" a disease, disorder, and/or condition has not been diagnosed with and/or may not exhibit symptoms of the disease, disorder, and/or condition. In some embodiments, an individual who is susceptible to a disease, disorder, and/or condition (for example, scleroderma) may be characterized by one or more of the following: (1) a genetic mutation associated with development of the disease, disorder, and/or condition; (2) a genetic polymorphism associated with development of the disease, disorder, and/or condition; (3) increased and/or decreased expression and/or activity of a protein associated with the disease, disorder, and/or condition; (4) habits and/or lifestyles associated with development of the disease, disorder, and/or condition; (5) a family history of the disease, disorder, and/or

condition; (6) reaction to certain bacteria or viruses; (7) exposure to certain chemicals. In some embodiments, an individual who is susceptible to a disease, disorder, and/or condition will develop the disease, disorder, and/or condition. In some embodiments, an individual who is susceptible to a disease, disorder, and/or condition will not develop the disease, disorder, and/or condition.

[0119] ***Treatment*:** As used herein, the term "*treatment*" (also "treat" or "treating") refers to any administration of a therapeutic molecule (e.g., bi-specific anti-CCL2/LOXL antibody or simultaneous or sequential co-administration of an anti-CCL2 monoclonal antibody or antigen binding fragment thereof and an anti-LOXL2 monoclonal antibody or antigen binding fragment thereof) that partially or completely alleviates, ameliorates, relieves, inhibits, delays onset of, reduces severity of and/or reduces incidence of one or more symptoms or features of a particular disease, disorder, and/or condition (e.g., scleroderma, fibrosis or inflammation). Such treatment may be of a subject who does not exhibit signs of the relevant disease, disorder and/or condition and/or of a subject who exhibits only early signs of the disease, disorder, and/or condition. Alternatively or additionally, such treatment may be of a subject who exhibits one or more established signs of the relevant disease, disorder and/or condition.

DETAILED DESCRIPTION OF THE INVENTION

[0120] The present invention provides, among other things, bi-specific molecules, including including, but not limited to, antibodies, fynomers, aptamers, fusion proteins, protein binding domains (e.g., those derived from receptors) against CCL2 and LOXL2 and uses thereof, in particular, for treatment of scleroderma and related fibrotic and/or inflammatory diseases, disorders and conditions. In some embodiments, the present invention further provides methods and compositions for treatment of scleroderma and related fibrotic and/or inflammatory diseases, disorders and conditions based on the combination of mono-specific anti-CCL2 and anti-LOXL2 molecules (e.g., antibodies).

[0121] The present invention is, in part, based on the unique insights observed by the present inventors, that is, bi-specific molecules, including antibodies or fusion proteins, allow tissue specific targeting of CCL2 without wasting anti-CCL2 molecules such as antibodies in plasma, resulting in highly effective treatment of scleroderma. Embodiments of the invention include bi-specific antibodies that bind to both CCL2 and LOXL2. Bi-specific antibodies capable of binding to CCL2 and LOXL2 are particularly

advantageous in that they possess a unique tissue selectivity profile and have the potential to arrest and clear development of scleroderma, fibrosis and inflammation. As LOXL2 is an important enzyme for the development of connective tissue, anti-LOXL2 binding activity may be used to preferentially target or identify tissues with relatively large amounts of connective tissue. Likewise, anti-LOXL2 binding activity may be used to target or identify tissues with aberrant connective tissue formation; e.g., as may be observed in scleroderma. Moreover, an anti-LOXL2 antibody provides synergistic therapeutic benefit in that inhibition or neutralization of LOXL2 reduces amine oxidase activity afflicted tissues, thus blocking an initiating step in the formation of connective tissue. The therapeutic benefit and tissue specificity of LOXL2 antibodies can be combined with the therapeutic efficacy of a neutralizing anti-CCL2 monoclonal antibody to synergistically target inflammation and reduce fibrotic formation. This synergistic targeting is particularly important in the treatment of more advanced cases of scleroderma because the LOX binding allows anti-CCL2 antibodies to be sequestered and compensates for decreased permeability due to fibrosis.

[0122] Various aspects of the invention are described in detail in the following sections. The use of sections is not meant to limit the invention. Each section can apply to any aspect of the invention. In this application, the use of "or" means "and/or" unless stated otherwise.

Scleroderma

[0123] Scleroderma, or systemic sclerosis, is generally considered a chronic systemic autoimmune disease characterized, among other things, fibrosis or hardening, vascular alterations, and autoantibodies. Without wishing to be bound by theory, it is thought that scleroderma is caused by a hyperactive autoimmune response trapped in a reinforcing amplification loop. For example, scleroderma is histologically characterized by inflammatory infiltrates of mononuclear cells, which in turn activate and are associated with increased collagen synthesis in the surrounding fibroblasts. In particular, activated macrophages produce TGF-beta and PDGF, which activate fibroblasts in the affected areas to produce high amounts of collagen.

[0124] T cells also appear to play a role in the disease process through activation of macrophages and the direct release of inflammatory pro-fibrogenic cytokines. In addition to collagen, the activated fibroblasts appear to secrete factors that recruit

additional inflammatory cells to the affected areas, which release cytokines, which recruit further cytokine-releasing inflammatory cells, thereby leading to unregulated inflammation and tissue fibrosis.

[0125] Typically, monocytes/macrophages and T cells increase in both numbers and activation in the circulation and tissues of scleroderma patients. Tissue accumulation is both a cause and effect of microvascular injury, which is one of the early events in the pathogenesis of scleroderma. The microvascular injury is characterized by endothelial-cell damage, the proliferation of basal-lamina layers, occasional entrapment of peripheral-blood mononuclear cells in the vessel wall, and initial perivascular mononuclear-cell infiltrates. As the inflammatory cascades worsen, it is dominated by fibrosis, derangement of visceral organ architecture, rarefaction of blood vessels, and consequently, hypoxia. All of these factors and the continual recruitment of monocytes contributes to the maintenance of fibrosis

[0126] In some embodiments, scleroderma is also considered a connective tissue disease generally characterized with an excessive accumulation of Extracellular Matrix proteins in the skin and internal organs, vascular injury, and immunological abnormalities.

[0127] Many of the clinical manifestations of the disease are thought to involve a misregulation of vascular remodeling. One of the earliest symptoms of scleroderma is microvascular injury. This microvascular injury is thought to cause increased endothelial cell activation. Activated endothelial cells are believed to express adhesion molecules resulting in altered capillary permeability allowing migration of inflammatory cells through the endothelium and entrapment in the vessel wall. The immune activation is thought to contribute to sustained endothelial activation, which results in the breakdown of endothelial cells. This process is believed to contribute to the loss of elasticity and narrowing of the vessels commonly observed in scleroderma patients. Furthermore, it is thought that microvascular injury contributes to perivascular infiltrates of mononuclear cells in the dermis which is thought to contribute to the activation of fibroblasts and may of the associated hallmark symptoms of scleroderma.

[0128] Many of the clinical manifestations of the disease are generally thought to involve the misregulation of fibroblasts. The main function of fibroblasts is to maintain the structural integrity of connective tissues by continuously secreting precursors of the extracellular matrix. Fibroblasts provide a structural framework (stroma) for many tissues,

play an important role in wound healing and are the most common cells of connective tissue in animals. Fibroblasts are morphologically heterogeneous with diverse appearances depending on their location and activity.

[0129] There are two major forms of scleroderma: limited systemic sclerosis/scleroderma and diffuse systemic sclerosis/scleroderma. In limited cutaneous scleroderma, the fibrosis of the skin is generally confined to the area proximal to the elbow. Patients with limited cutaneous scleroderma generally experience vascular impairment. Cutaneous and organ fibrosis generally progresses slowly in patients with limited scleroderma. Patients with diffuse scleroderma generally experience fibrosis of skin and organs that progresses more rapidly than in limited scleroderma and/or widespread inflammation and/or more severe internal organ involvement than is seen in limited scleroderma.

[0130] It is generally thought that interstitial lung disease, resulting in pulmonary fibrosis, is the leading cause of scleroderma related deaths (Ludwicka-Bradley, A., et al. Coagulation and autoimmunity in scleroderma interstitial lung disease. Semin Arthritis Rheum, 41(2), 212-22, 2011). Further complications resulting in scleroderma-related deaths include but are not limited to cancer, heart failure, pulmonary hypertension, kidney failure, and malabsorption, or any combination thereof.

[0131] Scleroderma is most commonly diagnosed by inspection of skin symptoms. Tests to diagnosis include but are not limited to visual and/or manual inspection of the skin, blood pressure testing, chest x-ray, lung CT, echocardiogram, urinalysis, skin biopsy, and blood tests including antinuclear antibody testing, antitopoisomerase antibody testing, anticentromere antibody testing, anti-U3 antibody testing, anti-RNA antibody testing, other types of antibody testing, erythrocyte sedimentation rate, and rheumatoid factor.

Bi-specific Anti-CCL2 and Anti-LOXL2 Molecules

[0132] The present invention provides methods and compositions for treating scleroderma, and related fibrotic and/or inflammatory diseases, disorders and conditions based on administration of molecules that bind both CCL2 and LOXL2, in particular, bi-specific anti-CCL2 and LOXL2 molecules. In some embodiments, bi-specific molecules are nucleic acids, such as, bi-specific nucleic acid aptamers. In some embodiments, bi-specific molecules are proteins, such as, bi-specific fusion proteins, protein aptamers, and protein binding domains. In some embodiments, bi-specific molecules comprise bi-

specific fynomers. In some embodiments, bi-specific molecules comprise bi-specific antibodies. In some embodiments, a bi-specific antibody suitable for the present invention includes a first antigen-binding site that specifically binds to LOXL2 and a second antigen-binding site that specifically binds to CCL2 (see FIG. 1).

CCL2

[0133] CCL2 is a chemokine produced by a variety of cell types. It is also known as monocyte chemoattractant protein-1 (MCP-1). CCL2 is known to be a potent attractant for many cell types of the immune system, including but not limited to monocytes, CD4 and CD8 memory T lymphocytes and NK cells (Carulli, M. et al. Can CCL2 serum levels be used in risk stratification or to monitor treatment response in systemic sclerosis? Ann Rheum Dis, 67, 105-109, 2008, Yamamoto , T. Scleroderma – Pathophysiology. Eur J Dermatol, 19 (1), 14-24). CCL2 has been shown to promote leukocyte migration across endothelial monolayers, suggesting a role in the promotion of perivascular infiltrates of mononuclear cells (*Id.*). CCL2 has also been shown to promote activation of fibroblasts and to upregulate Collagen type I mRNA expression in rat fibroblasts in vitro. Elevated CCL2 levels have been shown in patients with scleroderma and also in animal models of scleroderma (*Id.*). Specifically, increased CCL2 expression levels have been shown in scleroderma skin and increased CCL2 RNA and protein has been shown in scleroderma fibroblasts (*Id.*).

[0134] Human CCL2 is an 8.6 kDa protein containing 76 amino acid residues, the amino acid sequence of which is shown in Table 1. It is expressed by a variety of cell types, including monocytes, vascular endothelial cells, smooth muscle cells, certain epithelial cells, among others and binds its receptor CCR2. CCL2 belongs to the family of the CC chemokines which contains two cysteine residues that are adjacent (the adjacent cysteine residues underlined in Table 1).

TABLE 1

Human CCL2 Protein Sequence (GeneBank: NP_002973)	MKVSAALLCLLLIAATFIPQGLAQPDAINAPVTCCYNFTN RKISVQRLASYRRITSSKCPKEAVIFKTIVAKEICADPKQK WVQDSMDHLDKQTQTPKT **(SEQ ID NO: 1)**

[0135] CCL2 has also been purified, characterized, cloned and sequenced from non-human sources and can be recombinantly produced or chemically synthesized. As used herein, the term CCL2 encompasses any CCL2 proteins naturally-occurring in other species including, but not limited to, mouse, rats, primates, pigs, chickens, dogs, goats, sheeps, horses, camels, llama, to name but a few, and any recombinant or synthetic CCL2 that is substantially homologous or identical to human CCL2. In some embodiments, a CCL2 protein as used herein has a sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more homologous to SEQ ID NO:1. In some embodiments, a CCL2 protein as used herein has a sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to SEQ ID NO:1. Typically, a CCL2 protein substantially homologous or identical to human CCL2 also retains substantial activity of human CCL2.

[0136] Any of the above described CCL2 proteins can be used to generate and identify mono-specific and/or bi-specific antibodies that specifically bind to CCL2. See the Anti-CCL2 Antibodies and Bi-specific Anti-CCL2 and LOXL2 Antibodies sections below.

LOXL2

[0137] LOXL2 is a member of the lysyl oxidase family of copper-dependent amine oxidases. Without wishing to be bound by theory, it is thought that LOXL2 catalyzes the covalent cross-link of the component side chains of collagen and those of elastin, thus stabilizing these proteins in the extracellular matrix (ECM). The polypeptide sequence of human LOXL2 is well characterized, as shown in Table 2.

TABLE 2

Human LOXL2 Protein Sequence (GeneBank: AAD34343)	MEGYVEVKEGKTWKQICDKHWTAKNSRVVCGMFGFPG ERTYNTKVYKMFASRRKQRYWPFSMDCTGTEAHISSCK LGPQVSLDPMKNVTCENGQPAVVSCVPGQVFSPDGPSRF RKAYKPEQPLVRLRGGAYIGEGRVEVLKNGEWGTVCDD KWDLVSASVVCRELGFGSAKEAVTGSRLGQGIGPIHLNEI QCTGNEKSIIDCKFNAESQGCNHEEDAGVRCNTPAMGLQ KKLRLNGGRNPYEGRVEVLVERNGSLVWGMVCGQNWG IVEAMVVCRQLGLGFASNAFQETWYWHGDVNSNKVVM SGVKCSGTELSLAHCRHDGEDVACPQGGVQYGAGVACS ETAPDLVLNAEMVQQTTYLEDRPMFMLQCAMEENCLSA

SAAQTDPTTGYRRLLRFSSQIHNNGQSDFRPKNGRHAWI
WHDCHRHYHSMEVFTHYDLLNLNGTKVAEGQKASFCL
EDTECEGDIQKNYECANFGDQGITMGCWDMYRHDIDCQ
WVDITDVPPGDYLFQVVINPNFEVAESDYSNNIMKCRSR
YDGHRIWMYNSHIGGSFSEETEKKFEHFSGLLNNQLSPPV
KKPAWSTPVFRPHHIFHGTSPQQLSLNECHVPSPSPAPTLS
RPLQLCLSSGGKGPSHHSWGAAT **(SEQ ID NO: 2)**

[0138] LOXL2 has also been purified, characterized, cloned and sequenced from non-human sources and can be recombinantly produced or chemically synthesized. As used herein, the term LOXL2 encompasses any LOXL2 proteins naturally-occurring in other species including, but not limited to, mouse, rats, primates, pigs, chickens, dogs, goats, sheeps, horses, camels, llama, to name but a few, and any recombinant or synthetic LOXL2 that is substantially homologous or identical to human LOXL2. In some embodiments, a LOXL2 protein as used herein has a sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more homologous to SEQ ID NO:2. In some embodiments, a LOXL2 protein as used herein has a sequence at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to SEQ ID NO:2. Typically, a LOXL2 protein substantially homologous or identical to human LOXL2 also retains substantial activity of human LOXL2.

[0139] Any of the above described LOXL2 proteins can be used to generate and identify desired mono-specific and/or bi-specific antibodies that specifically bind to LOXL2. See the Anti-LOXL2 Antibodies and Bi-specific Anti-CCL2 and LOXL2 Antibodies sections below.

[0140] "Percent (%) amino acid sequence identity" with respect to the CCL2 and LOXL2 sequences identified herein is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the CCL2 or LOXL2 sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to

achieve maximal alignment over the full length of the sequences being compared. Preferably, the WU-BLAST-2 software is used to determine amino acid sequence identity (Altschul *et al.*, Methods in Enzymology 266, 460-480 (1996); http://blast.wustl/edu/blast/README.html). WU-BLAST-2 uses several search parameters, most of which are set to the default values. The adjustable parameters are set with the following values: overlap span=1, overlap fraction=0.125, world threshold (T)=11. HSP score (S) and HSP S2 parameters are dynamic values and are established by the program itself, depending upon the composition of the particular sequence, however, the minimum values may be adjusted and are set as indicated above.

Anti-CCL2 and Anti-LOXL2 Mono-specific Antibodies

[0141] CCL2 and LOXL2 proteins described herein, or fragments thereof, can be used to generate antibodies by methods well known to those of skill in the art. As used herein, anti-CCL2 mono-specific antibodies include any antibodies or fragments of antibodies that bind specifically to any epitopes of CCL2 and anti-LOXL2 mono-specific antibodies include any antibodies or fragments of antibodies that bind specifically to any epitopes of LOXL2. As used herein, the term "antibodies" is intended to include immunoglobulins and fragments thereof which are specifically reactive to the designated protein or peptide, or fragments thereof. For example, the term "antibodies" includes intact monoclonal antibodies, polyclonal antibodies, single domain antibodies (e.g., shark single domain antibodies (e.g., IgNAR or fragments thereof)), and antibody fragments so long as they exhibit the desired biological activity. Suitable antibodies also include, but are not limited to, human antibodies, primatized antibodies, chimeric antibodies, bi-specific antibodies, humanized antibodies, conjugated antibodies (*i.e.*, antibodies conjugated or fused to other proteins, radiolabels, cytotoxins), Small Modular ImmunoPharmaceuticals ("SMIPs™"), and antibody fragments.

[0142] As used herein, an "antibody fragment" includes a portion of an intact antibody, such as, for example, the antigen-binding or variable region of an antibody. Examples of antibody fragments include Fab, Fab', F(ab')2, and Fv fragments; triabodies; tetrabodies; linear antibodies; single-chain antibody molecules. The term "antibody fragment" also includes any synthetic or genetically engineered protein that acts like an antibody by binding to a specific antigen to form a complex. For example, antibody fragments include isolated fragments, "Fv" fragments, consisting of the variable regions of

the heavy and light chains, recombinant single chain polypeptide molecules in which light and heavy chain variable regions are connected by a peptide linker ("ScFv proteins"), and minimal recognition units consisting of the amino acid residues that mimic the hypervariable region.

[0143] Mono-specific antibodies can be generated using methods well known in the art. For example, protocols for antibody production are described by Harlow and Lane, *Antibodies: A Laboratory Manual,* (1988). Typically, antibodies can be generated in mouse, rat, guinea pig, hamster, camel, llama, shark, or other appropriate host. Alternatively, antibodies may be made in chickens, producing IgY molecules (Schade *et al.*, (1996) *ALTEX* 13(5):80-85). In some embodiments, antibodies suitable for the present invention are subhuman primate antibodies. For example, general techniques for raising therapeutically useful antibodies in baboons may be found, for example, in Goldenberg *et al.*, international patent publication No. WO 91/11465 (1991), and in Losman *et al.*, *Int. J. Cancer* 46: 310 (1990). In some embodiments, monoclonal antibodies may be prepared using hybridoma methods (Milstein and Cuello, (1983) *Nature* 305(5934):537-40.). In some embodiments, monoclonal antibodies may also be made by recombinant methods (U.S. Pat. No. 4,166,452, 1979).

[0144] Many of the difficulties associated with generating monoclonal antibodies by B-cell immortalization can be overcome by engineering and expressing antibody fragments in E. coli, using phage display. To ensure the recovery of high affinity, monoclonal antibodies a combinatorial immunoglobulin library must typically contain a large repertoire size. A typical strategy utilizes mRNA obtained from lymphocytes or spleen cells of immunized mice to synthesize cDNA using reverse transcriptase. The heavy- and light-chain genes are amplified separately by PCR and ligated into phage cloning vectors. Two different libraries are produced, one containing the heavy-chain genes and one containing the light-chain genes. Phage DNA is isolated from each library, and the heavy- and light-chain sequences are ligated together and packaged to form a combinatorial library. Each phage contains a random pair of heavy- and light-chain cDNAs and upon infection of E. coli directs the expression of the antibody chains in infected cells. To identify an antibody that recognizes the antigen of interest, the phage library is plated, and the antibody molecules present in the plaques are transferred to filters. The filters are incubated with radioactively labeled antigen and then washed to remove excess unbound ligand. A radioactive spot on the autoradiogram identifies a

plaque that contains an antibody that binds the antigen. Cloning and expression vectors that are useful for producing a human immunoglobulin phage library can be obtained, for example, from STRATAGENE Cloning Systems (La Jolla, Calif.).

[0145] A similar strategy can be employed to obtain high-affinity scFv. See, e.g., Vaughn et al., Nat. Biotechnol., 14: 309 314 (1996). An scFv library with a large repertoire can be constructed by isolating V-genes from non-immunized human donors using PCR primers corresponding to all known V_H, V_k and V_λ gene families. Following amplification, the V_k and V_λ pools are combined to form one pool. These fragments are ligated into a phagemid vector. The scFv linker, $(Gly_4, Ser)_3$, is then ligated into the phagemid upstream of the V_L fragment. The V_H and linker-V_L fragments are amplified and assembled on the JH region. The resulting V_H-linker-V_L fragments are ligated into a phagemid vector. The phagemid library can be panned using filters, as described above, or using immunotubes (Nunc; Maxisorp). Similar results can be achieved by constructing a combinatorial immunoglobulin library from lymphocytes or spleen cells of immunized rabbits and by expressing the scFv constructs in P. pastoris. See, e.g., Ridder et al., Biotechnology, 13: 255 260 (1995). Additionally, following isolation of an appropriate scFv, antibody fragments with higher binding affinities and slower dissociation rates can be obtained through affinity maturation processes such as CDR3 mutagenesis and chain shuffling. See, e.g., Jackson et al., Br. J. Cancer, 78: 181 188 (1998); Osbourn et al., Immunotechnology, 2: 181 196 (1996).

[0146] Another form of an antibody fragment is a peptide coding for a single CDR. CDR peptides ("minimal recognition units") can be obtained by constructing genes encoding the CDR of an antibody of interest. Such genes are prepared, for example, by using the polymerase chain reaction to synthesize the variable region from RNA of antibody-producing cells. See, for example, Larrick et al., Methods: A Companion to Methods in Enzymology 2:106 (1991); Courtenay-Luck, "Genetic Manipulation of Monoclonal Antibodies," in MONOCLONAL ANTIBODIES: PRODUCTION, ENGINEERING AND CLINICAL APPLICATION, Ritter et al. (eds.), pages 166 179 (Cambridge University Press 1995); and Ward et al., "Genetic Manipulation and Expression of Antibodies," in MONOCLONAL ANTIBODIES: PRINCIPLES AND APPLICATIONS, Birch et al., (eds.), pages 137 185 (Wiley-Liss, Inc. 1995).

[0147] In some embodiments, antibodies suitable for the invention may include humanized or human antibodies. Humanized forms of non-human antibodies are chimeric

Igs, Ig chains or fragments (such as Fv, Fab, Fab', F(ab')2 or other antigen-binding subsequences of Abs) that contain minimal sequence derived from non-human Ig. Generally, a humanized antibody has one or more amino acid residues introduced from a non-human source. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization is accomplished by substituting rodent complementarity determining regions (CDRs) or CDR sequences for the corresponding sequences of a human antibody (Riechmann *et al.*, *Nature* 332(6162):323-7, 1988; Verhoeyen *et al.*, *Science.* 239(4847):1534-6, 1988.). Such "humanized" antibodies are chimeric Abs (U.S. Pat. No. 4,816,567, 1989), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In some embodiments, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent Abs. Humanized antibodies include human Igs (recipient antibody) in which residues from a CDR of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit, having the desired specificity, affinity and capacity. In some instances, corresponding non-human residues replace Fv framework residues of the human Ig. Humanized antibodies may comprise residues that are found neither in the recipient antibody nor in the imported CDR or framework sequences. In general, the humanized antibody comprises substantially all of at least one, and typically two, variable domains, in which most if not all of the CDR regions correspond to those of a non-human Ig and most if not all of the FR regions are those of a human Ig consensus sequence. The humanized antibody optimally also comprises at least a portion of an Ig constant region (Fc), typically that of a human Ig (Riechmann *et al.*, *Nature* 332(6162):323-7, 1988; Verhoeyen *et al.*, *Science.* 239(4847):1534-6, 1988.).

[0148] Human antibodies can also be produced using various techniques, including phage display libraries (Hoogenboom *et al.*, *Mol Immunol.* (1991) 28(9):1027-37; Marks *et al.*, *J Mol Biol.* (1991) 222(3):581-97) and the preparation of human monoclonal antibodies (Reisfeld and Sell, 1985, *Cancer Surv.* 4(1):271-90). Similarly, introducing human Ig genes into transgenic animals in which the endogenous Ig genes have been partially or completely inactivated can be exploited to synthesize human antibodies. Upon challenge, human antibody production is observed, which closely resembles that seen in

humans in all respects, including gene rearrangement, assembly, and antibody repertoire (Fishwild *et al.*, High-avidity human IgG kappa monoclonal antibodies from a novel strain of minilocus transgenic mice, *Nat Biotechnol.* 1996 July; 14(7):845-51; Lonberg *et al.*, Antigen-specific human antibodies from mice comprising four distinct genetic modifications, *Nature* 1994 April 28;368(6474):856-9; Lonberg and Huszar, Human antibodies from transgenic mice, *Int. Rev. Immunol.* 1995;13(1):65-93; Marks *et al.*, By-passing immunization: building high affinity human antibodies by chain shuffling. *Biotechnology* (N Y). 1992 July; 10(7):779-83).

[0149] In some embodiments, a mono-specific anti-CCL2 antibody or fragment thereof suitable for the present invention has a binding affinity of or greater than approximately 500nM, 100nM, 10nM, 1nM, 500pM, 100pM, 50pM, 10pM, 1pM, 500fM, 400fM, 300fM, 200fM, 100fM, 50fM, 10fM, 1fM. In some embodiments, a mono-specific anti-CCL2 antibody or fragment thereof suitable for the present invention has a binding affinity ranging between approximately 500nM and 1fM, between 500nM and 10fM, between 500nM and 100fM, between 500nM and 1pM, between 10nM and 1fM, between 10nM and 100fM, between 10nM and 1pM, between 1nM and 1fM, between 1nM and 100fM, between 1nM and 500fM, between 1nM and 1pM, between 1nM and 10pM, between 1nM and 50pM, between 1nM and 100pM, between 1nM and 500pM.

[0150] In some embodiments, a mono-specific anti-LOXL2 antibody or fragment thereof suitable for the present invention has a binding affinity of or greater than approximately 10nM, 1nM, 500pM, 100pM, 50pM, 10pM, 1pM, 500fM, 400fM, 300fM, 200fM, 100fM, 50fM, 10fM, 1fM. In some embodiments, a mono-specific anti-LOXL2 antibody or fragment thereof suitable for the present invention has a binding affinity ranging between approximately 10nM and 1fM, between 10nM and 100fM, between 10nM and 1pM, between 1nM and 1fM, between 1nM and 100fM, between 1nM and 500fM, between 1nM and 1pM, between 1nM and 10pM, between 1nM and 50pM, between 1nM and 100pM, between 1nM and 500pM.

Bi-specific Anti-CCL2 and Anti-LOXL2 Antibodies and Fusion Proteins

[0151] In some embodiments, the present invention provides bi-specific anti-CCL2 and anti-LOXL2 antibodies and/or fusion proteins. As used herein, the term "bi-specific antibodies or fusion proteins" encompasses any antibodies, fusion proteins, or fragments thereof, that contain at least two antigen-binding sites or antigen-binding arms with

distinct specificities. For example, a bi-specific anti-CCL2 and anti-LOXL2 antibody or fusion protein suitable for the present invention contains at least a first antigen-binding site or arm that specifically binds to LOXL2 and at least a second antigen-binding site or arm that specifically binds to CCL2.

[0152] Each individual antigen-binding sites or arms of a bi-specific antibody may have desired binding affinity against its specific binding target (e.g., CCL2 or LOXL2). In some embodiments, an antigen-binding site or arm specifically binds to CCL2 with a binding affinity of or greater than approximately 500nM, 100nM, 10nM, 1nM, 500pM, 100pM, 50pM, 10pM, 1pM, 500fM, 400fM, 300fM, 200fM, 100fM, 50fM, 10fM, 1fM. In some embodiments, an antigen-binding site or arm specifically binds to CCL2 with a binding affinity ranging between approximately 500nM and 1fM, between 500nM and 10fM, between 500nM and 100fM, between 500nM and 1pM, between 10nM and 1fM, between 10nM and 100fM, between 10nM and 1pM, between 1nM and 1fM, between 1nM and 100fM, between 1nM and 500fM, between 1nM and 1pM, between 1nM and 10pM, between 1nM and 50pM, between 1nM and 100pM, between 1nM and 500pM. In some embodiments, an antigen-binding site or arm specifically binds to LOXL2 with a binding affinity of or greater than approximately 10nM, 1nM, 500pM, 100pM, 50pM, 10pM, 1pM, 500fM, 400fM, 300fM, 200fM, 100fM, 50fM, 10fM, 1fM. In some embodiments, an antigen-binding site or arm specifically binds to LOXL2 with a binding affinity ranging between approximately 10nM and 1fM, between 10nM and 100fM, between 10nM and 1pM, between 1nM and 1fM, between 1nM and 100fM, between 1nM and 500fM, between 1nM and 1pM, between 1nM and 10pM, between 1nM and 50pM, between 1nM and 100pM, between 1nM and 500pM. The present invention encompasses bi-specific antibodies with combinations of the anti-CCL2 and anti-LOXLs antigen binding sites or arms with any of the above described binding affinities. In particular, a bi-specific antibody may contain a first antigen-binding site or arm that specifically binds to LOXL2 with a binding affinity of 1pM or greater and a second antigen-binding site or arm that binds to CCL2 with a binding affinity ranging between 500nM and 1fM. In particular, a bi-specific antibody may contain a first antigen-binding site or arm that specifically binds to LOXL2 with a binding affinity of 1pM or greater and a second antigen-binding site or arm that binds to CCL2 with a binding affinity greater than 1pM.

[0153] Each antigen-binding site of a bi-specific antibody can be independently a complete antigen-binding arm including a full length heavy chain and a full length light

chain, an Fab fragment, a single-chain variable fragments (scFvs), or other forms of antibody fragments. In some embodiments, desired antigen-binding sites or arms may be prepared from mono-specific antibodies against CCL2 and LOXL2 produced using the techniques described above and then associating such desired anti-CCL2 and anti-LOXL2 antigen-binding sites or arms to produce desired bi-specific antibodies. For example, a desired anti-CCL2 or anti-LOXL2 antigen–binding site or arm may be isolated, separated or enzymatically digested from mono-specific monoclonal antibodies described above. Antigen-binding sites or arms of a bi-specific antibody can be arranged in various configurations that allow the two sites or arms to associate while retaining their antigen binding ability.

[0154] Suitable bi-specific antibodies or fusion proteins can be in various bi-specific antibody formats including, but not limited to, quadromas, chemical heteroconjugates, recombinant constructs using selected heterodimerization domains and recombinant constructs of minimal size consisting of two minimal antigen-binding sites. In general, quadromas look like monoclonal antibodies but have two different antigen-binding arms. Their classical way of production is based on the somatic fusion of two different hybridoma (fused between a tumor cell and an antibody-making normal cell) cells, each producing a unique monoclonal antibody (e.g., anti-CCL2 or anti-LOXL2 monoclonal antibody). Bi-specific antibodies with desired antigen-binding arms (e.g., anti-CCL2 and anti-LOXL2) can be produced by random pairing of two different antibody heavy and light chains. Various preferential pairing methods are available to reduce mispaired by-products and increase bi-specific antibody yield. For example, a murine and a rat hybridoma cell line can be fused that express monoclonal antibodies of particular IgG subclasses preferentially pairing with each other. Additionally, the preferential pairing of two different antibody heavy chains can be achieved by certain mutations in the CH3-domain of human IgG1, so-called “knobs-into-holes” strategy.

[0155] Quadroma format typically contains an Fc region, which can interact with Fc receptors. Therefore, bi-specific antibodies with an Fc part are also referred to as trispecific antibodies. In some embodiments, the Fc part of a quadroma can be enzymatic removed, resulting in bi-specific $F(ab')_2$ (the two antigen binding arms of an antibody chemically linked through (a) disulfide bond(s)). In addition, two antigen-binding sites or arms can be linked with thioether bonding or through one or more functional groups on the antibody or fragment including amine, carboxyl, phenyl, thiol, or hydroxyl groups.

[0156] In some embodiments, a bi-specific antibody can be produced by chemical coupling of two different monoclonal antibodies or antibody fragments with, e.g., a hetero-bifunctional crosslinker. For example, two different Fab's (monovalent antigen binding arm(s) of an antibody) can be chemically crosslinked at their hinge cysteine residues in a site-directed way. Examples of chemicals appropriate for chemical crosslinking or coupling include but are not limited to N-succinimidyl-3-(2-pyridyldithio)propionate) (SPDP), 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC), 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), *o*-phenylene dimaleimide, carbodiimides, diisocyanates, diazobenzenes, hexamethylene diamines, dimaleimide, glutaraldehyde, 4-succinimidyl-oxycarbonyl-.alpha.-methyl .alpha.(2-pyridylthio)toluene (SMPT), N-succinimidyl-S acetyl-thioacetate (SATA), and combinations thereof.

[0157] In some embodiments, certain protein domains that naturally form heterodimers are used to construct heterodimeric bi-specific antibodies suited for large-scale expression. One example is the leucine zipper domains of transcription factors Fos and Jun which can be fused to the carboxy-terminus of two different Fab's or single-chain (sc) Fv (fragment of the variable region) antibody fragments. In some embodiments, antibody constant region domains Cκ and CH1 can be used instead of Fos and Jun dimerization domains for expression of the bi-specific antibody in bacteria such as *E. coli.* In certain embodiments, the two antigen-binding sites or arms can be associated via GST (glutathione S-transferase) fusion proteins, or a dimerization motifs thereof, PDZ dimerization domains, FK-506 BP (binding protein) or dimerization motifs thereof, natural or artificial helix-turn-helix dimerization domains (e.g., p53), Protein A or its dimerization domain, domain B, among others. In certain embodiments, two antigen-binding sites or arms may be associated via interaction with an exogenous component. For example, the two antigen-binding sites or arms may contain avidin motifs and both interact with added biotin.

[0158] In some embodiments, bi-specific antibodies include so called diabodies and tandem single-chain Fv constructs. Typically, these forms of bi-specific antibodies are made up from two different antigen-binding sites with minimal additional protein sequences acting as linker sequences. Each antigen binding site uses the minimal V_H and V_L domains from two antibody heavy and light chains, respectively. In diabodies, the V_L domain of one antigen binding site is connected by a short peptide linker with the V_H

domain of the other antigen binding site and vice versa. Bi-specific antibodies in the tandem scFv format typically include two different V_L/V_H pairs connected by a flexible peptide linker on a single protein chain. In some embodiments, tandem scFv constructs can be expressed in mammalian host cells which are capable of properly folding the four consecutively aligned antibody V regions. The fully functional bi-specific tandem single-chain antibodies are secreted into the cell culture supernatant and can be efficiently purified by affinity chromatography via a poly-histidine tag followed by size exclusion chromatography. Suitable peptide linkers may include any sequence that does not interfere with the conformation of the antigen binding sites or arms. In certain embodiments, a suitable peptide linker is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more amino acids long.

[0159] Antibodies produced by various methods described herein typically contain both homospecific and bi-specific molecules. Methods to assay for the presence of bi-specific monoclonals are known in the art, including bridge ELISA assays (see, e.g., Suresh et al. (1986) Proc. Natl. Acad. Sci. USA 83, 7989-93; Koolwijk et al. (1988) Hybridoma 7, 217-225; and De Lau et al. (1989) J. Immunol. 149, 1840-46). Double-antigen ELISA may be employed if sufficient quantities of the respective antigens are available.

[0160] The particular methods of bi-specific antibody preparation described above occasional result in the formation of monospecific as well as bi-specific antibodies (e.g., following procedures of chemical coupling). When this occurs, the desired bi-specific antibodies can be separated from the monospecific ones by any of a variety of procedures which allow differentiation between the two forms. Such procedures include but are not limited to passive elution from preparative, non-denaturing acrylamide gels or various conventional chromatographic techniques, e.g., anion-exchange, HPLC, or thiophilic adsorption chromatography (see,e.g., Kreutz et al. (1998). J. Chromatography 14, 161-170). Additionally, each of the antigen-binding sites or arms may be tagged with a different tag, and doubly tagged, bi-specific antibodies are separated from singly tagged monospecific antibodies by dual affinity chromatography.

[0161] Additional methods of generating, purifying and characterizing bi-specific antibodies are known in the art; for example, as disclosed in U.S. Pat. Nos. 5,601,819; 6,004,555, 5,762,930; 6,060,285; 6,010,902; 5,959,083; 5,807,706, and U.S. Patent Publication No. 2002/0025317, each of which is incorporated by reference herein.

[0162] Desired bi-specific anti-CCL2 and anti-LOXL2 antibodies can be further modified to produce chimeric, humanized, or fully human bi-specific antibodies using standard methods known in the art including various methods described herein.

Treatment of Scleroderma and Related Diseases, Disorders or Conditions

[0163] Bi-specific and/or mono-specific anti-CCL2 and anti-LOXL2 molecules (e.g., antibodies, fynomers, aptamers, fusion proteins, or protein binding domains) described herein may be used to effectively treat individuals suffering from or susceptible to scleroderma or related fibrotic, inflammatory diseases, disorders or conditions. The terms, "treat" or "treatment," as used herein, refers to amelioration of one or more symptoms, prevention or delay of the onset of one or more symptoms, and/or lessening of the severity or frequency of one or more symptoms of the relevant disease, disorder or condition.

[0164] Various molecules of the invention may be administered alone or in combination. In some embodiments, a method of treatment according to the present invention involves administering a bi-specific molecule described herein into a subject in need of treatment. In some embodiments, a method of treatment according to the present invention involves administering an anti-CCL2 and an anti-LOXL2 mono-specific antibodies, or fragments thereof, described herein into a subject in need of treatment. Anti-CCL2 and anti-LOXL2 mono-specific antibodies, or fragments thereof, can be administered simultaneously or sequentially, via same or different administration routes.

[0165] In some embodiments, molecules described herein may be administered alone or in conjunction with other therapeutic agents, such as those that are useful in treating fibrotic or inflammatory diseases, disorders or conditions. Such therapeutic agents include, but are not limited to, corticosteroids, NSAIDs, immune-suppressing drugs (e.g., Metotrexate and Cytoxan), small molecule immunomodulators, interferon receptor antibodies, anti-fibrotic drugs including D-penicillamine, colchicine, PUVA, relaxin, and cyclosporine and anti-TGFbeta treatments, and endothelin receptor antagonists.

[0166] In some embodiments, molecules described herein can be administered using conventional doses and delivery methods, such as those described for other, comparable therapeutic agents. Dosages to be administered can be determined by conventional procedures known to those of skill in the art. See, e.g., The Pharmacological Basis of Therapeutics, Goodman and Gilman, eds., Macmillan Publishing Co., New York.

In general, effective dosages are those which are large enough to produce the desired effect, e.g., neutralizing CCL2 and/or LOXL2 and/or blocking the binding of CCL2 and/or LOXL2 to their cognate receptors. The dosage should not be so large as to cause adverse side effects, such as unwanted cross-reactions, anaphylactic reactions, and the like. Factors to be considered include the activity of the specific antibody/agent involved, its metabolic stability and length of action, mode and time of administration, drug combination, rate of excretion, and the age, body weight, general health, sex, diet, and severity of the particular disease-states of the host undergoing therapy.

[0167] Molecules described herein can be administered in any dosing regimen that is therapeutically effective. In some embodiments, anti-CCL2/LOXL2 bi-specific or mono-specific antibodies are administered at bimonthly, monthly, triweekly, biweekly, weekly, daily, or at variable intervals.

[0168] Molecules described herein can be administered using any method of administration including parenteral and non-parenteral routes of administration. Parenteral routes include, e.g., intravenous, intraarterial, intraportal, intramuscular, subcutaneous, intraperitoneal, intraspinal, intrathecal, intracerebroventricular, intracranial, intrapleural or other routes of injection. Non-parenteral routes include, e.g., oral, nasal, transdermal, pulmonary, rectal, buccal, vaginal, ocular. Administration may also be by continuous infusion, local administration, sustained release from implants (gels, membranes or the like), and/or intravenous injection. When a combination of anti-CCL2 and anti-LOXL2 antibodies are used, anti-CCL2 and anti-LOXL2 antibodies can be administered via the same administration route or via different administration routes.

Scleroderma

[0169] In some embodiments, methods and compositions described herein can be used to treat a subject who is suffering or susceptible to all forms of scleroderma, including, the limited systemic sclerosis/scleroderma, the diffuse systemic sclerosis/scleroderma, and other forms of scleroderma. Limited systemic sclerosis/scleroderma typically involves cutaneous manifestations that mainly affect the hands, arms and face. It is also known as CREST syndrome in reference to the following complications: Calcinosis, Raynaud's phenomenon, Esophageal dysfunction, Sclerodactyly, and Telangiectasias. Additionally, pulmonary arterial hypertension may occur in up to one-third of patients, and is the most serious complication for this form of

scleroderma. Diffuse systemic sclerosis/scleroderma is rapidly progressing and affects a large area of the skin and one or more internal organs, frequently the kidneys, esophagus, heart and lungs. Other forms of scleroderma include systemic sine scleroderma, which lacks skin changes, but has systemic manifestations, and two localized forms which affect the skin, but not the internal organs: morphea and linear scleroderma.

[0170] In some embodiments, treatment refers to partially or completely alleviation, amelioration, relief, inhibition, delaying onset, reducing severity and/or incidence of one or more symptoms associated with scleroderma, including but not limited to, endothelial-cell damage, proliferation of basal-lamina layers, perivascular mononuclear-cell infiltration, fibrosis, derangement of visceral-organ architecture, rarefaction of blood vessels, hypoxia, swelling of the fingers, dorsa, and forearms, sensations of coldness in the extremities, digital ulcers, elongation of nail folds, pitted bleeding of the nails, pitting scars on the nails, pulmonary hypertension, skin fibrosis, hair loss, skin tightness, skin hardness, hyperpigmentation, hypopigmentation, itching of the skin, carpal tunnel syndrome, muscle weakness, joint pain, joint stiffness, kidney fibrosis, esophageal fibrosis, mouth fibrosis, heart fibrosis, and lung fibrosis, liver fibrosis, muscle fibrosis, dry cough, shortness of breath, difficulty breathing, alveolitis, pneumonia, wheezing, bloating after meals, constipation, diarrhea, difficulty swallowing, gastric antral vascular ectasia, esophageal reflux, heartburn, fecal incontinence, flat white patches in the mouth, loss of attached gingival mucosa, gingival recession, diffuse widening of the periodontal ligament, dysphagia, inelasticity of the mouth, resorption of posterior ramus of the mandible, coronoid process, and condyle, cancer, heart failure, pulmonary hypertension, kidney failure, malabsorption, or any combination thereof, as compared to an untreated control or the pre-treatment state.

[0171] In some embodiments, treatment refers to partially or completely alleviation, amelioration, relief, inhibition, delaying onset, reducing severity and/or incidence of fibrosis. As used herein, the term “fibrosis” refers to the formation of an excess fibrous connective tissue in an organ or tissue. Without wishing to be bound by particular theory, it is thought that fibrosis may be caused by activation of certain fibroblast. Different subtypes of fibroblasts are known to perform diverse functions, even within a single tissue. For example, papillary fibroblasts of the upper layers of the skin produce thin collagen bundles and have a high rate of proliferation, whereas reticular fibroblasts from the deeper dermal layer of the skin produce thick collagen bundles and

abundant versican, and promote rapid lattice contraction. Fibroblasts can be in a quiescent state or at varying stages of activation. During normal cellular function, fibroblasts become activated, for example, in response to injury to facilitate wound healing. Activated fibroblasts produce increased components of the extracellular matrix, including collagen and collagen modifying enzymes. In individuals with scleroderma, an increase in fibroblast activation is generally observed, accompanied by an overproduction of the ECM. This overproduction of the ECM is generally believed to cause fibrosis, the formation of excess fibrous connective tissue in an organ or tissue, that is a characteristic of scleroderma.

[0172] In some embodiments, treatment refers to partially or completely alleviation, amelioration, relief, inhibition, delaying onset, reducing severity and/or incidence of fibrosis in skin, kidney, liver, lung and/or oesophagus.

[0173] In some embodiments, treatment results in partially or completely alleviation, amelioration, relief, inhibition, delaying onset, reducing severity and/or incidence of skin fibrosis. Typically, skin fibrosis is associated with skin thickening, hardening, or formation of scars (e.g., keloid or burn scar, etc.). In some embodiments, skin fibrosis is assessed by Modified Rodnan Skin Score. For example, as illustrated in FIG. 2 uninvolved skin is given a score 0; mild thickening is given a score 1; moderate thickening is given a score 2; and severe thickening is given a score 3. In some embodiments, treatment results in a reduction of Modified Rodnan Skin Score by more than 10%, more than 15%, more than 20%, more than 25%, more than 30%, more than 35%, more than 40%, more than 45%, more then 50%, more than 55%, more than 60%, more than 65%, more than 70%, more than 75%, more than 80%, more than 85%, more than 90%, more than 95%, or more, as compared to the pre-treatment state. In some embodiments, treatment results in substantial elimination of skin fibrosis.

[0174] Without wishing to be bound by theory, it is also thought that activation of fibroblasts in scleroderma patients may be caused by the activation of the immune response by the production of cytokines. Examples of cytokines include but are not limited to TGF-β, CCL2, CTGF, ET-1, Fibroblast Growth Factor, IL-1, IL-4, IL-6, IL-12, IL-13, IL-17, MCP-1, MCP-3, and PDGF. Cytokines can be produced by pro-inflammatory cells of the immune system, for example activated T-cells, monocytes, or macrophages or, alternatively, cytokines can be produced by epithelial cells. One factor contributing to the activation of fibroblasts may be perivascular infiltrates of mononuclear

cells in the dermis associated with increased capillary permeability. Alternative or additional means of fibroblast activation include interaction with the extracellular matrix and/or mechanical tension. Thus, in some embodiments, treatment of scleroderma patients according to the present invention results in reduction of the production of one or more pro-inflammatory cytokines, such as those described herein. In some embodiments, treatment results in a reduction of a pro-inflammatory cytokine (e.g., TGF-β, CCL2, CTGF, ET-1, Fibroblast Growth Factor, IL-1, IL-4, IL-6, IL-12, IL-13, IL-17, MCP-1, MCP-3, and/or PDGF) by more than 10%, more than 15%, more than 20%, more than 25%, more than 30%, more than 35%, more than 40%, more than 45%, more then 50%, more than 55%, more than 60%, more than 65%, more than 70%, more than 75%, more than 80%, more than 85%, more than 90%, more than 95%, or more, as compared to the pre-treatment state. Various methods for determining the level of cytokines are known in the art and can be used to practice the present invention.

[0175] In some embodiments, treatment results in reduced CCL2 serum levels. In some embodiments, treatment results in a reduction of CCL2 serum levels by more than 10%, more than 15%, more than 20%, more than 25%, more than 30%, more than 35%, more than 40%, more than 45%, more then 50%, more than 55%, more than 60%, more than 65%, more than 70%, more than 75%, more than 80%, more than 85%, more than 90%, more than 95%, or more, as compared to the pre-treatment state. In some embodiments, treatment results in a CCL2 serum level of less than about 800 pg/ml, 700 pg/ml, 600 pg/ml, 500 pg/ml, 400 pg/ml, 350 pg/ml, 300 pg/ml, 250 pg/ml, 200 pg/ml, 150 pg/ml, or 100 pg/ml. In some embodiments, treatment results in a CCL2 serum level comparable to that of a healthy control of substantially same age or developmental stage.

Fibrotic diseases, disorders or conditions

[0176] In addition to Scleroderma, methods and compositions according to the present invention can be used to treat fibrotic diseases, disorders or conditions in general including, but not limited to, multifocal fibrosclerosis, sclerodermatous graft-vs-host-disease, nephrogenic systemic fibrosis, organ specific fibrosis, and the like. Illustrative organ specific fibrotic disorders include, but are not limited to, pulmonary fibrosis, pulmonary hypertension, cystic fibrosis, asthma, chronic obstructive pulmonary disease, liver fibrosis, kidney fibrosis, NASH, and the like. Many fibrotic diseases, disorders or conditions have disordered and/or exaggerated deposition of extracellular matrix in

affected tissues. Fibrosis may be associated with inflammation, occur as a symptom of underlying disease, and/or caused by surgical procedure or wound healing process. Unchecked fibrosis can result in destruction of the architecture of the underlying organ or tissue, commonly referred to as scarring.

[0177] NASH is usually a silent disease with few or no symptoms. Patients generally feel well in the early stages and only begin to have symptoms—such as fatigue, weight loss, and weakness—once the disease is more advanced or cirrhosis develops. The progression of NASH can take years, even decades. The process can stop and, in some cases may even begin to reverse on its own without specific therapy. Or NASH can slowly worsen, causing scarring or fibrosis to appear and accumulate in the liver. As fibrosis worsens, cirrhosis develops in which the liver becomes seriously scarred, hardened, and unable to function normally. Not every person with NASH develops cirrhosis, but once serious scarring or cirrhosis is present, few treatments can halt the progression. A person with cirrhosis experiences fluid retention, muscle wasting, bleeding from the intestines, and liver failure. Liver transplantation is the only treatment for advanced cirrhosis with liver failure, and transplantation is increasingly performed in people with NASH. NASH ranks as one of the major causes of cirrhosis in America, behind hepatitis C and alcoholic liver disease.

[0178] Kidney (renal) fibrosis results from excessive formation of fibrous connective tissue in the kidney. Kidney fibrosis causes significant morbidity and mortality and leads to a need for dialysis or kidney transplantation. Fibrosis can occur in either the filtering or reabsorptive component of the nephron, the functional unit of the kidney. A number of factors may contribute to kidney scarring, particularly derangements of physiology involved in the autoregulation of glomerular filtration. This in turn leads to replacement of normal structures with accumulated extracellular matrix. A spectrum of changes in the physiology of individual cells leads to the production of numerous peptide and non-peptide fibrogens that stimulate alterations in the balance between extracellular matrix synthesis and degradation to favor scarring.

Inflammatory diseases, disorders or conditions

[0179] In some embodiments, methods and compositions according to the present invention are used to treat inflammatory diseases, disorders or conditions including, but not limited to: Systemic Inflammatory Response (SIRS); Alzheimer's Disease (and

associated conditions and symptoms including: chronic neuroinflammation, glial activation; increased microglia; neuritic plaque formation; and response to therapy); Amyotropic Lateral Sclerosis (ALS), arthritis (and associated conditions and symptoms including, but not limited to: acute joint inflammation, antigen-induced arthritis, arthritis associated with chronic lymphocytic thyroiditis, collagen-induced arthritis, juvenile arthritis; rheumatoid arthritis, osteoarthritis, prognosis and streptococcus-induced arthritis, spondyloarthopathies, gouty arthritis), asthma (and associated conditions and symptoms, including: bronchial asthma; chronic obstructive airway disease; chronic obstructive pulmonary disease, juvenile asthma and occupational asthma); cardiovascular diseases (and associated conditions and symptoms, including atherosclerosis; autoimmune myocarditis, chronic cardiac hypoxia, congestive heart failure, coronary artery disease, cardiomyopathy and cardiac cell dysfunction, including: aortic smooth muscle cell activation; cardiac cell apoptosis; and immunomodulation of cardiac cell function; diabetes and associated conditions and symptoms, including autoimmune diabetes, insulin-dependent (Type 1) diabetes, diabetic periodontitis, diabetic retinopathy, and diabetic nephropathy); gastrointestinal inflammations (and related conditions and symptoms, including celiac disease, associated osteopenia, chronic colitis, Crohn's disease, inflammatory bowel disease and ulcerative colitis); gastric ulcers; hepatic inflammations such as viral and other types of hepatitis, cholesterol gallstones and hepatic fibrosis, HIV infection (and associated conditions and symptoms, including degenerative responses, neurodegenerative responses, and HIV associated Hodgkin's Disease), Kawasaki's Syndrome (and associated diseases and conditions, including mucocutaneous lymph node syndrome, cervical lymphadenopathy, coronary artery lesions, edema, fever, increased leukocytes, mild anemia, skin peeling, rash, conjunctiva redness, thrombocytosis; multiple sclerosis, nephropathies (and associated diseases and conditions, including diabetic nephropathy, endstage renal disease, acute and chronic glomerulonephritis, acute and chronic interstitial nephritis, lupus nephritis, Goodpasture's syndrome, hemodialysis survival and renal ischemic reperfusion injury), neurodegenerative diseases (and associated diseases and conditions, including acute neurodegeneration, induction of IL-1 in aging and neurodegenerative disease, IL-1 induced plasticity of hypothalamic neurons and chronic stress hyperresponsiveness), opthalmopathies (and associated diseases and conditions, including diabetic retinopathy, Graves' opthalmopathy, and uveitis, osteoporosis (and associated diseases and conditions, including alveolar, femoral, radial, vertebral or wrist bone loss or fracture incidence, postmenopausal bone loss, mass,

fracture incidence or rate of bone loss), otitis media (adult or pediatric), pancreatitis or pancreatic acinitis, periodontal disease (and associated diseases and conditions, including adult, early onset and diabetic); pulmonary diseases, including chronic lung disease, chronic sinusitis, hyaline membrane disease, hypoxia and pulmonary disease in SIDS; restenosis of coronary or other vascular grafts; rheumatism including rheumatoid arthritis, rheumatic Aschoff bodies, rheumatic diseases and rheumatic myocarditis; thyroiditis including chronic lymphocytic thyroiditis; urinary tract infections including chronic prostatitis, chronic pelvic pain syndrome and urolithiasis. Immunological disorders, including autoimmune diseases, such as alopecia aerata, autoimmune myocarditis, Graves' disease, Graves opthalmopathy, lichen sclerosis, multiple sclerosis, psoriasis, systemic lupus erythematosus, systemic sclerosis, thyroid diseases (e.g. goiter and struma lymphomatosa (Hashimoto's thyroiditis, lymphadenoid goiter), sleep disorders and chronic fatigue syndrome and obesity (non-diabetic or associated with diabetes). Resistance to infectious diseases, such as Leishmaniasis, Leprosy, Lyme Disease, Lyme Carditis, malaria, cerebral malaria, meningitis, tubulointerstitial nephritis associated with malaria), which are caused by bacteria, viruses (e.g. cytomegalovirus, encephalitis, Epstein-Barr Virus, Human Immunodeficiency Virus, Influenza Virus) or protozoans (e.g., Plasmodium falciparum, trypanosomes). Response to trauma, including cerebral trauma (including strokes and ischemias, encephalitis, encephalopathies, epilepsy, perinatal brain injury, prolonged febrile seizures, SIDS and subarachnoid hemorrhage), low birth weight (e.g. cerebral palsy), lung injury (acute hemorrhagic lung injury, Goodpasture's syndrome, acute ischemic reperfusion), myocardial dysfunction, caused by occupational and environmental pollutants (e.g. susceptibility to toxic oil syndrome silicosis), radiation trauma, and efficiency of wound healing responses (e.g. burn or thermal wounds, chronic wounds, surgical wounds and spinal cord injuries). Hormonal regulation including fertility/fecundity, likelihood of a pregnancy, incidence of preterm labor, prenatal and neonatal complications including preterm low birth weight, cerebral palsy, septicemia, hypothyroidism, oxygen dependence, cranial abnormality, early onset menopause. A subject's response to transplant (rejection or acceptance), acute phase response (e.g. febrile response), general inflammatory response, acute respiratory distress response, acute systemic inflammatory response, wound healing, adhesion, immunoinflammatory response, neuroendocrine response, fever development and resistance, acute-phase response, stress response, disease susceptibility, repetitive motion stress, tennis elbow, and pain management and response.

Biomarkers or Indicators for Patient Stratification, Treatment Monitoring and/or Optimization

[0180] In some embodiments, methods and compositions based on anti-CCL2/LOXL2 bi-specific or mono-specific molecules (e.g., antibodies, fynomers, aptamers, fusion proteins, or protein binding domains) described herein can be used with biomarkers for patient stratification, treatment monitoring and/or optimization. In some embodiments, suitable biomarkers are differentially expressed biomarkers. As used herein, the term "*differentially expressed biomarker*" refers to a biomarker whose level of expression is different in a subject (or a population of subjects) afflicted with scleroderma relative to its level of expression in a healthy or normal subject (or a population of healthy or normal subjects). The term also encompasses a biomarker whose level of expression is different for a different disease subtype (*i.e.*, Limited cutaneous or diffuse cutaneous). The term further encompasses a biomarker whose level of expression is different at different stages of the disease (*e.g.*, mild or early scleroderma, severe or late scleroderma). Differential expression includes quantitative, as well as qualitative, differences in the temporal or cellular expression pattern of the biomarker. As described in greater details below, a differentially expressed biomarker, alone or in combination with other differentially expressed biomarkers, is useful in a variety of different applications in diagnostic, staging, therapeutic, drug development and related areas. The expression patterns of the differentially expressed biomarkers disclosed herein can be described as a fingerprint or a signature of scleroderma, scleroderma subtype, scleroderma stage and scleroderma disease severity and/or progression. They can be used as a point of reference to compare and characterize unknown samples and samples for which further information is sought. The term "*decreased level of expression*", as used herein, refers to a decrease in expression of at least 10% or more, for example, 20%, 30%, 40%, or 50%, 60%, 70%, 80%, 90% or more, or a decrease in expression of greater than 1-fold, 2-fold, 3-fold, 4-fold, 5-fold, 10-fold, 50-fold, 100-fold or more as measured by one or more methods described herein. The term "*increased level of expression*", as used herein, refers to an increase in expression of at least 10% or more, for example, 20%, 30%, 40%, or 50%, 60%, 70%, 80%, 90% or more or an increase in expression of greater than 1-fold, 2-fold, 3-fold, 4-fold, 5-fold, 10-fold, 50-fold, 100-fold or more as measured by one or more methods, such as method described herein.

Skin gene expression analysis

[0181] Various methods for identifying differentially expressed biomarkers in scleroderma patients are known in the art and can be used to practice the present invention. For example, skin gene expression analysis can be a powerful tool for subsetting patients, identifying protein biomarkers and indicators of responsive patient subsets. In some embodiments, genes that are differentially regulated in patients with scleroderma can be identified by comparing transcriptional profiles of skin samples of healthy individuals with those having scleroderma. Further, gene transcripts that associate with severity of disease can be identified by including scleroderma patients at various stages of degree progression. Transcriptional profiles can be analyzed by microarray analysis, as has been described, for example, by Milano et al. in "Molecular Subsets in the Gene Expression Signatures of Scleroderma Skin" (PLOS One, 3:7, 1-18, 2008), the entirety of which is herein incorporated by reference. For example, microarray analysis can be performed on skin samples (e.g., forearm and back samples) from patients with diffuse scleroderma, limited scleroderma, morphea (a disease similar to scleroderma with no internal organ involvement) and healthy controls. To identify genes most highly associated with scleroderma, the genes that are most internally consistent between replicates and sample sites, while being the most variable between individuals, are selected for further analysis. Cluster analysis based on differential gene expression correlated with severity of scleroderma can be used to select genes affected by scleroderma.

[0182] It has been reported that differentially expressed exemplary genes in scleroderma can be clustered into 6 groups. The first group includes immunoglobulin genes expressed highly in a subset of patients with diffuse scleroderma and in patients with morphea, including but not limited to CCR2, CCL4, and IGLL1. The second group includes proliferation signature, including genes that are expressed only when the cell is dividing. Genes showing increased expression in this cluster include the cell-cycle regulated genes such as CKS1B, CDKS2, CDC2, MCM8 and E2F7. The existence of a proliferation signature is consistent with reports from skin biopsies demonstrating that cells of diffuse scleroderma tissue undergoing increased proliferation. The third group includes collagen and extracelluar matrix components, including but not limited to COL5A2, COL8A1, COL10A1, COL12A1. The fourth group includes genes typically associated with the presence of T-lymphocyes and macrophages, which are similarly

expressed to the third group and include PTPRC, which is required for T-cell activation, as well as CD2 and CDW52, that are expressed on the surface of T lymphocytes. The fifth group includes genes showing low expression in diffuse scleroderma. These genes show higher expression levels in other biopsies and include WIF1, Tetranectin, IGFBP6, and IGFBP5, among others. The final group is a heterogeneous gene expression cluster that is high in limited scleroderma and a subset of diffuse scleroderma, including but not limited to, UTS2R, GALR3, PARD6G, PSEN1, PHOX2A, CENTG3, HCN4, KLF16, and GPR15G. Additional differentially expressed exemplary genes are described in Milano et al. in "Molecular Subsets in the Gene Expression Signatures of Scleroderma Skin" (PLOS One, 3:7, 1-18, 2008), the entirety of which is herein incorporated by reference.

Multi-gene signature as surrogate markers

[0183] Combinations of genes may be used as biomarkers. Exemplary methods for biomarker identification is provided in, for example, Farina et al., in "A Four-Gene Biomarker Predicts Skin Disease in Patients with Diffuse Cutaneous Systemic Sclerosis" (Arthritis Rheum. 62(2), 580-588, 2010), the entirety of which is incorporated herein by reference. Starting with targets such as TGFß and interferon known to be regulated in scleroderma, Farina identified a four-gene biomarker, including the genes CTGF, THS1, COL4, and PAI1. The transcription of these four genes in combination was found to be highly correlated with Modified Rodnan Skin Score (mRSS) and highly predictive of diffuse scleroderma.

[0184] mRSS is used as one clinical marker of scleroderma. Typically, mRRS is assigned as shown in FIG. 2: uninvolved skin is assigned a score 0; mild thickening is given a score 1; moderate thickening is given a score 2; and severe thickening is given a score 3. Typically, a total mRSS score ranging from 0-51 can be determined based on a grading of 0-3 at 17 skin areas of a patient. mRSS can be used as indicators for diagnosis and monitoring treatment alone or in combination with other biomarkers..

[0185] Similar strategy can be used to identify and validate potential signature biomarkers for scleroderma. Specifically, gene transcripts identified as positively or negatively regulated in scleroderma are tested alone or in combination to identify biomarkers comprised of gene transcript(s) or combinations of gene transcripts that are most highly correlated with clinical markers of scleroderma. In addition to mRSS, other clinical markers can be used, such as the HAQ - DI, DLCO, or FVC.

CCL2 levels

[0186] CCL2 levels, for example, CCL2 serum levels, can be used as biomarker or indicators for determining disease severity, organ involvement, selecting appropriate treatment, monitoring disease progression and patient response. To determine CCL2 levels as biomarkers or indicators, CCL2 levels in the serum of patients at a variety of stages of scleroderma and unaffected individuals are determined. This can be done by assaying CCL2 protein levels in serum by, e.g., ELISA, and correlated with skin and other organ (e.g., lung, liver, kidney, oesophagus) involvement. Exemplary methods are described in Carulli et al. Ann Rheum Dis. 67:105-109, 2008.

[0187] CCL2 levels present in skin, such as from a biopsy, and/or serum can also be correlated with mRSS or other clinical markers, such as the Health Assessment Questionnaire (HAQ - DI), Diffusing capacity of the lung for carbon monoxide (DLCO), or Forced Vital Capacity (FVC).

[0188] Various biomarkers can be used alone or in combination, or alternatively, together with clinical diagnostic markers, such as mRSS, to stratify patients based on severity of scleroderma, selecting proper therapy or dosing regimen, evaluating the effectiveness of a therapy, monitoring responsiveness to therapy, prognosis for disease course, and measurement of disease progression in a subject. Typically, in such methods, levels of suitable biomarkers (e.g., such as those selected from various differentially expressed genes described herein and other known markers such as CCL2 levels) determined for a biological sample obtained from the subject from one or more time points are compared to the levels from the subject from one or more other time points. For example, biomarker levels may be measured before or at the beginning of a treatment course. Biomarker levels may be measured at one or more time points throughout the course of treatment and compared with the level before the treatment or from an earlier time point of a treatment course. Identification or selection of appropriate treatment, determining if a patient has positive response to a treatment and/or optimization of the treatment can be determined based on the evaluation of biomarkers.

Pharmaceutical Compositions

[0189] The present invention also provides compositions comprising one or more provided molecules (e.g., antibodies, fynomers, aptamers, fusion proteins, protein binding domains). In some embodiments the present invention provides at least one molecule and at least one pharmaceutically acceptable excipient. Such pharmaceutical compositions may optionally comprise and/or be administered in combination with one or more additional therapeutically active substances. In some embodiments, provided pharmaceutical compositions are useful in medicine. In some embodiments, provided pharmaceutical compositions are useful as prophylactic agents (i.e., vaccines) in the treatment or prevention of scleroderma or of negative ramifications associated or correlated with scleroderma. In some embodiments, provided pharmaceutical compositions are useful in therapeutic applications, for example in individuals suffering from or susceptible to scleroderma. In some embodiments, pharmaceutical compositions are formulated for administration to humans.

[0190] For example, pharmaceutical compositions provided here may be provided in a sterile injectable form (e.g., a form that is suitable for subcutaneous injection or intravenous infusion). For example, in some embodiments, pharmaceutical compositions are provided in a liquid dosage form that is suitable for injection. In some embodiments, pharmaceutical compositions are provided as powders (e.g., lyophilized and/or sterilized), optionally under vacuum, which are reconstituted with an aqueous diluent (e.g., water, buffer, salt solution, etc.) prior to injection. In some embodiments, pharmaceutical compositions are diluted and/or reconstituted in water, sodium chloride solution, sodium acetate solution, benzyl alcohol solution, phosphate buffered saline, etc. In some embodiments, powder should be mixed gently with the aqueous diluent (e.g., not shaken).

[0191] In some embodiments, provided pharmaceutical compositions comprise one or more pharmaceutically acceptable excipients (e.g., preservative, inert diluent, dispersing agent, surface active agent and/or emulsifier, buffering agent, etc.). In some embodiments, pharmaceutical compositions comprise one or more preservatives. In some embodiments, pharmaceutical compositions comprise no preservative.

[0192] In some embodiments, pharmaceutical compositions are provided in a form that can be refrigerated and/or frozen. In some embodiments, pharmaceutical compositions are provided in a form that cannot be refrigerated and/or frozen. In some

embodiments, reconstituted solutions and/or liquid dosage forms may be stored for a certain period of time after reconstitution (e.g., 2 hours, 12 hours, 24 hours, 2 days, 5 days, 7 days, 10 days, 2 weeks, a month, two months, or longer). In some embodiments, storage of antibody compositions for longer than the specified time results in molecular degradation.

[0193] Liquid dosage forms and/or reconstituted solutions may comprise particulate matter and/or discoloration prior to administration. In some embodiments, a solution should not be used if discolored or cloudy and/or if particulate matter remains after filtration.

[0194] Compositions of the pharmaceutical compositions described herein may be prepared by any method known or hereafter developed in the art of pharmacology. In some embodiments, such preparatory methods include the step of bringing active ingredient into association with one or more excipients and/or one or more other accessory ingredients, and then, if necessary and/or desirable, shaping and/or packaging the product into a desired single- or multi-dose unit.

[0195] A pharmaceutical composition in accordance with the invention may be prepared, packaged, and/or sold in bulk, as a single unit dose, and/or as a plurality of single unit doses. As used herein, a "unit dose" is discrete amount of the pharmaceutical composition comprising a predetermined amount of the active ingredient. The amount of the active ingredient is generally equal to a dose which would be administered to a subject and/or a convenient fraction of such a dose such as, for example, one-half or one-third of such a dose.

[0196] Relative amounts of active ingredient, pharmaceutically acceptable excipient, and/or any additional ingredients in a pharmaceutical composition in accordance with the invention may vary, depending upon the identity, size, and/or condition of the subject treated and/or depending upon the route by which the composition is to be administered. By way of example, the composition may comprise between 0.1% and 100% (w/w) active ingredient.

[0197] Pharmaceutical compositions of the present invention may additionally comprise a pharmaceutically acceptable excipient, which, as used herein, may be or comprise solvents, dispersion media, diluents, or other liquid vehicles, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents,

preservatives, solid binders, lubricants and the like, as suited to the particular dosage form desired. Remington's The Science and Practice of Pharmacy, 21st Edition, A. R. Gennaro, (Lippincott, Williams & Wilkins, Baltimore, MD, 2006) discloses various excipients used in formulating pharmaceutical compositions and known techniques for the preparation thereof. Except insofar as any conventional excipient medium is incompatible with a substance or its derivatives, such as by producing any undesirable biological effect or otherwise interacting in a deleterious manner with any other component(s) of the pharmaceutical composition, its use is contemplated to be within the scope of this invention.

EXAMPLES

[0198] The present invention will be further illustrated by the following non-limiting examples. These Examples are set forth to aid in the understanding of the invention but are not intended to, and should not be construed to, limit its scope in any way. The Examples do not include detailed descriptions of conventional methods that would be well known to those of ordinary skill in the art. Unless indicated otherwise, parts are parts by weight, molecular weight is average molecular weight, temperature is indicated in Celsius, and pressure is at or near atmospheric.

Example 1. Preparation of Bi-specific Anti-CCL2/LOXL2 Antibodies

[0199] This example illustrates preparation of bi-specific anti-CCL2/LOXL2 antibodies. As described above, various methods are available to generate and select bi-specific antibodies with desired specificities and binding affinities.

[0200] In this particular example, the bi-specific antibody is composed of a complete antigen-binding arm against CCL2 and a complete antigen-binding arm against LOLX2. Specifically, a mouse cell line producing a humanized CCL2-specific monoclonal antibody is fused to a rat cell line producing a humanized LOXL2-specific monoclonal antibody to produce a quadroma. Supernatants of quadroma cells are tested for binding to target cells by FACS analysis. Antibodies are purified from quadroma cell culture by protein A affinity followed by ion exchange chromatography.

Example 2. Dose range testing

[0201] This example illustrates a dose response study designed to evaluate effective dose ranges of bi-specific anti-CCL2 and anti-LOXL2 antibody for treatment of scleroderma.

[0202] A bleomycin induced scleroderma mouse model is used in this example. Typically, fibrosis is induced in mice by repeated subcutaneous injection of bleomycin, polyinosinic-polycytidylic acidand/or LPS into the dorsal skin. Specifically, osmotic pumps (7-day) containing either bleomycin at concentration of 10-110 μg and up to 200 μg, LPS at a concentration of 300 μg, polycytidylic acid at a concentration of 100 μg or PBS alone are implanted subcutaneously into groups of 10 B6 mice. In this mouse model, histopathological changes in the skin closely resembles that seen in scleroderma. Early mononuclear cell accumulation and upregulated TGF-β and chemokine expression is followed by dermal fibrosis characterized by thick collagen bundles and accumulation of activated fibroblasts. Mice also manifest evidence of pulmonary and renal fibrosis.

[0203] Dose(s) of bi-specific CCL2/LOXL2 antibody or a control antibody escalating concentrations are administered into the mice via intraperitoneal injection.

Example 3. *In vivo* efficacy of bi-specific anti-CCL2/LOXL2 antibody

[0204] This example illustrates a study designed to evaluate the effect of treatment with anti-CCL2/LOXL2 antibodies on inflammation and fibrosis in the bleomycin mouse model for scleroderma.

[0205] 7 or 28-day osmotic pumps containing either PBS alone or 10-110 μg and up to 200 μg bleomycin in PBS will be implanted subcutaneously into B6 mice. Every two days, mice will be treated via intraperitoneal injection with anti-CCL2/LOXL2 bi-specific antibody at suitable concentrations, as determined in example 2, or with a control antibody.

[0206] After 7 days, in the case of a 7 day osmotic pump, or 28 days, in the case of a 28 day osmotic pump, skin and lung tissue will be harvested for transcriptional and histological analysis. Levels of CCL2 protein in tissue samples is measured by ELISA. For transcriptional analysis, RNA is extracted from skin tissue and the isolated RNA is subject to and semi-quantitative or quantitative reverse transcriptase-PCR using techniques commonly known in the art. Levels of TGFβ gene expression and gene expression levels

of pro-inflammatory genes, including but not limited to PAI1, COMP, COL1a1, F4/80, IL-6, and TNFα is measured using commercially available primers (TaqMan®). For histological analysis, skin fibrosis is analyzed by microscopic examination of tissue sections stained with hematoxylin and eosin (H&E). The use of H&E staining to visualize tissue morphology is well known in the art. Immunohistochemistry is used to quantify monocyte infiltration by microscopic examination of tissue sections probed with the monocyte specific anti-F4/80 antibody using techniques well known in the art.

[0207] It is anticipated that treatment with anti-CCL2/LOXL2 antibody will reduce infiltration of monocytes and macrophages, will reduce inflammatory gene expression (ex., IL-6, TNFα), and will decrease TGFβ-induced marker gene expression. This is expected to result in a general decrease in fibrosis.

Example 4. Therapeutic modeling

[0208] This example illustrates a model of CCL2 production and turnover in various tissues and plasma to predict tissue target levels. The illustrated model represents an extreme presentation of high CCL2 levels.

[0209] Typically, CCL2 is produced in disease tissues and secreted into plasma. In healthy individuals, CCL2 synthesis in skin is low or undetectable. CCL2 synthesis increases with involvement of total skin in both non-affected and affected skin, leading to increased serum CCL2 levels. Serum CCL2 levels further increase with organ involvement. Typically, healthy individuals have an average serum CCL2 level of less than about 100 pg/ml. Individuals having so called Raynaud's phenomenon has slightly increased average serum CCL2 levels. Patients suffering from sclerosis typically have an average serum CCL2 level of about 250 pg/ml. Patients suffering from limited cutaneous systemic sclerosis typically have an average serum CCL2 level of about 250 pg/ml. Patients suffering from diffuse cutaneous systemic sclerosis typically have an average serum CCL2 level of about 380 pg/ml. Patients suffering from limited cutaneous systemic sclerosis typically have an average serum CCL2 level of about 250 pg/ml.

[0210] The molecular weight of CCL2 is about 8.6kDa, which is much smaller than the glomerular filtration threshold of about 50 kDa, resulting in rapid kidney clearance. CCL2 is internalized by active receptor mediated internalization. Typical kd for CCL2 to bind its receptor CCR2 is about 60pM- 2nM. CCR2 is primarily present on

lymphoid-origin cells and lymphatic endothelium. It is contemplated that scleroderma causes increased vascular permeability early in disease progression, which permits substantial equilibration of CCL2 and any therapeutic antibodies between interstitium and serum. Therefore, serum half-life of CCL2 is about 10 minutes based on data from mice and rabbits. It is expected that CCL2 serum half-life in humans is similar. Relatively permeable tissue allows CCL2 reach equilibration from tissue to serum (half-max) quickly, for example in about 2 hours. In some cases, serum CCL2 level may reach 1000 pg/ml (~ 75pM) with whole skin involvement but without organ involvement. A target profile showing serum and tissue CCL2 equilibration is shown in FIG. 3, which predicts the desired amount of antibodies need to neutralize 3nM of tissue CCL2 and compete it off its receptor.

[0211] Monoclonal antibodies injected intravenously typically binds CCL2 in plasma and forms a complex before they reach diseased tissues, resulting in wasted monoclonal antibodies. Bi-specific mAbs allow us to sequester mAb in diseased tissue with a "free" anti-CCL2 arm to bind to tissue CCL2, which provides tissue specific targeting of CCL2 (See FIG. 4). We can design the affinity for CCL2 such that it does not bind to serum CCL2 but binds tissue CCL2. Furthermore, we can also compete with the 60pM affinity for CCR2 by increasing dose. Thus, this approach allows us preferentially inhibit tissue CCL2 as opposed to plasma CCL2, resulting in highly effective treatment of scleroderma.

[0212] A preliminary set of results from modeling bi-specific binding proteins are shown in FIG. 5 based on the following assumptions: Anti-LOXL2 arm has a kd of 1pM or better; anti-CCL2 arm has a kd ranging between 500pM and 1nM; and LOXL2 bound mAb is not internalized or degraded.

Example 5: Clinical design

[0213] Based upon the success of animal treatments, Phase I-III dose ranging and single dose studies of anti-CCL2/LOXL2 bi-specific antibody detailed in Tables 3-7 are designed in healthy individuals and individuals with different stages of scleroderma to evaluate the safety, tolerability, efficacy, and pharmacokinetics of anti-CCL2/LOXL2.

[0214] A primary objective of Human Clinical Trial 1 includes determining the safety of 4 dose levels of anti-CCL2/LOXL2 antibody administered in healthy individuals. Secondary objectives include evaluating the pharmacokinetics of 4 different dose levels of

anti-CCL2/LOXL2 antibody administered in healthy individuals. A detailed protocol synopsis of this clinical trial is shown in Table 3.

TABLE 3: Human Clinical Trial 1

Phase	Phase 1
# of Trials	1
Patient Population	Healthy volunteers
Trial Design and Endpoints	Single dose, dose escalation Primary: Safety Secondary: PK
# of Subjects	4 dose groups n=4 each 16 subjects total
Trial Length (FPI to LPV)	0.5 years ~ 6 weeks to dose ~ 15 weeks follow up for PK
Comments	Single Phase 1 unit

[0215] A primary objective of Human Clinical Trial 2 includes determining the safety of 4 dose levels of anti-CCL2/LOXL2 antibody administered in individuals with early symptoms of scleroderma. Secondary objectives include (1) to determine the pharmacokinetics of 4 different dose levels of anti-CCL2/LOXL2 antibody administered in individuals with early symptoms of scleroderma (2) to determine the pharmacodynamic (PD) response of individuals with early symptoms of scleroderma to 4 different dose levels of anti-CCL2/LOXL2 antibody by assaying gene expression in sequential skin biopsies and (3) to determine the clinical response of individuals with early symptoms of scleroderma to 4 different dose levels of anti-CCL2/LOXL2 antibody as measured by the Modified Rodnan Skin Score (mRSS). A detailed protocol synopsis of this clinical trial is shown in Table 4.

TABLE 4: Human Clinical Trial 2

Phase	Phase 1/2
# of Trials	1
Patient Population	Early (<2 yrs since non- Raynaud's Phenomenon (RP) symptom onset) diffuse SSc mRSS ≥ 15
Trial Design and Endpoints	Multiple Dose Escalation

	Double-blind placebo-controlled Treatment duration: 6 months 4 Dose levels Primary: Safety Secondary: PK PD response (sequential skin biopsy gene expression – baseline, 4 wks, 6 months) Clinical response (mRSS)
# of Subjects	4 dose groups n = 10 each (8 active / 2 placebo) 40 subjects total
Trial Length (FPI to LPV)	1.5 years
Comments	Up to 8 sites to recruit within 1 yr

[0216] A primary objective of Human Clinical Trial 3 includes determining the efficacy of a single dose level of anti-CCL2/LOXL2 antibody administered in individuals with early symptoms of scleroderma as measured by the Modified Rodnan Skin Score (mRSS). Secondary objectives include (1) determining the efficacy of a single dose level of anti-CCL2/LOXL2 antibody administered in individuals with early symptoms of scleroderma as measured by the Health Assessment Questionnaire – Disability Index (HAQ - DI) and (2) determining the efficacy of a single dose level of anti-CCL2/LOXL2 antibody administered in individuals with early symptoms of scleroderma as measured by organ specific assessments. A detailed protocol synopsis of this clinical trial is shown in Table 5.

TABLE 5: Human Clinical Trial 3

Phase	Phase 2
# of Trials	1
Patient Population	Early (<2 yrs since non- Raynaud's Phenomenon (RP) symptom onset) diffuse SSc mRSS ≥ 15
Trial Design and Endpoints	1 dose level Double-blind Placebo Controlled Parallel Group Treatment duration 6 months Open-label extension Primary: mRSS Secondary: HAQ DI, organ-specific assessments
# of Subjects	2:1 randomization 120 subjects total
Trial Length (FPI to LPV)	1.5 years
Comments	Up to 20 sites to recruit within 1 yr

[0217] A primary objective of Human Clinical Trial 4 includes determining the efficacy relative to oral cyclophosphamide of a single dose level of anti-CCL2/LOXL2 antibody administered in individuals with limited or diffuse scleroderma with lung disease as measured by Forced Vital Capacity (FVC). Secondary objectives include (1) determining the efficacy relative to oral cyclophosphamide of a single dose level of anti-CCL2/LOXL2 antibody administered in individuals with limited or diffuse scleroderma with lung disease as measured by the HAQ - DI, (2) determining the efficacy relative to oral cyclophosphamide of a single dose level of anti-CCL2/LOXL2 antibody administered in individuals with limited or diffuse scleroderma with lung disease as measured by the mRSS, and (3) determining the efficacy relative to oral cyclophosphamide of a single dose level of anti-CCL2/LOXL2 antibody administered in individuals with limited or diffuse scleroderma with lung disease as measured by diffusing capacity of the lung for carbon monoxide (DLCO). A detailed protocol synopsis of this clinical trial is shown in Table 6.

TABLE 6: Human Clinical Trial 4

Phase	Phase 2
# of Trials	1
Patient Population	Limited or Diffuse SSc with lung disease: Active alveolitis by HRCT <7 yrs since non-RP symptom onset FVC <85%>45% predicted
Trial Design and Endpoints	1 dose level Double-blind Controlled Parallel Group Comparator: SoC (oral cyclophosphamide) Treatment duration 12 months Open-label extension Primary: FVC Secondary: DLCO, HAQ DI, mRSS
# of Subjects	2:1 randomization 120 subjects total
Trial Length (FPI to LPV)	1.5 years
Comments	Up to 10 sites to recruit within 6 months

[0218] Objective of Human Clinical Trial 5 include (1) determining the efficacy relative to oral cyclophosphamide of a single dose level of anti-CCL2/LOXL2 antibody

administered in individuals with early symptoms of scleroderma and/or limited or diffuse scleroderma with lung disease as measured by Forced Vital Capacity (FVC), (2) determining the efficacy relative to oral cyclophosphamide of a single dose level of anti-CCL2/LOXL2 antibody administered in individuals with early symptoms of scleroderma and/or limited or diffuse scleroderma with lung disease as measured by the HAQ - DI, (3) determining the efficacy relative to oral cyclophosphamide of a single dose level of anti-CCL2/LOXL2 antibody administered in individuals with early symptoms of scleroderma and/or limited or diffuse scleroderma with lung disease as measured by mRSS, and (4) determining the efficacy relative to oral cyclophosphamide of a single dose level of anti-CCL2/LOXL2 antibody administered in individuals with early symptoms of scleroderma and/or limited or diffuse scleroderma with lung disease as measured by DLCO. A detailed protocol synopsis of this clinical trial is shown in Table 7.

TABLE 7: Human Clinical Trial 5

Phase	Phase 3
# of Trials	1 each
Trial Design and Endpoints	Single dose level, double-blind head-to- head comparison with SoC in either or both early dSSc or SSc Lung Disease, depending on outcome of Phase 2s Endpoints as in Phase 2
# of Subjects	120 patients each
Trial Length (FPI to LPV)	2.0 years 0.5 to 1 year enrollment period
Comments	Treatment duration 12 months

[0219] Patients exhibiting early symptoms of scleroderma treated with anti-CCL2/LOXL2 antibody are expected to demonstrate significant improvement of symptoms as measured by the mRSS and HAQ - DI. Patients with limited or diffuse scleroderma with lung disease treated with anti-CCL2/LOXL2 antibody are expected to demonstrate significant improvement of symptoms as measured by the mRSS , HAQ - DI, and FVC. Anti-CCL2/LOXL2 antibody is expected to be more effective than cyclophosphamide in treatment of patients either with early symptoms of scleroderma or with limited or diffuse scleroderma with lung disease as measured by mRSS , HAQ - DI, and/or FVC.

Example 6: *In vivo* efficacy of anti-CCL2 and anti-LOXL2 Combination Therapy in Bleomycin-induced Fibrosis

[0220] This example describes the effect of treatment of inflammation and fibrosis with a combination of monospecific anti-CCL2 and anti-LOXL2 antibodies in an animal model of scleroderma over a two-week time course. The evaluation of monotherapy using either an anti-CCL2 or an anti-LOXL2 antibody and combination therapy (both anti-CCL2 and anti-LOXL2 antibodies) in a murine model of fibrosis was performed. A chronic bleomycin 14-day mini-osmotic subcutaneous pump was used with skin and lung fibrosis as outcomes for drug efficacy. As shown below, combination therapy with both antibodies demonstrated a significant effect in both skin and lung fibrosis.

[0221] Briefly, a bleomycin 14-day pump murine SSc-skin and lung fibrosis model was used to test the efficacy of the drugs. Groups (n=5; 8-10 weeks) of female C57BL/6 mice were exposed subcutaneously to bleomycin (90 U/Kg) or PBS (n=3 mice) via osmotic pump for 7 days with skin and lungs harvested on day 14. Bleomycin exposed mice were treated intraperitoneally with anti-CCL2 (dose 2 mg/Kg/2x/week), anti-LOXL2 (dose 15 mg/Kg/2x/week) or IgG-control (dose 17 mg/Kg/2x/week) twice a week starting on the day of the pump insertion until day 14. Treatment groups are set forth in Table 8.

TABLE 8

Treatment Group	Description
PBS	mini-osmotic pumps with PBS only
IgG	mini-osmotic pumps with bleomycin, IgG/IP/2x/week
anti-CCL2	mini-osmotic pumps with bleomycin, anti-CCL2/IP/2x/week
anti-LOXL2	mini-osmotic pumps with bleomycin, anti-LOXL2/IP/2x/week
Both	mini-osmotic pumps with bleomycin, anti-CCL2 and anti-LOXL2/IP/2x/week

Outcomes measures for cutaneous fibrosis

[0222] To determine drug efficacy in dermal fibrosis, skin was analyzed by hematoxylin-eosin (H&E) and collagen deposition by Masson's trichrome staining. In addition, the presence of ulcers was assessed clinically and the skin thickness was

measured by the maximal distance between the epidermal-dermal junction and the dermal-subcutaneous fat junction in four different skin sections from each mouse using Olympus DP70 camera and OLYMPUS® Micro Suite Basic software. Tissue sections were analyzed by a blinded investigator.

Outcome measures for lung fibrosis

[0223] To determine drug efficacy in lung fibrosis, histology and gene expression were analyzed. Insufflated lungs were fixed in formalin, embedded in paraffin, and stained with H&E, Masson's trichrome staining, and Arginase-1. The Aschroft score (Ashcroft *et al.*, 1988, *J. Clin. Pathol.* 41:467-470) was blindly analyzed in all groups after Masson's staining. Arginase-1 staining was also blindly scored from zero to four in at least four sections per slides. The average of Aschroft and Arginase-1 scores in each mouse was used as the final score.

Statistical analysis

[0224] Comparison of gene expressions, histological analysis of specific staining and lung score was analyzed by One-Way ANOVA and Bonferroni's multiple comparison post-tests. Two-group comparisons was analyzed by Student T-test. Microarray analysis followed standard false discovery rate (FDR) of less than 10%, comparing treated samples with controls. P-values less than or equal to 0.05 were considered statistically significant.

Results

Cutaneous fibrosis

[0225] The surgical procedure to implant the mini-osmotic pumps was overall well tolerated in mice. One mouse in the PBS treatment group died after 18 hours, which was likely attributed to anesthesia. FIG. 6 shows the percentage of skin ulcers observed in each treatment group. FIG. 7 shows the thickness of skin tissue samples measured for each treatment group.

[0226] As shown in FIG. 6, none of the mice treated with PBS developed skin ulcers. In contrast, all the mice exposed to bleomycin and treated with IgG (control) or anti-CCL2 developed skin ulcers, while only two mice exposed to bleomycin and treated

with anti-LOXL2 developed skin ulcers. In the combination treatment group, none of the mice developed skin ulcers.

[0227] As shown in FIG. 7, skin thickness was strongly suppressed in both mono- and combination therapy (anti-CCL2, anti-LOX2, Both) groups as compared to control (IgG). The average fold-change for each group is set forth in Table 9.

TABLE 9

Treatment group	Average fold-change skin thickness
PBS	1.07
IgG	1.64
anti-CCL2	1.30
anti-LOXL2	1.34
Both	1.21 ($p<0.001$)

Lung fibrosis

[0228] Lung tissue samples were scored using the Ashcroft method (as described above; FIG. 8). The Aschroft score was blindly analyzed in the lungs of all five treatment groups (Table 8). The average of the Aschroft score for each treatment group is set forth in Table 10. Using the ANOVA analysis for all treatment groups, only the combination treatment group (Both) was statistically reduced as compared to control (IgG; $p<0.01$). A Student T-test comparing control (IgG) and anti-CCL2 groups was not significant, whereas control as compared to anti-LOXL2 treatment group showed a trend ($p=0.07$). Control versus combination treatment group was reduced ($p< 0.01$).

TABLE 10

Treatment group	Average Aschroft Score
PBS	1.5
IgG	5.76
anti-CCL2	4.87
anti-LOXL2	3.38
Both	2.64 ($p<0.01$)

Arginase-1 lung staining

[0229] Staining for Arginase 1 (Arg1), a specific marker for macrophage activation, was also performed on lung tissue samples for each treatment group (FIG. 9). The inventors have previously observed that the bleomycin chronic model deomstrates a strong activation of macrophages in the lungs based on CD163 staining, which is almost abolished in CCL2-deficient mice. Therefore, since the peak of cell influx into the lungs is known to happen on day-14 of the bleomycin model, analysis of $CD163^+$ expression in lung cells from all bleomycin-exposed groups after treatments was performed. Strong expression of $CD163^+$ was confirmed in the lungs after exposure to bleomycin.

[0230] Arginase-1 expression was blindly quantified in at least four sections in the lungs of each mouse. The average Arginase-1 score for each treatment group is set forth in Table 11. Arginase-1 staining was strongly correlated with Aschroft score (FIG. 10). FIG. 11 shows histological sections of lung tissue samples stained with Trichrome. FIGs. 12 and 13 shows histological sections of lung tissue samples stained with Arginase 1 (Arg1).

TABLE 10

Treatment group	Average Arg1 Score
PBS	0
IgG	2.82
anti-CCL2	3.32
anti-LOXL2	3.05
Both	2.01 (p=0.06)

[0231] As shown in this example, all mice in the IgG and anti-CCL2 treatment groups contained skin ulcers. However, only 2 of 5 mice in the anti-LOXL2 treatment group contained skin ulcers. Interestingly, the treatment group that received both anti-CCL2 and anti-LOXL2 antibodies did not show any ulcers in the harvested skin tissue (see FIG. 6). Analysis of variance (ANOVA) confirmed that the reduction in skin thickness for both mono-treatment (either anti-CCL2 or anti-LOXL2 alone) and combination treatment (anti-CCL2 and anti-LOXL2 together) was significant as compared to the IgG treatment group (see FIG. 7). Therefore, treatment of fibrotic disease by combination therapy with anti-CCL2 and anti-LOXL2 antibodies was effective to stop the formation of skin ulcers, and effective to reduce the thickening of skin tissue, in mice treated with bleomycin.

[0232] Further, ANOVA also confirmed that combination therapy with anti-CCL2 and anti-LOXL2 antibodies significantly reduced the degree of fibrosis in lung tissue samples (Aschroft score of about 2) as compared to the IgG treatment group (Aschroft score of about 6) (see FIG. 8). Combination therapy with anti-CCL2 and anti-LOXL2 antibodies demonstrated the lowest level of macrophage activation in lungs of all four treatment groups (Arg1-staining, see FIG. 9).

[0233] Taken together, these data demonstrate that treatment with anti-CCL2, anti-LOXL2 alone or in combination in the bleomycin 14-day murine model showed reduction of cutaneous and/or lung fibrosis with the strongest effect in the skin and in the lungs observed when treating with an anti-LOXL2 antibody or a combination of anti-CCL2 and anti-LOXL2 antibodies. Thus, anti-CCL2 and anti-LOXL2 antibodies can be administered in combination to effectively treat and/or ameliorate one or more symptoms of a fibrotic or related inflammatory disease (e.g., scleroderma), disorder or condition.

Equivalents and Scope

[0234] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. The scope of the present invention is not intended to be limited to the above Description, but rather is as set forth in the appended claims.

[0235] In the claims articles such as "a", "an" and "the" may mean one or more than one unless indicated to the contrary or otherwise evident from the context. Thus, for example, reference to "an antibody" includes a plurality of such antibodies, and reference to "the cell" includes reference to one or more cells known to those skilled in the art, and so forth. Claims or descriptions that include "or" between one or more members of a group are considered satisfied if one, more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process unless indicated to the contrary or otherwise evident from the context. The invention includes embodiments in which exactly one member of the group is present in, employed in, or otherwise relevant to a given product or process. The invention includes embodiments in which more than one, or all of the group members are presenting, employed in, or otherwise relevant to a given product or process. Furthermore, it is to be understood that the invention encompasses all variations, combinations, and permutations in which one or more limitation, elements, clauses, descriptive terms, etc., from one or more of the listed

claims is introduced into another claim. For example, any claim that is dependent on another claim can be modified to include one or more limitations found in any other claim that is dependent on the same base claim. Furthermore, where the claims recite a composition, it is to be understood that methods of using the composition for anyone of the purposes disclosed herein are included, and methods of making the composition according to any of the methods of making disclosed herein or other methods known in the art are included, unless otherwise indicated or unless it would be evident to one of ordinary skill in the art that a contradiction or inconsistency would arise.

[0236] Where elements are presented as lists, e.g., in Markush group format, it is to be understood that each subgroup of the elements is also disclosed, and any element(s) can be removed from the group. It should be understood that, in general, where the invention, or aspects of the invention, is/are referred to as comprising particular elements, features, etc., certain embodiments of the invention or aspects of the invention consist, or consist essentially of, such elements, features, etc. For purposes of simplicity those embodiments have not been specifically set forth in haec verba herein. It is noted that the term "comprising" is intended to be open and permits the inclusion of additional elements or steps.

[0237] Where ranges are given, endpoints are included. Furthermore, it is to be understood that unless otherwise indicated or otherwise evident from the context and understand of one of ordinary skill in the art, values that are expressed as ranges can assume any specific value or sub-range within the state ranges in different embodiments of the invention, to the tenth of the unit of the lower limit of the range, unless the context clearly dictates otherwise.

[0238] In addition, it is to be understood that any particular embodiment of the present invention that falls within the prior art may be explicitly excluded from any one or more of the claims. Since such embodiments are deemed to be known to one of ordinary skill in the art, they may be excluded even if the exclusion is not set forth explicitly herein. Any particular embodiment of the compositions of the invention (e.g., any HCV genotype/subtype, any HCV antibody, any epitope, any pharmaceutical composition, any method of administration, any therapeutic application, etc.) can be excluded from any one or more claims, for any reason, whether or not related to the existence of prior art.

[0239] The publications discussed above and throughout the text are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the inventors are not entitled to antedate such disclosure by virtue of prior disclosure.

Other Embodiments

[0240] Those of ordinary skill in the art will readily appreciate that the foregoing represents merely certain preferred embodiments of the invention. Various changes and modifications to the procedures and compositions described above can be made without departing from the spirit or scope of the present invention, as set forth in the following claims.

We claim:

1. A bi-specific antibody comprising a first antigen-binding site that specifically binds to LOXL2 and a second antigen-binding site that specifically binds to CCL2.

2. The bi-specific antibody of claim 1, wherein the first antigen-binding site specifically binds to LOXL2 with a binding affinity of 1pM or greater.

3. The bi-specific antibody of claim 1 or 2, wherein the second antigen-binding site specifically binds to CCL2 with a binding affinity ranging between 500nM and 1fM.

4. The bi-specific antibody of claim 1 or 2, wherein the second antigen-binding site specifically binds to CCL2 with a binding affinity greater than 500pM.

5. The bi-specific antibody of claim 4, wherein the second antigen-binding site specifically binds to CCL2 with a binding affinity greater than 1pM.

6. The bi-specific antibody of any one of the preceding claims, wherein the first antigen-binding site comprises a first full length heavy chain and a first full length light chain.

7. The bi-specific antibody of any one of claims 1-5, wherein the first antigen-binding site comprises a first Fab fragment.

8. The bi-specific antibody of any one of claims 1-5, wherein the first antigen-binding site comprises a first single-chain variable fragments (scFvs).

9. The bi-specific antibody of any one of the preceding claims, wherein the second antigen-binding site comprises a second full length heavy chain and a second full length light chain.

10. The bi-specific antibody of any one of claims 1-8, wherein the second antigen-binding site comprises a second Fab fragment.

11. The bi-specific antibody of any one of claims 1-8, wherein the second antigen-binding site comprises a second single-chain variable fragments (scFvs).

12. The bi-specific antibody of any one of the preceding claims, wherein the first and second antigen-binding sites are linked by a peptide linker.

13. The bi-specific antibody of claim 12, wherein the peptide linker is ≥ 5 amino acid long.

14. The bi-specific antibody of any one of the preceding claims, wherein the first and second antigen binding sites are configured such that they form a single polypeptide chain.

15. The bi-specific antibody of any one of the preceding claims, wherein the first and second antigen-binding sites are associated via chemical cross-linking.

16. The bi-specific antibody of any one of the preceding claims, wherein the bi-specific antibody comprises an Fc region.

17. The bi-specific antibody of any one of the preceding claims, wherein the bi-specific antibody is humanized.

18. A pharmaceutical composition comprising a bi-specific antibody of any one of claims 1-17 and a pharmaceutically acceptable carrier.

19. A method of treating scleroderma comprising administering to an individual who is suffering from or susceptible to scleroderma a bi-specific antibody of any one of claims 1-17.

20. The method of claim 19, wherein the bi-specific antibody is administered at a therapeutically effective dose and an administration interval such that at least one symptom or feature of scleroderma on a target tissue is reduced in intensity, severity, or frequency, or has delayed onset.

21. The method of claim 20, wherein the at least one pathological feature of scleroderma is ameliorated, including but not limited to, endothelial-cell damage, proliferation of basal-lamina layers, perivascular mononuclear-cell infiltration, fibrosis, derangement of visceral-organ architecture, rarefaction of blood vessels, hypoxia, and combination thereof.

22. The method of claim 20 or 21, wherein the target tissue is selected from the group consisting of skin, blood vessels, lung, heart, kidney, gastrointestinal tract (including liver), musculoskeletal system and combinations thereof.

23. The method of any one of claims 20-22, wherein the target tissue is lung.

24. The method of any one of claims 20-22, wherein the target tissue is heart.

25. The method of any one of claims 19-24, wherein the individual is suffering from or susceptible to limited cutaneous scleroderma.

26. The method of any one of claims 19-24, wherein the individual is suffering from or susceptible to diffuse cutaneous scleroderma.

27. The method of any one of claims 19-26, wherein the bi-specific antibody is administered parenterally.

28. The method of claim 27, wherein the parenteral administration is selected from intravenous, intradermal, inhalation, transdermal (topical), subcutaneous, and/or transmucosal administration.

29. The method of claim 28, wherein the parenteral administration is intravenous administration.

30. The method of any one of claims 19-26, wherein the bi-specific antibody is administered orally.

31. The method of any one of claims 19-30, wherein the bi-specific antibody is administered bimonthly, monthly, triweekly, biweekly, weekly, daily, or at variable intervals.

32. The method of any one of claims 19-31, wherein the bi-specific antibody is co-administered with one or more anti-fibrotic agents.

33. The method of any one of claims 19-32, wherein the bi-specific antibody is co-administered with one or more anti-inflammatory agents.

34. A method of treating a fibrotic disease, disorder or condition comprising administering to an individual who is suffering from or susceptible to a fibrotic disease, disorder or condition a bi-specific antibody of any one of claims 1-17.

35. The method of claim 34, wherein the fibrotic disease, disorder or condition is selected from the group consisting of skin fibrosis, kidney fibrosis, liver fibrosis, lung fibrosis, heart fibrosis, muscle fibrosis, and combination thereof.

36. A method of treating an inflammatory disease, disorder or condition comprising administering to an individual who is suffering from or susceptible to an inflammatory disease, disorder or condition a bi-specific antibody of any one of claims 1-17.

37. The method of claim 36, wherein the inflammatory disease, disorder or condition is selected from a group consisting of psoriasis, rheumatoid arthritis, atherosclerosis, epilepsy, Alzheimer's disease, obesity, lupus nephritis, general kidney inflammation,

multiple sclerosis, Crohn's disease, asthma, discoid lupus erythematosus, inflammatory bowel disease, or systemic lupus erythematosus.

38. A method of treating scleroderma comprising administering to an individual who is suffering from or susceptible to scleroderma

an anti-CCL2 antibody, or fragment thereof, and

an anti-LOXL2 antibody, or fragment thereof.

39. The method of claim 38, wherein the anti-CCL2 antibody, or fragment thereof, and the anti-LOXL2 antibody, or fragment thereof, are administered simultaneously.

40. The method of claim 38, wherein the anti-CCL2 antibody, or fragment thereof, and the anti-LOXL2 antibody, or fragment thereof, are administered sequentially.

41. The method of any one of claims 38-40, wherein the anti-CCL2 antibody, or fragment thereof, has a binding affinity of 1nM or greater.

42. The method of any one of claims 38-40, wherein the anti-CCL2 antibody, or fragment thereof, has a binding affinity of 1pM or greater.

43. The method of any one of claims 38-42, wherein the anti-LOXL2 antibody, or fragment thereof, has a binding affinity of 1pM or greater.

44. The method of any one of claims 38-43, wherein the anti-CCL2 antibody, or fragment thereof, is selected from the group consisting of intact IgG, F(ab')2, F(ab)2, Fab', Fab, ScFvs, diabodies, triabodies and tetrabodies.

45. The method of any one of claims 38-44, wherein the anti-LOXL2 antibody, or fragment thereof, is selected from the group consisting of intact IgG, F(ab')2, F(ab)2, Fab', Fab, ScFvs, diabodies, triabodies and tetrabodies.

46. The method of any one of claims 38-45, wherein one or both of the anti-CCL2 antibody, or fragment thereof, and the anti-LOXL2 antibody, or fragment thereof, are humanized.

47. The method of any one of claims 38-46, wherein the anti-CCL2 antibody, or fragment thereof, and the anti-LOXL2 antibody, or fragment thereof, are administered via same administration route.

48. The method of any one of claims 38-46, wherein the anti-CCL2 antibody, or fragment thereof, and the anti-LOXL2 antibody, or fragment thereof, are administered via different administration route.

49. The method of any one of claims 38-48, wherein the anti-CCL2 antibody, or fragment, is administered intravenously, intradermally, by inhalation, transdermally (topically), subcutaneously, transmucosally, and/or orally.

50. The method of any one of claims 38-49, wherein the anti-CCL2 antibody, or fragment thereof, is administered bimonthly, monthly, triweekly, biweekly, weekly, daily, or at variable intervals.

51. The method of any one of claims 38-50, wherein the anti-LOXL2 antibody, or fragment, is administered intravenously, intradermally, by inhalation, transdermally (topically), subcutaneously, transmucosally, and/or orally.

52. The method of any one of claims 38-51, wherein the anti-LOXL2 antibody, or fragment thereof, is administered bimonthly, monthly, triweekly, biweekly, weekly, daily, or at variable intervals.

53. A method of treating a fibrotic disease, disorder or condition comprising administering to an individual who is suffering from or susceptible to a fibrotic disease, disorder or condition

an anti-CCL2 antibody, or fragment thereof, and

an anti-LOXL2 antibody, or fragment thereof.

54. A method of treating an inflammatory disease, disorder or condition comprising administering to an individual who is suffering from or susceptible to an inflammatory disease, disorder or condition

an anti-CCL2 antibody, or fragment thereof, and

an anti-LOXL2 antibody, or fragment thereof.

55. A kit comprising

an anti-CCL2 antibody, or fragment thereof, and

an anti-LOXL2 antibody, or fragment thereof.

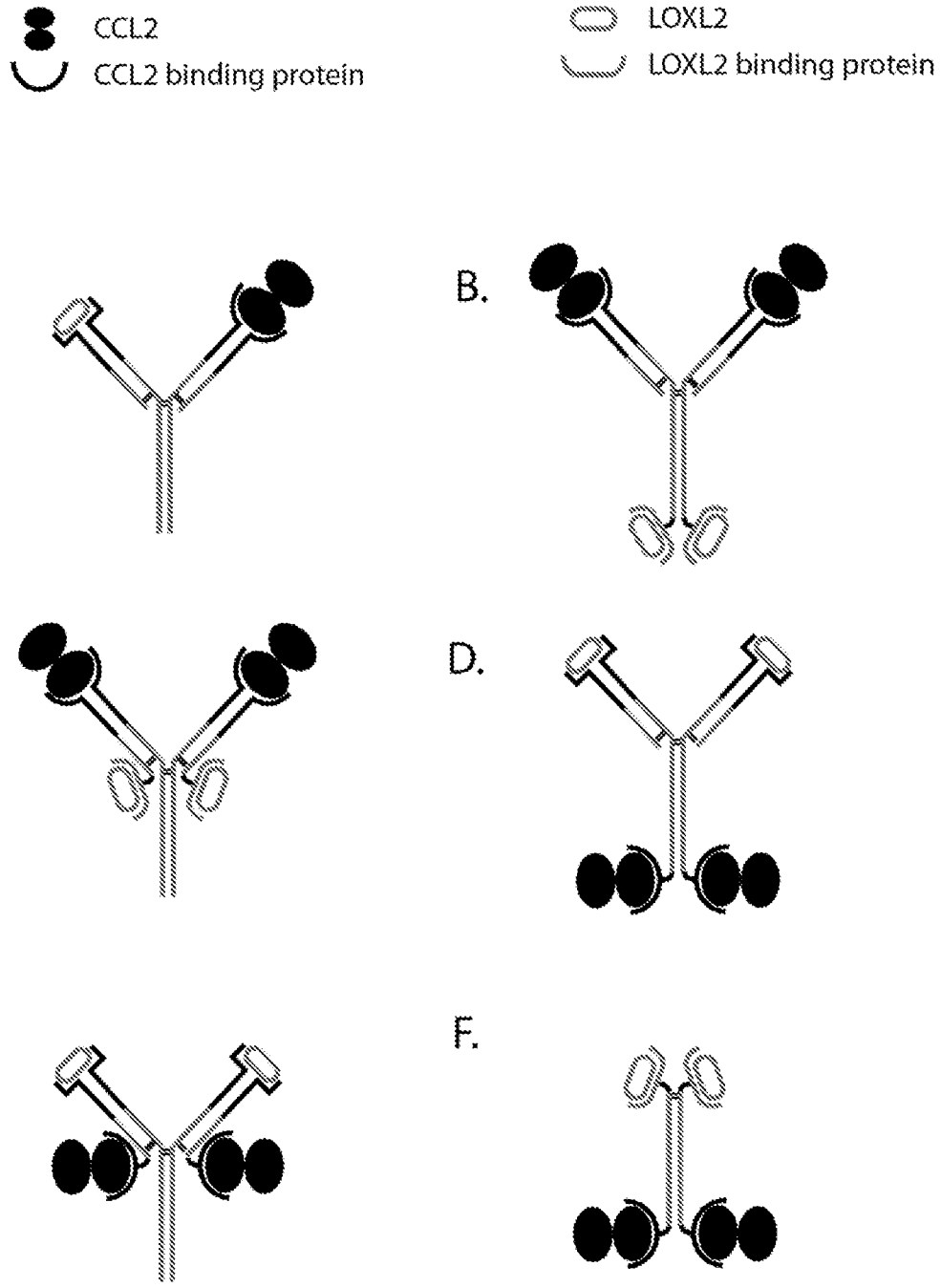
CCL2
CCL2 binding protein
LOXL2
LOXL2 binding protein
A.
B.
C.
D.
E.
F.

FIG. 1

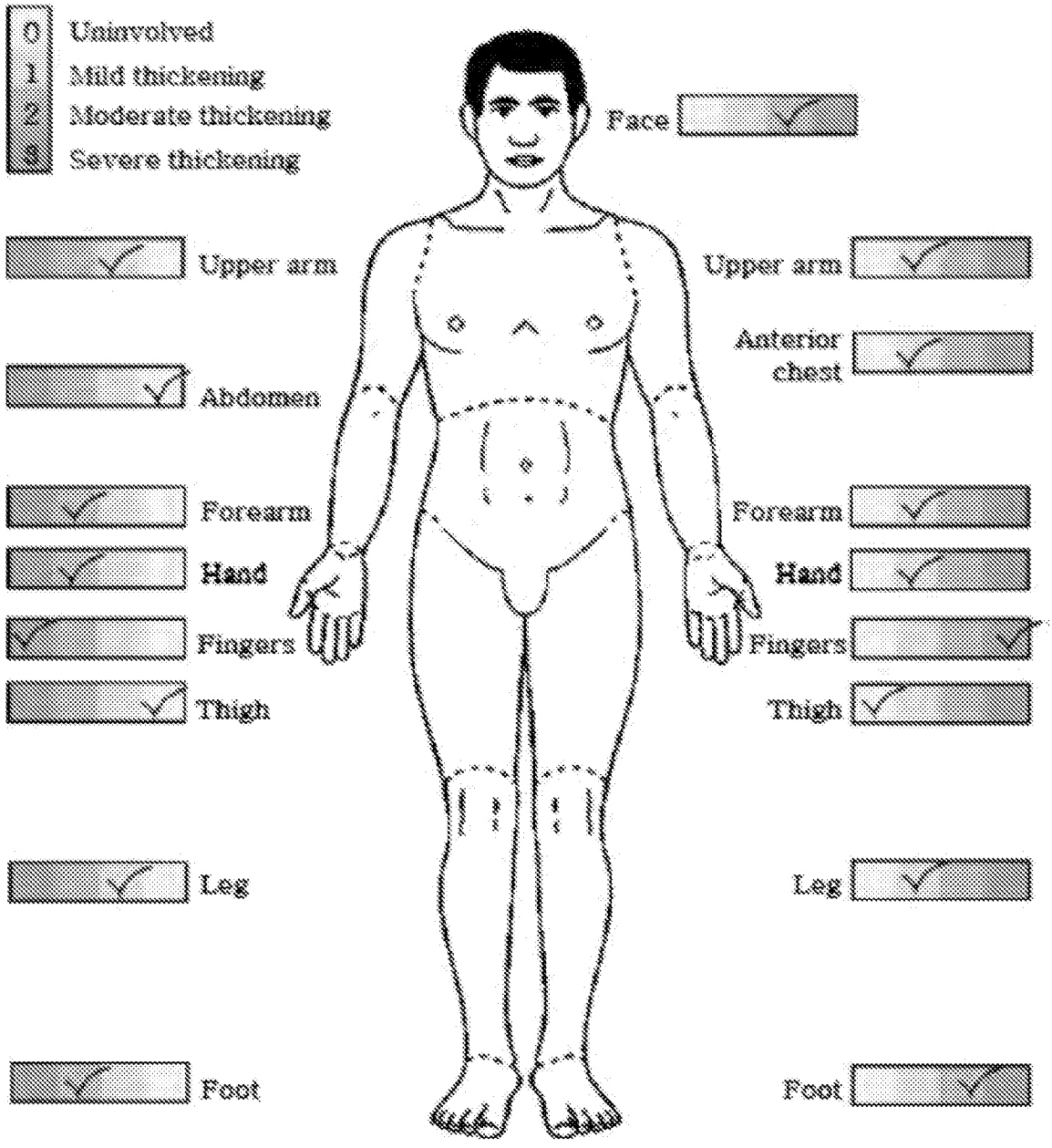
0 Uninvolved
1 Mild thickening
2 Moderate thickening
3 Severe thickening
Face
Upper arm
Upper arm
Anterior chest
Abdomen
Forearm
Forearm
Hand
Hand
Fingers
Fingers
Thigh
Thigh
Leg
Leg
Foot
Foot

FIG. 2

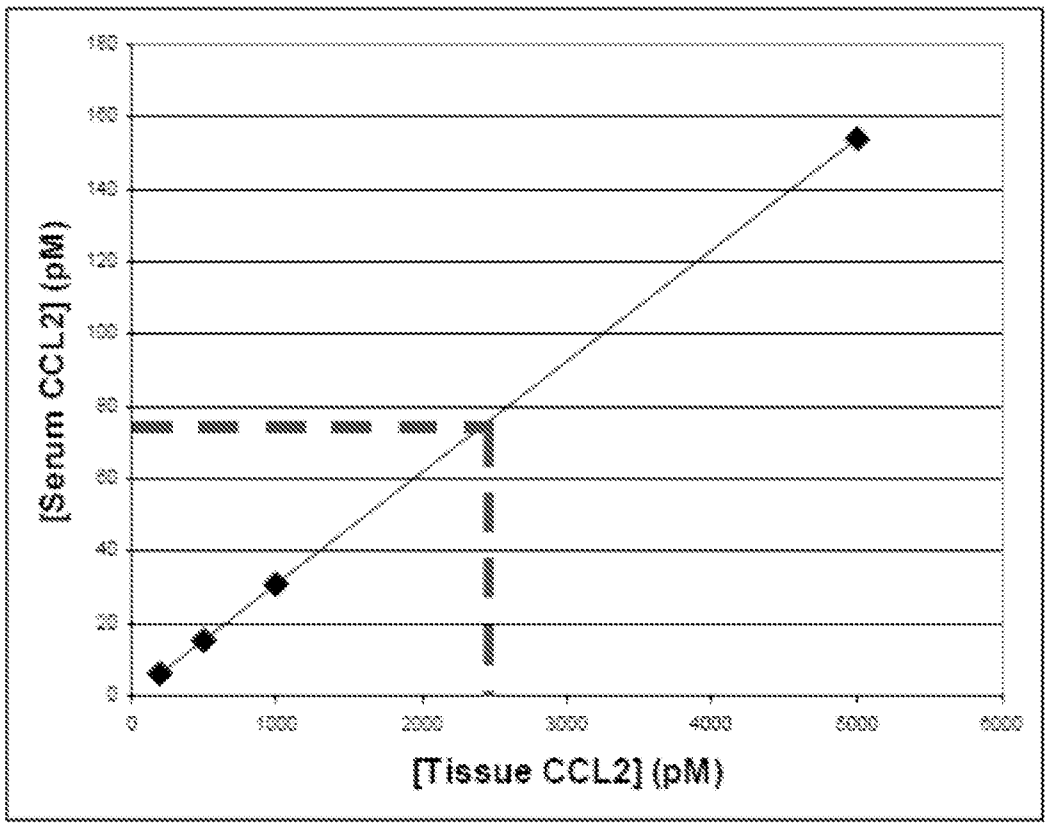

FIG. 3

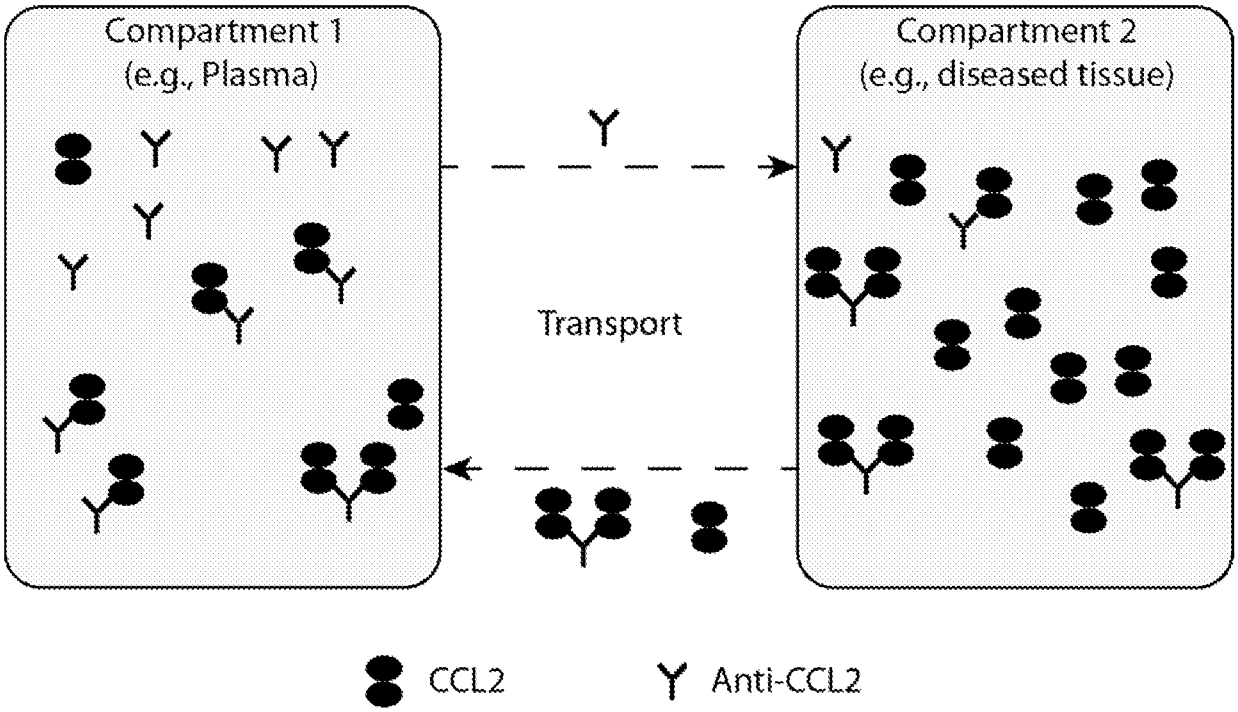
Compartment 1
(e.g., Plasma)
Compartment 2
(e.g., diseased tissue)
Transport
CCL2
Anti-CCL2

FIG. 4

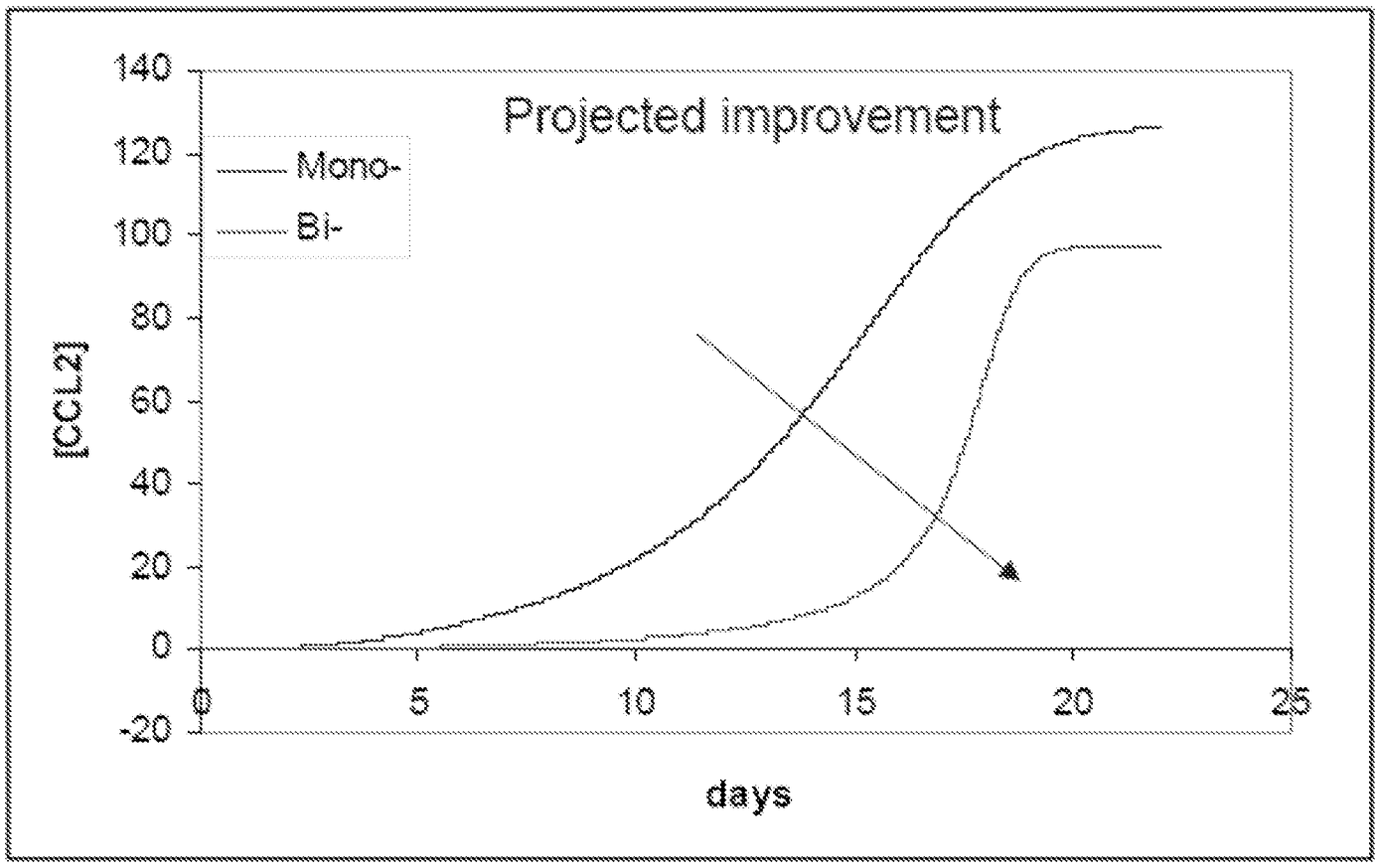
Projected improvement
Mono-
Bi-
[CCL2]
140
120
100
80
60
40
20
0
-20
0
5
10
15
20
25
days

FIG. 5

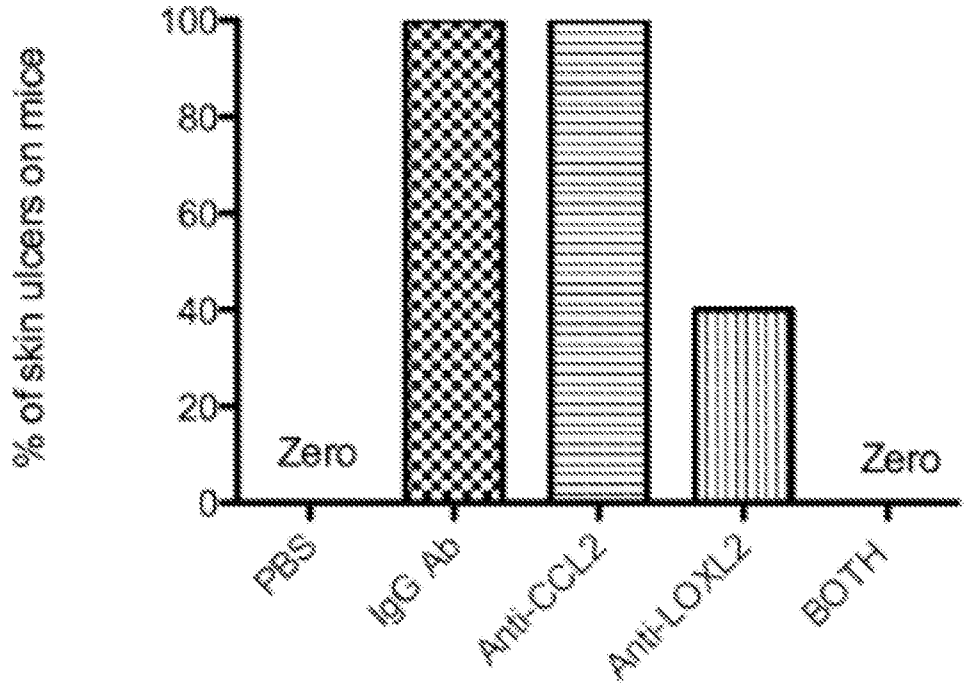
% of skin ulcers on mice
100
80
60
40
20
0
Zero
Zero
PBS
IgG Ab
Anti-CCL2
Anti-LOXL2
BOTH

FIG. 6

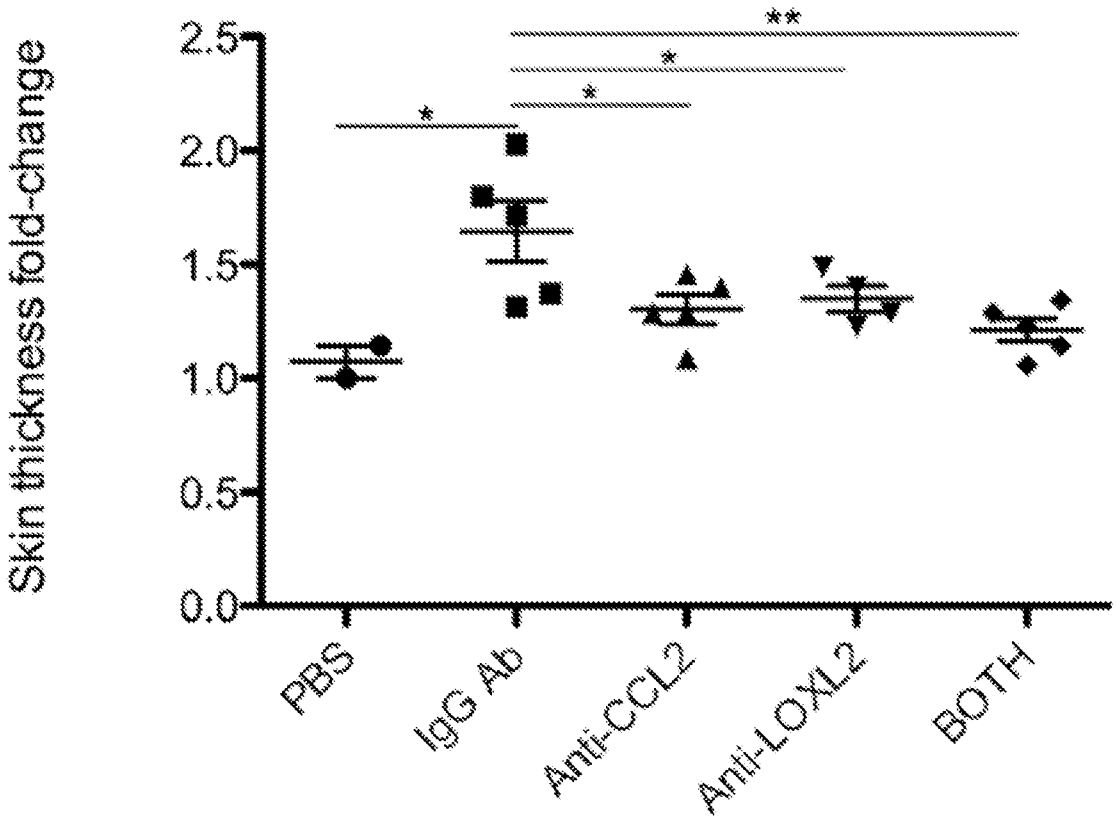
Skin thickness fold-change
2.5
2.0
1.5
1.0
0.5
0.0
*
*
*
**
PBS
IgG Ab
Anti-CCL2
Anti-LOXL2
BOTH

FIG. 7

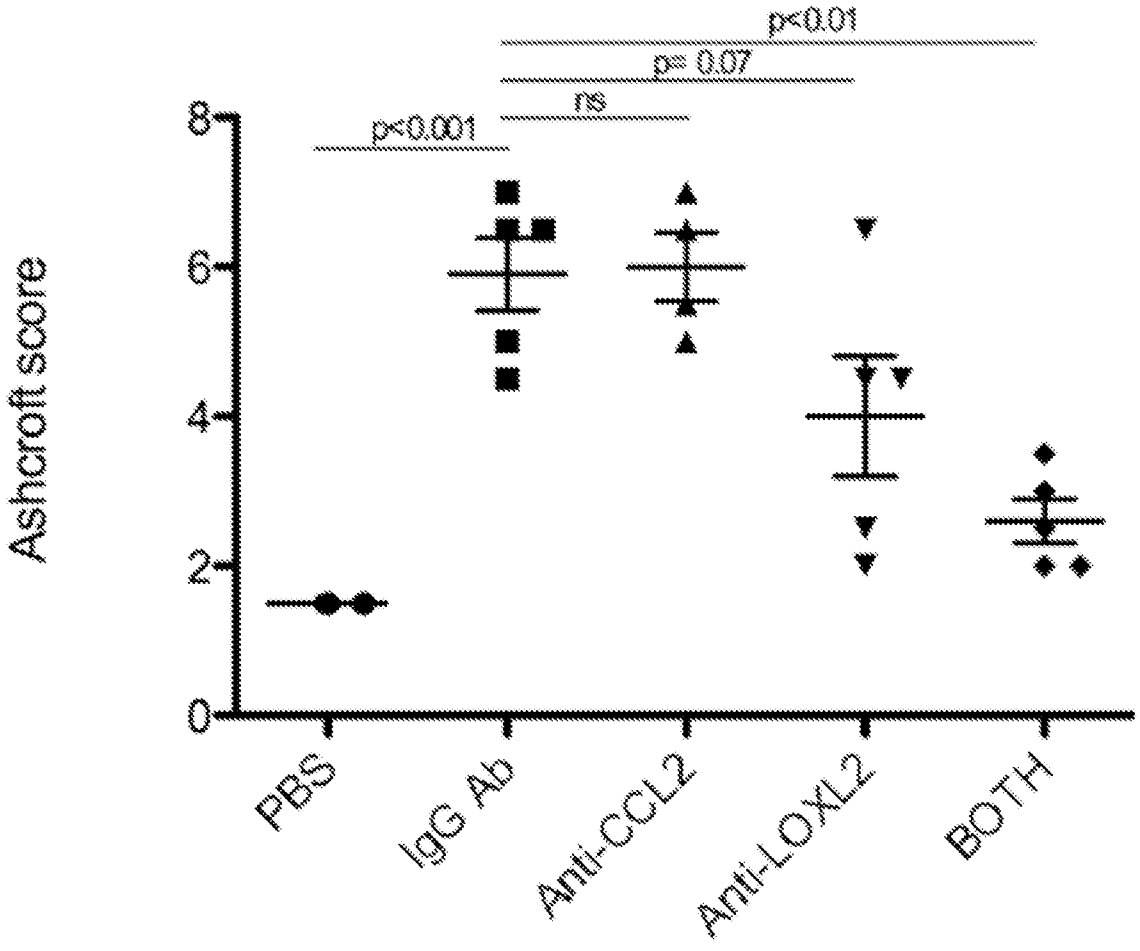
p<0.01
p= 0.07
ns
p<0.001
Ashcroft score
8
6
4
2
0
PBS
IgG Ab
Anti-CCL2
Anti-LOXL2
BOTH

FIG. 8

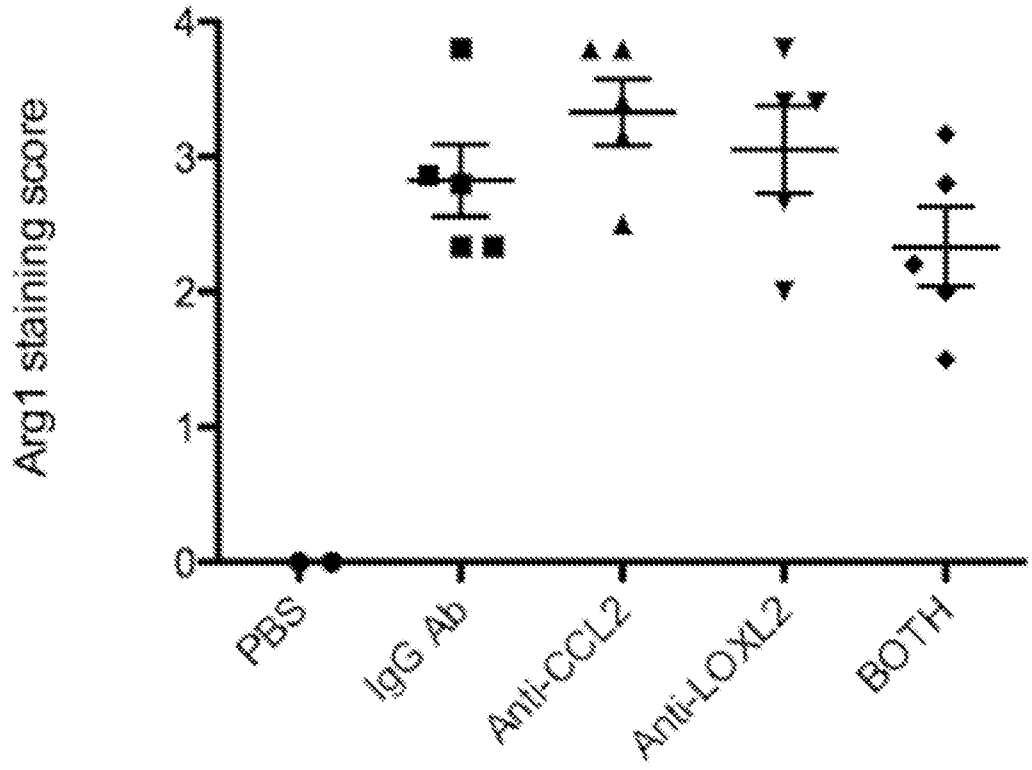
Arg1 staining score
0
1
2
3
4
PBS
IgG Ab
Anti-CCL2
Anti-LOXL2
BOTH

FIG. 9

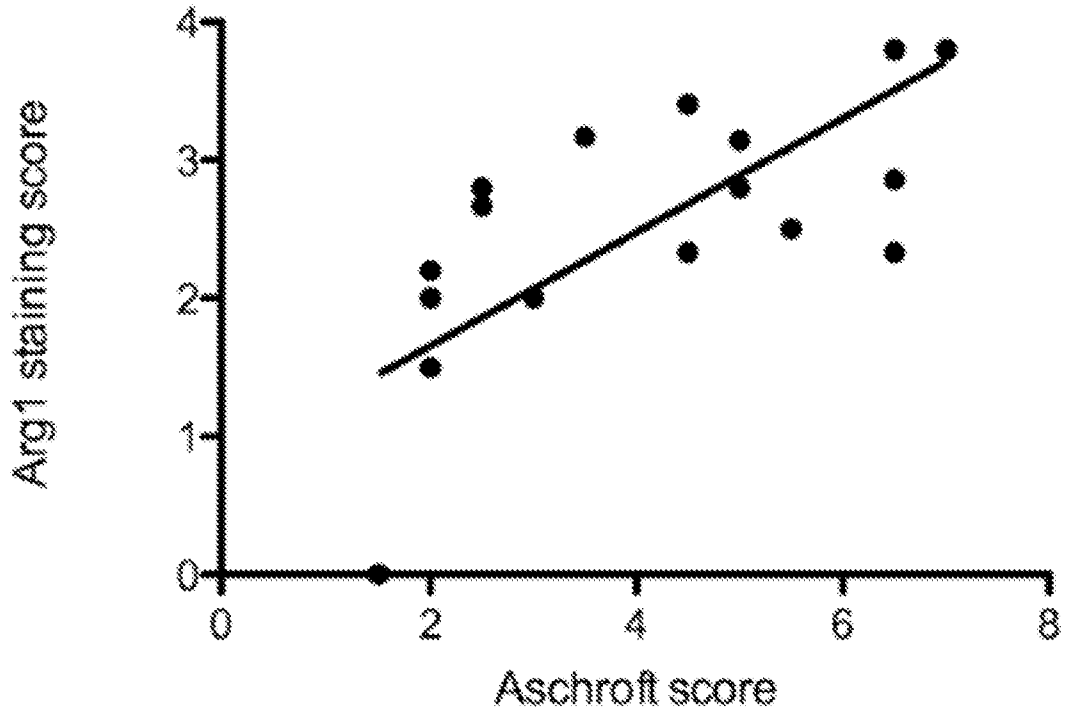

FIG. 10

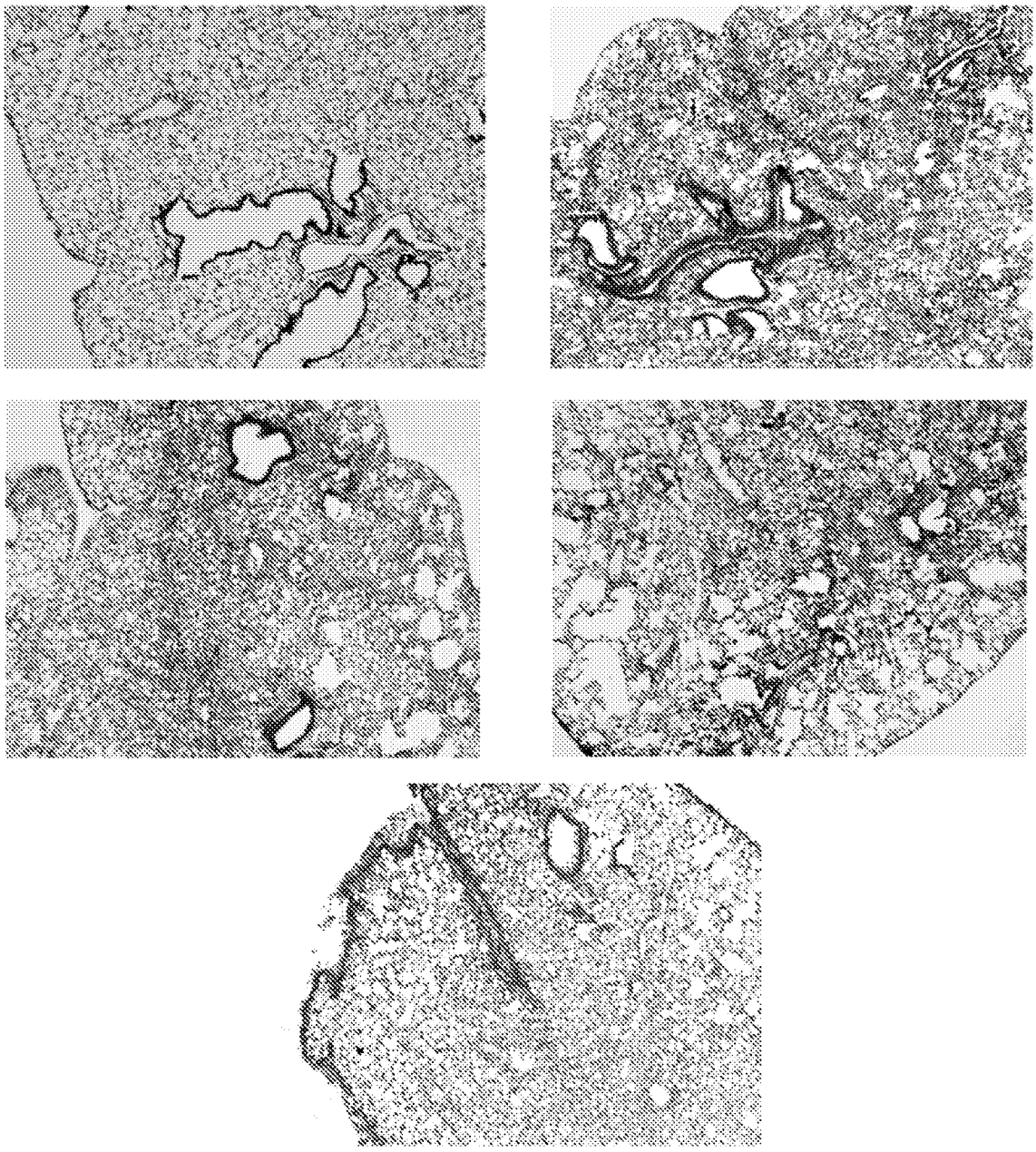

FIG. 11

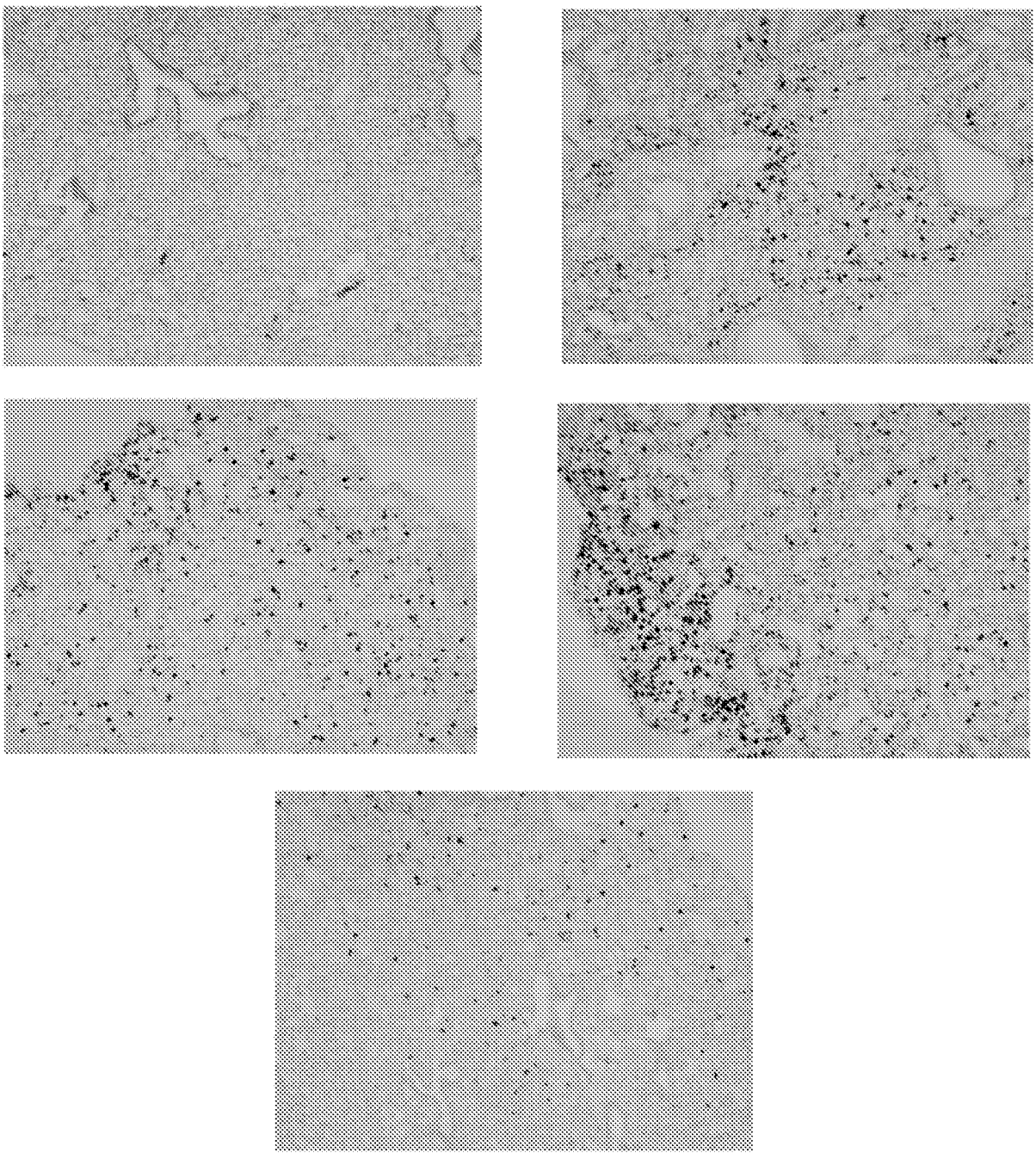

FIG. 12

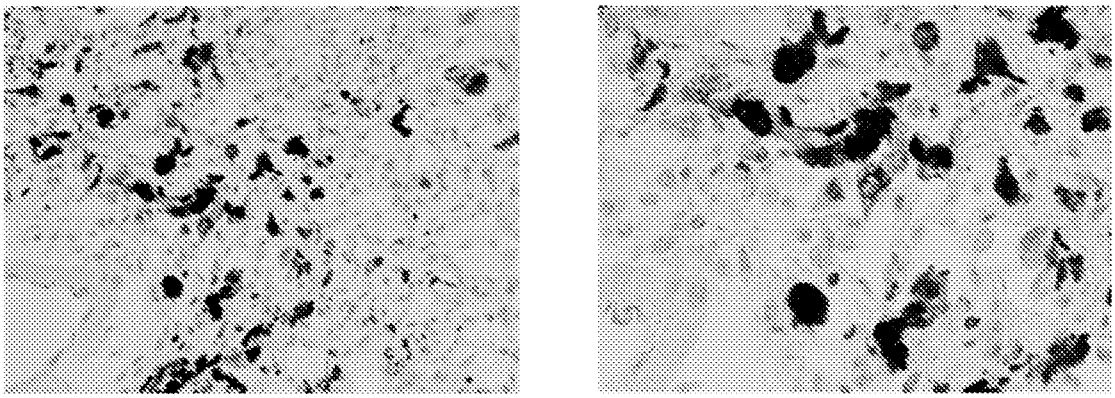

FIG. 13

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/039437

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/US2014/039437

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K39/00 C07K16/24 C07K16/40 C07K16/46
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)
A61K C07K

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, WPI Data, EMBASE, FSTA, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	VIVIAN BARRY-HAMILTON ET AL: "Allosteric inhibition of lysyl oxidase-like-2 impedes the development of a pathologic microenvironment", NATURE MEDICINE, vol. 16, no. 9, 1 September 2010 (2010-09-01), pages 1009-1017, XP055019972, ISSN: 1078-8956, DOI: 10.1038/nm.2208 The whole document, see e.g. the abstract ----- -/--	1-18,55

[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
10 September 2014	25/09/2014

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	Valcárcel, Rafael

2

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	LOBERG ROBERT D ET AL: "Targeting CCL2 with systemic delivery of neutralizing antibodies induces prostate cancer tumor regression in vivo", CANCER RESEARCH, AMERICAN ASSOCIATION FOR CANCER RESEARCH, US, vol. 67, no. 19, 1 October 2007 (2007-10-01), pages 9417-9424, XP007910910, ISSN: 0008-5472, DOI: 10.1158/0008-5472.CAN-07-1286 The whole document, see e.g. the abstract	1-18,55
Y	WO 2009/035791 A1 (ARRESTO BIOSCIENCES [US]; SMITH VICTORIA [US]; OGG SCOTT [US]; VAN VLA) 19 March 2009 (2009-03-19) For the use of anti-LOXL2 antibodies in the treatment of scleroderma see e.g. paragraph [00444]; for the use of the same antibodies in the treatment of other fibrotic disesses see e.g. paragraph [00443], for the use of the same antibodies in ther treatment of inflammatory diseases see paragraphs [004449] to [00452]; combination threpay is contemplated e.g. on paragraph [00453].	1-55
Y	YAMAMOTO T ET AL: "Role of cytokines in scleroderma: Use of animal models", CLINICAL AND APPLIED IMMUNOLOGY REVIEWS, ELSEVIER, AMSTERDAM, NL, vol. 6, no. 1, 1 January 2006 (2006-01-01) , pages 1-19, XP024969626, ISSN: 1529-1049, DOI: 10.1016/J.CAIR.2006.04.001 [retrieved on 2006-01-01] See in particular the section bridging pages 11 and 12	1-55
Y	MATTHEW B. GREENBLATT ET AL: "Interspecies Comparison of Human and Murine Scleroderma Reveals IL-13 and CCL2 as Disease Subset-Specific Targets", THE AMERICAN JOURNAL OF PATHOLOGY, vol. 180, no. 3, 1 March 2012 (2012-03-01) , pages 1080-1094, XP055138329, ISSN: 0002-9440, DOI: 10.1016/j.ajpath.2011.11.024 See e.g. the abstract	1-55

-/--

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2014/039437

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	SAHIN HACER ET AL: "Chemokines in tissue fibrosis", BIOCHIMICA ET BIOPHYSICA ACTA. MOLECULAR BASIS OF DISEASE, AMSTERDAM, NL, vol. 1832, no. 7, 15 November 2012 (2012-11-15), pages 1041-1048, XP028589912, ISSN: 0925-4439, DOI: 10.1016/J.BBADIS.2012.11.004 See e.g. page 1045, right column, last paragraph -----	1-55
Y	ZU-YAU LIN ET AL: "Cancer-associated fibroblasts up-regulate CCL2, CCL26, IL6 and LOXL2 genes related to promotion of cancer progression in hepatocellular carcinoma cells", BIOMEDICINE & PHARMACOTHERAPY, vol. 66, no. 7, 1 October 2012 (2012-10-01), pages 525-529, XP055138519, ISSN: 0753-3322, DOI: 10.1016/j.biopha.2012.02.001 See e.g. the abstract -----	1-18,55
Y,P	WO 2013/177264 A1 (SHIRE HUMAN GENETIC THERAPIES [US]) 28 November 2013 (2013-11-28) the whole document -----	1-55

2

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2014/039437

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2009035791 A1	19-03-2009	AU 2008282739 A1	05-02-2009
		AU 2008299784 A1	19-03-2009
		AU 2014204536 A1	07-08-2014
		CA 2693208 A1	05-02-2009
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		CN 101835490 A	15-09-2010
		CN 101842114 A	22-09-2010
		DK 2182981 T3	02-04-2013
		EP 2182981 A2	12-05-2010
		EP 2185198 A1	19-05-2010
		EP 2537529 A1	26-12-2012
		EP 2543389 A2	09-01-2013
		ES 2402334 T3	30-04-2013
		HK 1143736 A1	05-07-2013
		HR P20130287 T1	31-05-2013
		JP 5312459 B2	09-10-2013
		JP 2010535205 A	18-11-2010
		JP 2010535219 A	18-11-2010
		JP 2013231035 A	14-11-2013
		PT 2182981 E	18-04-2013
		US 2009053224 A1	26-02-2009
		US 2009104201 A1	23-04-2009
		US 2013095101 A1	18-04-2013
		US 2013324705 A1	05-12-2013
		US 2014079707 A1	20-03-2014
		US 2014128284 A1	08-05-2014
		WO 2009017833 A2	05-02-2009
		WO 2009035791 A1	19-03-2009
WO 2013177264 A1	28-11-2013	NONE	

(19)中华人民共和国国家知识产权局

(12)发明专利申请

(10)申请公布号 CN 105358175 A
(43)申请公布日 2016.02.24

(21)申请号 201480036627.0

(22)申请日 2014.05.23

(30)优先权数据
61/826,692 2013.05.23 US

(85)PCT国际申请进入国家阶段日
2015.12.25

(86)PCT国际申请的申请数据
PCT/US2014/039437 2014.05.23

(87)PCT国际申请的公布数据
WO2014/190316 EN 2014.11.27

(71)申请人 夏尔人类遗传性治疗公司
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(72)发明人 P·G·V·马丁尼 M·纳塔拉詹
P·A·J·哈斯利特 A·B·西摩

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代理人 邬玥 葛强

(51)Int.Cl.
A61K 39/00(2006.01)
C07K 16/24(2006.01)
C07K 16/40(2006.01)
C07K 16/46(2006.01)

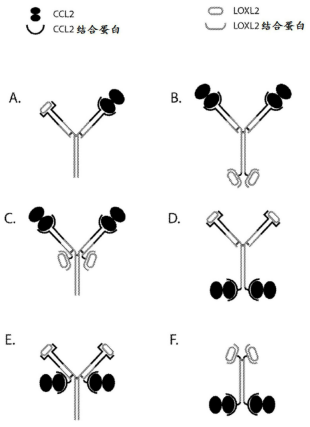
权利要求书3页 说明书38页
序列表5页 附图11页

(54)发明名称

用于治疗硬皮病的抗CCL2和抗LOXL2组合疗法

(57)摘要

本发明尤其提供了结合CCL2和LOXL2两者的双特异性分子,包括但不限于抗体、fynomer、适配体、融合蛋白和蛋白结合结构域;及其用途,具体来说,用于治疗硬皮病和相关纤维化和/或炎性疾病、病症和病状的用途。在一些实施方案中,本发明还提供了基于单特异性抗CCL2和抗LOXL2分子的组合的用于治疗硬皮病和相关纤维化和/或炎性疾病、病症和病状的方法和组合物。



CN 105358175 A

1.一种双特异性抗体，其包含与LOXL2特异性结合的第一抗原结合位点和与CCL2特异性结合的第二抗原结合位点。

2.根据权利要求1所述的双特异性抗体，其中所述第一抗原结合位点以1pM或更高的结合亲和力与LOXL2特异性结合。

3.根据权利要求1或2所述的双特异性抗体，其中所述第二抗原结合位点以范围介于500nM和1fM之间的结合亲和力与CCL2特异性结合。

4.根据权利要求1或2所述的双特异性抗体，其中所述第二抗原结合位点以高于500pM的结合亲和力与CCL2特异性结合。

5.根据权利要求4所述的双特异性抗体，其中所述第二抗原结合位点以高于1pM的结合亲和力与CCL2特异性结合。

6.根据前述权利要求中任一项所述的双特异性抗体，其中所述第一抗原结合位点包含第一全长重链和第一全长轻链。

7.根据权利要求1-5中任一项所述的双特异性抗体，其中所述第一抗原结合位点包含第一Fab片段。

8.根据权利要求1-5中任一项所述的双特异性抗体，其中所述第一抗原结合位点包含第一单链可变区片段(scFv)。

9.根据前述权利要求中任一项所述的双特异性抗体，其中所述第二抗原结合位点包含第二全长重链和第二全长轻链。

10.根据权利要求1-8中任一项所述的双特异性抗体，其中所述第二抗原结合位点包含第二Fab片段。

11.根据权利要求1-8中任一项所述的双特异性抗体，其中所述第二抗原结合位点包含第二单链可变区片段(scFv)。

12.根据前述权利要求中任一项所述的双特异性抗体，其中所述第一和第二抗原结合位点通过肽接头连接。

13.根据权利要求12所述的双特异性抗体，其中所述肽接头为≥5个氨基酸长。

14.根据前述权利要求中任一项所述的双特异性抗体，其中所述第一和第二抗原结合位点被构造以使其形成单一多肽链。

15.根据前述权利要求中任一项所述的双特异性抗体，其中所述第一和第二抗原结合位点经由化学交联缔合。

16.根据前述权利要求中任一项所述的双特异性抗体，其中所述双特异性抗体包含Fc区。

17.根据前述权利要求中任一项所述的双特异性抗体，其中所述双特异性抗体经人源化。

18.一种药物组合物，其包含根据权利要求1-17中任一项所述的双特异性抗体和药学上可接受的载体。

19.一种治疗硬皮病的方法，其包括向患有或易感硬皮病的个体施用根据权利要求1-17中任一项所述的双特异性抗体。

20.根据权利要求19所述的方法，其中按治疗有效剂量和施用间隔施用所述双特异性抗体，以使目标组织上硬皮病的至少一种症状或特征在强度、严重程度或频率上降低，或已

延迟发作。

21. 根据权利要求 20 所述的方法，其中硬皮病的所述至少一种病理学特征得以改善，包括但不限于，内皮细胞损伤、基底层增殖、血管周单核细胞浸润、纤维化、内脏器官结构错乱、血管稀疏、缺氧及其组合。

22. 根据权利要求 20 或 21 所述的方法，其中所述目标组织选自皮肤、血管、肺部、心脏、肾脏、胃肠道（包括肝脏）、肌肉骨骼系统及其组合。

23. 根据权利要求 20-22 中任一项所述的方法，其中所述目标组织为肺部。

24. 根据权利要求 20-22 中任一项所述的方法，其中所述目标组织为心脏。

25. 根据权利要求 19-24 中任一项所述的方法，其中所述个体患有或易感局限型皮肤硬皮病。

26. 根据权利要求 19-24 中任一项所述的方法，其中所述个体患有或易感弥漫型皮肤硬皮病。

27. 根据权利要求 19-26 中任一项所述的方法，其中所述双特异性抗体经肠胃外施用。

28. 根据权利要求 27 所述的方法，其中所述肠胃外施用选自静脉内、皮内、吸入、经皮（局部）、皮下和 / 或经粘膜施用。

29. 根据权利要求 28 所述的方法，其中所述肠胃外施用为静脉内施用。

30. 根据权利要求 19-26 中任一项所述的方法，其中所述双特异性抗体经口服施用。

31. 根据权利要求 19-30 中任一项所述的方法，其中所述双特异性抗体每两个月、每月、每三周、每两周、每周、每日或以可变间隔施用一次。

32. 根据权利要求 19-31 中任一项所述的方法，其中所述双特异性抗体与一种或多种抗纤维化剂共同施用。

33. 根据权利要求 19-32 中任一项所述的方法，其中所述双特异性抗体与一种或多种抗炎剂共同施用。

34. 一种治疗纤维化疾病、病症或病状的方法，其包括

向患有或易感纤维化疾病、病症或病状的个体施用根据权利要求 1-17 中任一项所述的双特异性抗体。

35. 根据权利要求 34 所述的方法，其中所述纤维化疾病、病症或病状选自皮肤纤维化、肾脏纤维化、肝脏纤维化、肺部纤维化、心脏纤维化、肌肉纤维化及其组合。

36. 一种治疗炎性疾病、病症或病状的方法，其包括

向患有或易感炎性疾病、病症或病状的个体施用根据权利要求 1-17 中任一项所述的双特异性抗体。

37. 根据权利要求 36 所述的方法，其中所述炎性疾病、病症或病状选自银屑病、类风湿性关节炎、动脉粥样硬化、癫痫、阿尔茨海默病、肥胖、狼疮性肾炎、一般性肾脏炎症、多发性硬化、克罗恩氏病、哮喘、盘状红斑狼疮、炎性肠病或全身性红斑狼疮。

38. 一种治疗硬皮病的方法，其包括向患有或易感硬皮病的个体施用

抗 CCL2 抗体或其片段，和

抗 LOXL2 抗体或其片段。

39. 根据权利要求 38 所述的方法，其中所述抗 CCL2 抗体或其片段和所述抗 LOXL2 抗体或其片段同时施用。

40.根据权利要求38所述的方法，其中所述抗CCL2抗体或其片段和所述抗LOXL2抗体或其片段依次施用。

41.根据权利要求38-40中任一项所述的方法，其中所述抗CCL2抗体或其片段具有1nM或更高的结合亲和力。

42.根据权利要求38-40中任一项所述的方法，其中所述抗CCL2抗体或其片段具有1pM或更高的结合亲和力。

43.根据权利要求38-42中任一项所述的方法，其中所述抗LOXL2抗体或其片段具有1pM或更高的结合亲和力。

44.根据权利要求38-43中任一项所述的方法，其中所述抗CCL2抗体或其片段选自完整IgG、F(ab')2、F(ab)2、Fab'、Fab、ScFv、双链抗体、三链抗体和四链抗体。

45.根据权利要求38-44中任一项所述的方法，其中所述抗LOXL2抗体或其片段选自完整IgG、F(ab')2、F(ab)2、Fab'、Fab、ScFv、双链抗体、三链抗体和四链抗体。

46.根据权利要求38-45中任一项所述的方法，其中所述抗CCL2抗体或其片段和所述抗LOXL2抗体或其片段中的一种或两种经人源化。

47.根据权利要求38-46中任一项所述的方法，其中所述抗CCL2抗体或其片段和所述抗LOXL2抗体或其片段经由相同施用途径施用。

48.根据权利要求38-46中任一项所述的方法，其中所述抗CCL2抗体或其片段和所述抗LOXL2抗体或其片段经由不同施用途径施用。

49.根据权利要求38-48中任一项所述的方法，其中所述抗CCL2抗体或片段经静脉内、皮内、通过吸入、经皮（局部）、皮下、经粘膜和/或口服施用。

50.根据权利要求38-49中任一项所述的方法，其中所述抗CCL2抗体或其片段每两个月、每月、每三周、每两周、每周、每日或以可变间隔施用一次。

51.根据权利要求38-50中任一项所述的方法，其中所述抗LOXL2抗体或片段经静脉内、皮内、通过吸入、经皮（局部）、皮下、经粘膜和/或口服施用。

52.根据权利要求38-51中任一项所述的方法，其中所述抗LOXL2抗体或其片段每两个月、每月、每三周、每两周、每周、每日或以可变间隔施用一次。

53.一种治疗纤维化疾病、病症或病状的方法，其包括向患有或易感纤维化疾病、病症或病状的个体施用

抗CCL2抗体或其片段，和

抗LOXL2抗体或其片段。

54.一种治疗炎性疾病、病症或病状的方法，其包括向患有或易感炎性疾病、病症或病状的个体施用

抗CCL2抗体或其片段，和

抗LOXL2抗体或其片段。

55.一种试剂盒，其包含

抗CCL2抗体或其片段，和

抗LOXL2抗体或其片段。

用于治疗硬皮病的抗 CCL2 和抗 LOXL2 组合疗法

[0001] 相关申请的交叉引用

[0002] 本申请根据美国法典第 35 篇第 119 条 (e) 款要求 2013 年 5 月 23 日提交的美国临时专利申请序列号 61/826,692 的权益，该申请特此通过引用整体并入。

[0003] 序列表

[0004] 本说明书引用了 2014 年 5 月 23 日作为命名为“2006685-0569_ST25”的 ASCII.txt 文件以电子表格提交的序列表。该 .txt 文件在 2014 年 5 月 12 日生成并且大小为 7KB。

[0005] 发明背景

[0006] 系统性硬化（硬皮病）是结缔组织的临床异质性病症，导致皮肤变硬和变紧。系统性硬化是特征在于免疫活化、血管损伤和纤维化的自身免疫型疾病。累及肺部、心脏、肾脏和胃肠道的主要基于器官的并发症可促成死亡率和发病率。发病机理未知。

[0007] 最常与硬皮病相关的特征是纤维化—皮肤和器官中的胶原蛋白积累。胶原蛋白积累促成病症的症状，包括脱发、皮肤变硬和变紧、皮肤色素减退、关节疼痛、手指和关节僵硬、消化道问题和呼吸并发症（干咳、呼吸短促、喘息）。硬皮病可分为两个主要亚类：局限型皮肤硬皮病和弥漫型皮肤硬皮病。在局限型皮肤硬皮病中，纤维化主要限于手、臂和面部。弥漫型皮肤硬皮病是影响大面积皮肤并且危及一个或多个内部器官的急进性病症。有局限型皮肤硬皮病的患者具有比有弥漫型皮肤硬皮病的患者相对更好的长期预后。分布广泛的全身性硬皮病可损伤心脏、肾脏、肺部或胃肠道，这可导致死亡。在有硬皮病的患者中肺纤维化是最常见的死因。

[0008] 因此，硬皮病是具有潜在致命影响的极端致衰弱性疾病。在美国存在约 50,000 名患者。女性患者与男性患者的比率为约 4:1。当前的治疗方法仅仅基于对症治疗和对病程中出现的并发症的管理（例如，皮质类固醇、NSAID 和免疫抑制药，如氨甲蝶呤 (Metotrexate) 和环磷酰胺 (Cytoxan)）。没有治疗显示逆转或中断疾病的进展。因此，存在高度未满足的对硬皮病有效治疗的医疗需要。

[0009] 发明概述

[0010] 本发明提供了（除了其它以外）尤其基于可与赖氨酰氧化酶样 -2（“LOXL2”）和 C-C 趋化因子配体 -2（“CCL2”）特异性结合的双特异性结合分子，包括但不限于抗体、fynomer、适配体、融合蛋白、蛋白结合结构域（例如，源自受体的蛋白结合结构域），用于有效治疗硬皮病的改进的方法和组合物，和 / 或基于与 LOXL2 和 CCL2 特异性结合的此类分子的组合疗法。已知 CCL2 是硬皮病的验证靶标。几项研究已经表明硬皮病成纤维细胞展示出 CCL2mRNA 和蛋白质的组成型表达增加。在硬皮病皮肤切片中，在成纤维细胞、角化细胞和单核细胞中检测到 CCL2 表达，而在正常皮肤中未能检测到 (Galindo 等，Arthritis Rheum.2001 年 6 月 ;44(6):1382-6 ;Distler 等，Arthritis Rheum.2001 年 11 月 ;44(11):2665-78 ;Lioyd 等，Exp Med.1997 年 4 月 7 日 ;185(7):1371-80 ;Yamamoto 等，J Dermatol Sci.2001 年 6 月 ;26(2):133-9 ;Denton 等，Trends Immunol.2005 年 11 月 ;26(11):596-602.2005 年 9 月 15 日电子出版物）。然而，在本发明之前，尚未基于抗 CCL2 抗体开发出对于硬皮病的有效治疗。本发明人观察到血浆中高水平的 CCL2 隔绝静脉注射

的抗 CCL2 抗体，导致抗 CCL2 抗体浪费并且对患病组织中 CCL2 的靶向无效。为解决这个问题，本发明人考虑到使用允许以与组织 CCL2 结合的自由抗 CCL2 臂将抗 CCL2 活性隔绝在患病组织中，这样提供对 CCL2 的组织特异性靶向的双特异性分子。因此，本发明提供了优先抑制与血浆 CCL2 相对的组织 CCL2 的方法和组合物，导致对硬皮病的高效治疗。

[0011] 因此，一方面，本发明提供了包含与 LOXL2 特异性结合的第一抗原结合位点和与 CCL2 特异性结合的第二抗原结合位点的双特异性结合分子（例如，双特异性抗体、fynomer、适配体、融合蛋白或蛋白结合结构域）。

[0012] 在一些实施方案中，第一抗原结合位点以 100nM 或更高（例如，10nM 或更高、1nM 或更高、500pM 或更高、100pM 或更高、50pM 或更高、10pM 或更高、1pM 或更高、500fM 或更高、400fM 或更高、300fM 或更高、200fM 或更高、100fM 或更高、50fM 或更高、10fM 或更高、或 1fM 或更高）的结合亲和力与 LOXL2 特异性结合。

[0013] 在一些实施方案中，第二抗原结合位点以介于约 500nM 和 1fM 之间（例如，介于 500nM 和 10fM 之间、介于 500nM 和 100fM 之间、介于 500nM 和 1pM 之间、介于 10nM 和 1fM 之间、介于 10nM 和 100fM 之间、介于 10nM 和 1pM 之间、介于 1nM 和 1fM 之间、介于 1nM 和 100fM 之间、介于 1nM 和 500fM 之间、介于 1nM 和 1pM 之间、介于 1nM 和 10pM 之间、介于 1nM 和 50pM 之间、介于 1nM 和 100pM 之间、介于 1nM 和 500pM 之间）的结合亲和力与 CCL2 特异性结合。在一些实施方案中，第二抗原结合位点以高于约 500nM（例如，高于约 500nM、100nM、10nM、1nM、500pM、100pM、50pM、10pM、1pM、500fM、400fM、300fM、200fM、100fM、50fM、10fM、1fM）的结合亲和力与 CCL2 特异性结合。

[0014] 在一些实施方案中，第一抗原结合位点包含第一全长重链和第一全长轻链。在一些实施方案中，第一抗原结合位点包含第一 Fab 片段。在一些实施方案中，第一抗原结合位点包含第一单链可变区片段 (scFv)。

[0015] 在一些实施方案中，第二抗原结合位点包含第二全长重链和第二全长轻链。在一些实施方案中，第二抗原结合位点包含第二 Fab 片段。在一些实施方案中，第二抗原结合位点包含第二单链可变区片段 (scFv)。

[0016] 在一些实施方案中，第一和第二抗原结合位点通过肽接头连接。在一些实施方案中，肽接头为≥ 5 个（例如，6 个、7 个、8 个、9 个、10 个、11 个、12 个、13 个、14 个、15 个、20 个、25 个或更多个）氨基酸长。在一些实施方案中，第一和第二抗原结合位点被构造以使其形成单一多肽链。

[0017] 在一些实施方案中，第一和第二抗原结合位点经由化学交联缔合。

[0018] 在一些实施方案中，根据本发明的双特异性结合分子为双特异性抗体。在一些实施方案中，双特异性抗体包含 Fc 区。

[0019] 在一些实施方案中，双特异性抗体为人类的。在一些实施方案中，双特异性抗体经人源化。

[0020] 另一方面，本发明提供了包含如本文所述的双特异性结合分子（例如，双特异性抗体、fynomer、适配体、融合蛋白、蛋白结合结构域）和药学上可接受的载体的药物组合物。

[0021] 再一方面，本发明提供了治疗硬皮病的方法，其包括向患有或易感硬皮病的个体施用如本文所述的双特异性结合分子（例如，双特异性抗体、fynomer、适配体、融合蛋白、

蛋白结合结构域）。在一些实施方案中，按治疗有效剂量和施用间隔施用所述双特异性抗体，以使目标组织上硬皮病的至少一种症状或特征在强度、严重程度或频率上降低，或已延迟发作。

[0022] 在一些实施方案中，硬皮病的至少一种病理学特征得以改善，包括但不限于，内皮细胞损伤、基底层增殖、血管周单核细胞浸润、纤维化、内脏器官结构错乱、血管稀疏、缺氧及其组合。

[0023] 在一些实施方案中，目标组织选自皮肤、血管、肺部、心脏、肾脏、胃肠道（包括肝脏）、肌肉骨骼系统及其组合。在一些实施方案中，目标组织为肺部。在一些实施方案中，目标组织为心脏。

[0024] 在一些实施方案中，个体患有或易感局限型皮肤硬皮病。在一些实施方案中，个体患有或易感弥漫型皮肤硬皮病。

[0025] 在一些实施方案中，双特异性抗体经肠胃外施用。在一些实施方案中，肠胃外施用选自静脉内、皮内、吸入、经皮（局部）、皮下和/或经粘膜施用。在一些实施方案中，肠胃外施用为静脉内施用。

[0026] 在一些实施方案中，双特异性抗体经口服施用。

[0027] 在一些实施方案中，双特异性抗体每两个月、每月、每三周、每两周、每周、每日或以可变间隔施用一次。

[0028] 在一些实施方案中，双特异性抗体与一种或多种抗纤维化剂或抗炎剂共同施用。

[0029] 另一方面，本发明提供了如本文所述的双特异性结合分子在用于硬皮病治疗的药剂生产中的用途，其中所述治疗包括向患有或易感硬皮病的个体施用有效量的双特异性分子，其中所述双特异性结合分子包含与LOXL2特异性结合的第一抗原结合位点和与CCL2特异性结合的第二抗原结合位点。

[0030] 在一些实施方案中，第一抗原结合位点以100nM或更高（例如，10nM或更高、1nM或更高、500pM或更高、100pM或更高、50pM或更高、10pM或更高、1pM或更高、500fM或更高、400fM或更高、300fM或更高、200fM或更高、100fM或更高、50fM或更高、10fM或更高、或1fM或更高）的结合亲和力与LOXL2特异性结合。

[0031] 在一些实施方案中，第二抗原结合位点以介于约500nM和1fM之间（例如，介于500nM和10fM之间、介于500nM和100fM之间、介于500nM和1pM之间、介于10nM和1fM之间、介于10nM和100fM之间、介于10nM和1pM之间、介于1nM和1fM之间、介于1nM和100fM之间、介于1nM和500fM之间、介于1nM和1pM之间、介于1nM和10pM之间、介于1nM和50pM之间、介于1nM和100pM之间、介于1nM和500pM之间）的结合亲和力与CCL2特异性结合。在一些实施方案中，第二抗原结合位点以高于约500nM(例如，高于约500nM、100nM、10nM、1nM、500pM、100pM、50pM、10pM、1pM、500fM、400fM、300fM、200fM、100fM、50fM、10fM、1fM)的结合亲和力与CCL2特异性结合。

[0032] 在一些实施方案中，第一抗原结合位点包含第一全长重链和第一全长轻链。在一些实施方案中，第一抗原结合位点包含第一Fab片段。在一些实施方案中，第一抗原结合位点包含第一单链可变区片段(scFv)。

[0033] 在一些实施方案中，第二抗原结合位点包含第二全长重链和第二全长轻链。在一些实施方案中，第二抗原结合位点包含第二Fab片段。在一些实施方案中，第二抗原结合位

点包含第二单链可变区片段 (scFv)。

[0034] 在一些实施方案中，第一和第二抗原结合位点通过肽接头连接。在一些实施方案中，肽接头为≥ 5 个（例如，6 个、7 个、8 个、9 个、10 个、11 个、12 个、13 个、14 个、15 个、20 个、25 个或更多个）氨基酸长。在一些实施方案中，第一和第二抗原结合位点被构造以使其形成单一多肽链。

[0035] 在一些实施方案中，第一和第二抗原结合位点经由化学交联缔合。

[0036] 在一些实施方案中，根据本发明的双特异性结合分子为双特异性抗体。在一些实施方案中，双特异性抗体包含 Fc 区。

[0037] 在一些实施方案中，双特异性抗体经人源化。

[0038] 另一方面，本发明提供了用于治疗硬皮病的方法中的如本文所述的双特异性结合分子，所述方法包括向患有或易感硬皮病的受试者施用有效量的双特异性结合分子的步骤，其中所述双特异性结合分子包含与 LOXL2 特异性结合的第一抗原结合位点和与 CCL2 特异性结合的第二抗原结合位点。

[0039] 在一些实施方案中，第一抗原结合位点以 100nM 或更高（例如，10nM 或更高、1nM 或更高、500pM 或更高、100pM 或更高、50pM 或更高、10pM 或更高、1pM 或更高、500fM 或更高、400fM 或更高、300fM 或更高、200fM 或更高、100fM 或更高、50fM 或更高、10fM 或更高、或 1fM 或更高）的结合亲和力与 LOXL2 特异性结合。

[0040] 在一些实施方案中，第二抗原结合位点以介于约 500nM 和 1fM 之间（例如，介于 500nM 和 10fM 之间、介于 500nM 和 100fM 之间、介于 500nM 和 1pM 之间、介于 10nM 和 1fM 之间、介于 10nM 和 100fM 之间、介于 10nM 和 1pM 之间、介于 1nM 和 1fM 之间、介于 1nM 和 100fM 之间、介于 1nM 和 500fM 之间、介于 1nM 和 1pM 之间、介于 1nM 和 10pM 之间、介于 1nM 和 50pM 之间、介于 1nM 和 100pM 之间、介于 1nM 和 500pM 之间）的结合亲和力与 CCL2 特异性结合。在一些实施方案中，第二抗原结合位点以高于约 500nM（例如，高于约 500nM、100nM、10nM、1nM、500pM、100pM、50pM、10pM、1pM、500fM、400fM、300fM、200fM、100fM、50fM、10fM、1fM）的结合亲和力与 CCL2 特异性结合。

[0041] 在一些实施方案中，第一抗原结合位点包含第一全长重链和第一全长轻链。在一些实施方案中，第一抗原结合位点包含第一 Fab 片段。在一些实施方案中，第一抗原结合位点包含第一单链可变区片段 (scFv)。

[0042] 在一些实施方案中，第二抗原结合位点包含第二全长重链和第二全长轻链。在一些实施方案中，第二抗原结合位点包含第二 Fab 片段。在一些实施方案中，第二抗原结合位点包含第二单链可变区片段 (scFv)。

[0043] 在一些实施方案中，第一和第二抗原结合位点通过肽接头连接。在一些实施方案中，肽接头为≥ 5 个（例如，6 个、7 个、8 个、9 个、10 个、11 个、12 个、13 个、14 个、15 个、20 个、25 个或更多个）氨基酸长。在一些实施方案中，第一和第二抗原结合位点被构造以使其形成单一多肽链。

[0044] 在一些实施方案中，第一和第二抗原结合位点经由化学交联缔合。

[0045] 在一些实施方案中，根据本发明的双特异性结合分子为双特异性抗体。在一些实施方案中，双特异性抗体包含 Fc 区。

[0046] 在一些实施方案中，双特异性抗体为人类的。在一些实施方案中，双特异性抗体经

人源化。

[0047] 再一方面，本发明提供了治疗纤维化疾病、病症或病状的方法，其包括向患有或易感纤维化疾病、病症或病状的个体施用如本文所述的双特异性结合分子（例如，双特异性抗体、fynomer、适配体、融合蛋白、蛋白结合结构域）。

[0048] 另一方面，本发明提供了如本文所述的双特异性结合分子在用于纤维化疾病、病症或病状治疗的药剂生产中的用途，其中所述治疗包括向患有或易感纤维化疾病、病症或病状的个体施用双特异性结合分子，其中所述双特异性分子包含与 LOXL2 特异性结合的第一抗原结合位点和与 CCL2 特异性结合的第二抗原结合位点。

[0049] 另一方面，本发明提供了用于治疗纤维化疾病、病症或病状的方法中的双特异性分子，所述方法包括向患有或易感纤维化疾病、病症或病状的个体施用双特异性结合分子的步骤，其中所述双特异性分子包含与 LOXL2 特异性结合的第一抗原结合位点和与 CCL2 特异性结合的第二抗原结合位点。

[0050] 在各个实施方案中，纤维化疾病、病症或病状选自皮肤纤维化、肾脏纤维化、肝脏纤维化、肺部纤维化、心脏纤维化、肌肉纤维化及其组合。

[0051] 另一方面，本发明提供了治疗炎性疾病、病症或病状的方法，所述方法包括向患有或易感炎性疾病、病症或病状的个体施用如本文所述的双特异性结合分子。

[0052] 另一方面，本发明提供了如本文所述的双特异性结合分子在用于炎性疾病、病症或病状治疗的药剂生产中的用途，其中所述治疗包括向患有或易感炎性疾病、病症或病状的个体施用双特异性结合分子，其中所述双特异性分子包含与 LOXL2 特异性结合的第一抗原结合位点和与 CCL2 特异性结合的第二抗原结合位点。

[0053] 另一方面，本发明提供了用于治疗纤维化疾病、病症或病状的方法中的双特异性分子，所述方法包括向患有或易感纤维化疾病、病症或病状的个体施用双特异性结合分子的步骤，其中所述双特异性分子包含与 LOXL2 特异性结合的第一抗原结合位点和与 CCL2 特异性结合的第二抗原结合位点。

[0054] 在各个实施方案中，炎性疾病、病症或病状选自银屑病、类风湿性关节炎、动脉粥样硬化、癫痫、阿尔茨海默病 (Alzheimer's disease)、肥胖、狼疮性肾炎、一般性肾脏炎症、多发性硬化、克罗恩氏病 (Crohn's disease)、哮喘、盘状红斑狼疮、炎性肠病或全身性红斑狼疮。

[0055] 另一方面，本发明提供了治疗硬皮病的方法，其包括向患有或易感硬皮病的个体施用抗 CCL2 抗体或其片段和抗 LOXL2 抗体或其片段。

[0056] 另一方面，本发明提供了抗 CCL2 抗体或其片段和抗 LOXL2 抗体或其片段在用于硬皮病治疗的药剂生产中的用途，其中所述治疗包括向患有或易感硬皮病的个体施用抗 CCL2 抗体或其片段和抗 LOXL2 抗体或其片段的步骤。

[0057] 另一方面，本发明提供了用于治疗硬皮病的方法中的抗 CCL2 抗体或其片段和抗 LOXL2 抗体或其片段，所述方法包括向患有或易感硬皮病的个体施用抗 CCL2 抗体或其片段和抗 LOXL2 抗体或其片段的步骤。

[0058] 在一些实施方案中，抗 CCL2 抗体或其片段和抗 LOXL2 抗体或其片段同时施用。在一些实施方案中，抗 CCL2 抗体或其片段和抗 LOXL2 抗体或其片段依次施用。

[0059] 在一些实施方案中，抗 CCL2 抗体或其片段具有 1nM 或更高（例如，500pM 或更高、

100pM或更高、50pM或更高、10pM或更高、1pM或更高、500fM或更高、400fM或更高、300fM或更高、200fM或更高、100fM或更高、50fM或更高、10fM或更高、1fM或更高）的结合亲和力。

[0060] 在一些实施方案中，抗LOXL2抗体或其片段具有1pM或更高（例如，500fM或更高、400fM或更高、300fM或更高、200fM或更高、100fM或更高、50fM或更高、10fM或更高、1fM或更高）的结合亲和力。

[0061] 在一些实施方案中，抗CCL2抗体或其片段选自完整IgG、F(ab')2、F(ab)2、Fab'、Fab、ScFv、双链抗体、三链抗体和四链抗体。

[0062] 在一些实施方案中，抗LOXL2抗体或其片段选自完整IgG、F(ab')2、F(ab)2、Fab'、Fab、ScFv、双链抗体、三链抗体和四链抗体。

[0063] 在一些实施方案中，抗CCL2抗体或其片段和抗LOXL2抗体或其片段中的一种或两种经人源化。

[0064] 在一些实施方案中，抗CCL2抗体或其片段和抗LOXL2抗体或其片段经由相同施用途径施用。在一些实施方案中，抗CCL2抗体或其片段和抗LOXL2抗体或其片段经由不同施用途径施用。

[0065] 在一些实施方案中，抗CCL2抗体或片段经静脉内、皮内、通过吸入、经皮（局部）、皮下、经粘膜和/或口服施用。

[0066] 在一些实施方案中，抗CCL2抗体或其片段每两个月、每月、每三周、每两周、每周、每日或以可变间隔施用一次。

[0067] 在一些实施方案中，抗LOXL2抗体或片段经静脉内、皮内、通过吸入、经皮（局部）、皮下、经粘膜和/或口服施用。

[0068] 在一些实施方案中，抗LOXL2抗体或其片段每两个月、每月、每三周、每两周、每周、每日或以可变间隔施用一次。

[0069] 另一方面，本发明提供了治疗纤维化疾病、病症或病状的方法，其包括向患有或易感纤维化疾病、病症或病状的个体施用抗CCL2抗体或其片段和抗LOXL2抗体或其片段。

[0070] 另一方面，本发明提供了抗CCL2抗体或其片段和抗LOXL2抗体或其片段在用于纤维化疾病、病症或病状治疗的药剂生产中的用途，其中所述治疗包括向患有或易感纤维化疾病、病症或病状的个体施用抗CCL2抗体或其片段和抗LOXL2抗体或其片段的步骤。

[0071] 另一方面，本发明提供了用于治疗纤维化疾病、病症或病状的方法中的抗CCL2抗体或其片段和抗LOXL2抗体或其片段，所述方法包括向患有或易感纤维化疾病、病症或病状的个体施用抗CCL2抗体或其片段和抗LOXL2抗体或其片段的步骤。

[0072] 另一方面，本发明提供了治疗炎性疾病、病症或病状的方法，所述方法包括向患有或易感炎性疾病、病症或病状的个体施用抗CCL2抗体或其片段和抗LOXL2抗体或其片段。

[0073] 另一方面，本发明提供了抗CCL2抗体或其片段和抗LOXL2抗体或其片段在用于治疗炎性疾病、病症或病状的药剂生产中的用途，其中所述治疗包括向患有或易感炎性疾病、病症或病状的个体施用抗CCL2抗体或其片段和抗LOXL2抗体或其片段的步骤。

[0074] 另一方面，本发明提供了用于治疗炎性疾病、病症或病状的方法中的抗CCL2抗体或其片段和抗LOXL2抗体或其片段，所述方法包括向患有或易感炎性疾病、病症或病状的个体施用抗CCL2抗体或其片段和抗LOXL2抗体或其片段的步骤。

[0075] 另一方面，本公开提供了包含抗CCL2抗体或其片段和抗LOXL2抗体或其片段的试

剂盒。

[0076] 本发明的其它特征、目的和优点在以下详述、附图和权利要求中显而易见。然而，应理解详述、附图和权利要求，在指示本发明的实施方案的同时，仅以说明，而非限制的方式给出。在本发明范围内的各种变化和修改对于本领域中的技术人员将变得显而易见。

[0077] 附图简述

[0078] 本文包括的由以下图片组成的附图仅仅是为了说明的目的，而非限制。

[0079] 图 1A-1F 说明了描绘示例性抗 CCL2 和抗 LOXL2 双特异性抗体的简图。

[0080] 图 2 说明了描绘改良的 Rodnan 皮肤得分的示例性简图。指出了身体上评估皮肤纤维化的位置。

[0081] 图 3 描绘了绘制平衡后 CCL2 血清和组织浓度的示例性图表。

[0082] 图 4 说明了描绘血浆和患病组织中 CCL2 靶向的示例性简图。

[0083] 图 5 描绘了随着用抗 CCL2（单）或抗 CCL2/LOXL2（双）处理后天数的变化绘制 CCL2 浓度的示例性图表，说明了初步双特异性建模结果。

[0084] 图 6 示出了在经 IgG、抗 CCL2 抗体、抗 LOXL2 抗体或抗 CCL2 和抗 LOXL2 抗体处理的 C57BL/6 小鼠中观察到的皮肤溃疡百分比。PBS :阴性对照。BOTH :用抗 CCL2 抗体和抗 LOXL2 抗体组合处理。

[0085] 图 7 示出了在经 IgG、抗 CCL2 抗体、抗 LOXL2 抗体或抗 CCL2 和抗 LOXL2 抗体处理的 C57BL/6 小鼠中观察到的皮肤厚度倍数变化。PBS :阴性对照。BOTH :用抗 CCL2 抗体和抗 LOXL2 抗体组合处理。

[0086] 图 8 示出了经 IgG、抗 CCL2 抗体、抗 LOXL2 抗体或抗 CCL2 和抗 LOXL2 抗体处理的 C57BL/6 小鼠的肺组织样品的 Ashcroft 得分。PBS :阴性对照。BOTH :用抗 CCL2 抗体和抗 LOXL2 抗体组合处理。

[0087] 图 9 示出了经 IgG、抗 CCL2 抗体、抗 LOXL2 抗体或抗 CCL2 和抗 LOXL2 抗体处理的 C57BL/6 小鼠的肺组织样品中的精氨酸酶 1(Arg1) 染色。PBS :阴性对照。BOTH :用抗 CCL2 抗体和抗 LOXL2 抗体组合处理。

[0088] 图 10 示出了随着经 IgG、抗 CCL2 抗体、抗 LOXL2 抗体或抗 CCL2 和抗 LOXL2 抗体处理的 C57BL/6 小鼠的肺组织样品中的 Ashcroft 得分变化，精氨酸酶 1(Arg1) 染色的相关性图。PBS :阴性对照。BOTH :用抗 CCL2 抗体和抗 LOXL2 抗体组合处理。

[0089] 图 11 示出了对于各处理组的小鼠而言在 4X 放大倍数下经三色法 (Trichrome) 染色的肺组织样品的代表性组织切片。顶行 :PBS（左）、IgG（右）。中间行 :抗 CCL2（左）、抗 LOXL2（右）。底行 :抗 CCL2 和抗 LOXL2。

[0090] 图 12 示出了对于各处理组的小鼠而言经精氨酸酶 1(Arg1) 染色的肺组织样品的代表性组织切片。顶行 :PBS（左）、IgG（右）。中间行 :抗 CCL2（左）、抗 LOXL2（右）。底行 :抗 CCL2 和抗 LOXL2。

[0091] 图 13 示出了对于 IgG 处理组而言在 20X（左）和 40X（右）放大倍数下经精氨酸酶 1(Arg1) 染色的肺组织样品的代表性组织切片。

[0092] 定义

[0093] 为了本发明更易于理解，首先定义了某些术语。说明书全篇提出了以下术语和其它术语的附加定义。

[0094] 亲和力：正如本领域中所知，“亲和力”是对特定配体与其伴侣结合（例如，非共价缔合）的紧密性和/或特定配体与其伴侣解离的速率或频率的度量。正如本领域中所知，各种技术中的任一种均可用于测定亲和力。在许多实施方案中，亲和力表示对特异性结合的度量。

[0095] 亲和力成熟（或亲和力成熟抗体）：如本文中所用，是指在其一个或多个CDR中具有一个或多个改变，引起与不具有那些改变的亲本抗体相比，抗体对抗原的亲和力提高的抗体。在一些实施方案中，亲和力成熟抗体将对靶抗原具有纳摩尔级或甚至皮摩尔级亲和力。亲和力成熟抗体可通过本领域中已知的各种程序中的任何一种生成。Marks 等 BioTechnology 10:779-783(1992) 描述了通过 V_H 和 V_L 结构域改组的亲和力成熟。由 Barbas 等 Proc Nat.Acad.Sci,USA91:3809-3813(1994)；Schier 等 Gene 169:147-155(1995)；Yelton 等 J.Immunol.155:1994-2004(1995)；Jackson 等，J.Immunol.154(7):3310-9(1995)；和 Hawkins 等，J.Mol.Biol.226:889-896(1992) 描述了CDR 和/或框架残基的随机诱变。

[0096] 抗体：如本文中所用，术语“抗体”是指由基本上由免疫球蛋白基因或免疫球蛋白基因的片段编码的一个或多个多肽组成的多肽。公认的免疫球蛋白基因包括 κ、λ、α、γ、δ、ε 和 μ 恒定区基因，以及无数免疫球蛋白可变区基因。轻链通常分为 κ 或 λ。重链通常分为 γ、μ、α、δ 或 ε，其转而分别定义了免疫球蛋白类别，IgG、IgM、IgA、IgD 和 IgE。已知典型的免疫球蛋白（抗体）结构单元包含四聚体。每个四聚体由两对相同的多肽链组成，每一对具有一条“轻”链（约 25kD) 和一条“重”链（约 50-70kD)。每条链的 N- 端限定了主要负责抗原识别的约 100 至 110 个或更多个氨基酸的可变区。术语“可变轻链”(V_L) 和“可变重链”(V_H) 分别是指这些轻链和重链。抗体可对特定抗原有特异性。抗体或其抗原可为分析物或结合伴侣。抗体作为完整免疫球蛋白或作为通过各种肽酶消化生成的很多良好表征的片段存在。因此，例如，胃蛋白酶在铰链区内的二硫键下方消化抗体以生成 F(ab)’2，本身为通过二硫键与 V_H-C_H1 连接的轻链的 Fab 的二聚体。F(ab)’2 可在温和条件下还原以破坏铰链区内的二硫键，从而将 F(ab)’2 二聚体转化为 Fab’单体。Fab’单体基本上为具有部分铰链区的Fab（对于其它抗体片段更加详细的描述，参见，Fundamental Immunology,W.E.Paul 编，Raven Press,N.Y.(1993)）。虽然根据完整抗体的消化定义了各种抗体片段，但是本领域中的普通技术人员将意识到此类 Fab’片段可经化学方法或利用重组 DNA 方法重新合成。因此，术语“抗体”，如本文中所用，还包括通过完整抗体修饰生成的或使用重组 DNA 方法重新合成的抗体片段。在一些实施方案中，抗体为单链抗体，例如其中可变重链和可变轻链连接在一起（直接或通过肽接头）以形成连续多肽的单链 Fv(scFv) 抗体。单链 Fv(“scFv”) 多肽为共价连接的 V_H::V_L 杂二聚体，其可由包括直接连接或通过肽编码接头连接的 V_H- 和 V_L- 编码序列的核酸表达。（参见，例如，Huston 等 (1988)Proc.Nat.Acad.Sci.USA,85:5879-5883，其全部内容通过引用并入本文）。存在许多结构，以将来自于抗体 V 区的天然聚集，但经化学分离的多肽轻链和重链转化为将折叠成基本上类似于抗原结合位点的结构的三维结构的 scFv 分子。参见，例如，美国专利第 5,091,513 和 5,132,405 及 4,956,778 号。

[0097] 大约：如本文中所用，术语“大约”或“约”，应用到一个或多个目标值时，是指类似于规定参考值的值。在某些实施方案中，除非另有规定或另外从上下文中显而易见（除非

此数字将超过可能值的100％），否则术语“大约”或“约”是指在任何方向上（大于或小于）落在规定参考值的25％、20％、19％、18％、17％、16％、15％、14％、13％、12％、11％、10％、9％、8％、7％、6％、5％、4％、3％、2％、1％或更小范围内的一系列值。

[0098] 结合剂：如本文中所用，术语“结合剂”包括结合抗原或靶蛋白或肽的任何天然存在、合成或经基因工程化的试剂，例如蛋白。“结合剂”也称为“结合蛋白”。结合剂可源自天然存在的抗体或经合成工程化。结合蛋白或试剂可类似于抗体通过与特异性抗原结合以形成复合物并且引起生物反应（例如，激动或拮抗特定生物活性）起作用。结合剂或蛋白可包括分离的片段、由抗体重链和轻链的可变区组成的“Fv”片段、其中轻链和重链可变区通过肽接头连接的重组单链多肽分子（“ScFv蛋白”）、和由模拟高变区的氨基酸残基组成的最小识别单元。如本文中所用的术语结合剂还可包括通过完整抗体修饰生成的或使用重组DNA方法重新合成的抗体片段。在一些实施方案中，抗体为单链抗体，例如其中可变重链和可变轻链连接在一起（直接或通过肽接头）以形成连续多肽的单链Fv(scFv)抗体。单链Fv(“scFv”)多肽为共价连接的VH::VL杂二聚体，其可由包括直接连接或通过肽编码接头连接的VH-和VL-编码序列的核酸表达。（参见，例如，Huston等(1988)Proc.Nat.Acad.Sci.USA,85:5879-5883，其全部内容通过引用并入本文）。存在许多结构，以将来自于抗体V区的天然聚集，但经化学分离的多肽轻链和重链转化为将折叠成基本上类似于抗原结合位点的结构的三维结构的scFv分子。参见，例如美国专利第5,091,513和5,132,405及4,956,778号。在一些实施方案中，如本文中所用的术语结合剂也可包括抗体。参见抗体的定义。

[0099] 双特异性：如本文中所用的术语“双特异性”是指分子具有两种不同的结合特异性。通常，双特异性结合分子含有至少两个抗原结合位点，其每一个与不同的抗原或表位特异性结合。双特异性分子可为，例如，双特异性抗体、fynomer、适配体、融合蛋白、蛋白结合结构域。如本文中所用，双特异性分子涵盖具有较高效价（即，结合两个以上抗原或表位的能力）的分子（例如，抗体、fynomer、适配体、融合蛋白、蛋白结合结构域或其它结合剂），也称为多特异性分子。

[0100] 双特异性抗体：如本文中所用的术语“双特异性抗体”是指其中至少一个并且通常是两个结合部分是或包含抗体组分或片段的双特异性结合分子。各种不同的双特异性抗体结构在本领域中已知。在一些实施方案中，双特异性抗体中是或包含抗体组分或片段的每个结合部分包括V_H和/或V_L区；在一些此类实施方案中，V_H和/或V_L区是在特定单克隆抗体中发现的V_H和/或V_L区。在其中双特异性抗体含有两个抗体组分结合部分的一些实施方案中，每个结合部分包括来自于不同单克隆抗体的V_H和/或V_L区。

[0101] 双特异性结合分子：如本文中所用的术语“双特异性结合分子”是指具有两个离散结合部分，每个部分与不同靶标结合的多肽。在一些实施方案中，双特异性结合分子为单一多肽；在一些实施方案中，双特异性结合分子是或包含在一些此类实施方案中可以，例如通过交联彼此共价缔合的多个肽。在一些实施方案中，双特异性结合分子的两个结合部分识别相同靶标（例如，抗原）的不同位点（例如，表位）；在一些实施方案中，它们识别不同靶标。在一些实施方案中，双特异性结合分子能够与不同结构的两个靶标同时结合。

[0102] CDR：如本文中所用的术语“CDR”是指抗体可变区内的互补决定区。在重链和轻链的每个可变区内存在三个CDR，对于每个可变区而言，指定为CDR1、CDR2和CDR3。“一组

CDR”或“CDR 组”是指一组三个或六个在能够结合抗原的单可变区内出现的 CDR 或能够结合抗原的同源重链和轻链可变区的 CDR。已经根据其中几个在本领域已知的系统（例如，Kabat、Chothia 等）不同地定义了 CDR 的边界。

[0103] 嵌合：如本文中所用的“嵌合”抗体，是含有包括源自一个物种的抗体，优选啮齿动物抗体的互补决定区 (CDR) 的可变结构域，而抗体分子的恒定结构域源自人抗体的恒定结构域的重组蛋白。对于兽医应用而言，嵌合抗体的恒定结构域可源自其它物种，例如猫或狗的恒定结构域。

[0104] 组合：如本文中所用的“组合”是指使用一种以上预防和 / 或治疗剂（例如，抗 CCL2 抗体和抗 LOXL2 抗体）。使用术语“组合”不限制向患病受试者施用预防和 / 或治疗剂的顺序。第一预防或治疗剂（例如，抗 CCL2 抗体）可在向患病受试者施用第二预防或治疗剂（例如，抗 LOXL2 抗体）之前（例如，5 分钟、15 分钟、30 分钟、45 分钟、1 小时、2 小时、4 小时、6 小时、12 小时、24 小时、48 小时、72 小时、96 小时、1 周、2 周、3 周、4 周、5 周、6 周、8 周或 12 周之前）、同时或之后（例如，5 分钟、15 分钟、30 分钟、45 分钟、1 小时、2 小时、4 小时、6 小时、12 小时、24 小时、48 小时、72 小时、96 小时、1 周、2 周、3 周、4 周、5 周、6 周、8 周或 12 周之后）施用。

[0105] 化合物和试剂：术语“化合物”和“试剂”在本文中可交换使用。它们是指任何天然存在或非天然存在（即，合成或重组）的分子，例如生物大分子（例如，核酸、多肽或蛋白）、有机或无机分子或由生物材料如细菌、植物、真菌或动物（特别是哺乳动物，包括人）细胞或组织制备的提取物。所述化合物可为单种分子或至少两种分子的混合物或复合物。

[0106] 相当的：如本文中所用的术语“相当的”是指描述彼此足够类似以容许比较获得的结果或观察到的现象的两组（或更多组）条件或环境。在一些实施方案中，相当的多组条件或环境特征在于多种基本上相同的特征和一种或少量不同的特征。本领域中的普通技术人员将意识到多组条件在特征在于足够数量和类型的基本上相同的特征时是彼此相当的以保证在多组不同条件或环境下获得的结果或观察到的现象的差异由那些不同的特征的变化引起或表明了那些不同的特征的变化的合理结论。

[0107] 对照：如本文中所用，术语“对照”具有其领域所理解的作为将结果与之相比较的标准的含义。通常，对照用于通过分离变量而增加实验的完整性以便得出关于此类变量的结论。在一些实施方案中，对照是与试验反应或测定同时进行以提供比较值的反应或测定。在一个实验中，应用“试验”（即，被测变量）。在第二个实验中，“对照”，不应用被测变量。在一些实施方案中，对照为历史对照（即，先前进行的试验或测定的历史对照，或先前已知的量或结果）。在一些实施方案中，对照是或包含印刷或以其它方式保存的记录。对照可为阳性对照或阴性对照。

[0108] 给药方案：正如该术语在本文中所用，“给药方案”（或“治疗方案”）是通常隔开一段时间，向受试者单独施用的一组单位剂量（通常为一个以上）。在一些实施方案中，给定治疗剂具有可包括一个或多个剂量的推荐给药方案。在一些实施方案中，给药方案包括各自彼此隔开相同长度时间段的多个剂量；在一些实施方案中，给药方案包括多个剂量和间隔单独剂量的至少两个不同时间段。

[0109] 诊断：如本文中所用，术语“诊断”是指目的在于确定个体是否受疾病或小病所折磨的过程。在本发明的上下文中，“硬皮病的诊断”是指目的在于以下一项或多项的过程：

确定个体是否受硬皮病所折磨，鉴定硬皮病亚型（即，弥漫性或局限型皮肤硬皮病）和确定该疾病的严重程度。

[0110] 有效量：如本文中所用，术语“有效量”是指化合物或试剂足以实现其预期目的的量。在本发明的上下文中，所述目的可为，例如：调节硬皮病的起因或症状；和/或延迟或预防硬皮病发作；和/或减缓或终止硬皮病症状的进展、加重或恶化；和/或减轻与硬皮病相关的一种或多种症状；和/或引起硬皮病症状的改善，和/或治愈硬皮病。

[0111] 框架或框架区：如本文中所用，是指可变区减去CDR的序列。因为CDR序列可通过不同系统确定，所以同样地框架序列受到相应的不同解释。6个CDR将重链和轻链上的框架区分为每条链上的4个子区(FR1、FR2、FR3和FR4)，其中CDR1位于FR1和FR2之间，CDR2位于FR2和FR3之间，并且CDR3位于FR3和FR4之间。无需将特定子区指定为FR1、FR2、FR3或FR4，其他人所称的框架区表示天然存在的免疫球蛋白单链的可变区内的组合FR。如本文中所用，FR表示4个子区之一，例如FR1表示最靠近可变区的氨基末端和相对于CDR1的5’的第一框架区，并且FR表示构成框架区的两个或更多个子区。

[0112] 人抗体：如本文中所用，其旨在包括具有由人免疫球蛋白序列生成（或组装）的可变区和恒定区的抗体。在一些实施方案中，抗体（或抗体组分）即使其氨基酸序列，例如在一个或多个CDR中并且尤其是在CDR3中包括并非由人生殖系免疫球蛋白序列编码的残基或元件（例如，包括序列变异，例如可能（最初）已经通过体外随机或位点特异性诱变或通过体内体细胞突变引入的序列变异），也可被视为“人类的”。

[0113] 人源化：正如本领域中所知，术语“人源化”通常用于指其氨基酸序列包括来自于在非人物种（例如，小鼠）中产生的参考抗体的V_H和V_L区序列，但是还包括相对于参考抗体在那些序列中旨在致使其更“似人”，即更类似于人生殖系可变序列的修饰的抗体（或抗体组分）。在一些实施方案中，“人源化”抗体（或抗体组分）是与目标抗原免疫特异性结合并且具有有与人抗体的氨基酸序列基本上相同的氨基酸序列的框架(FR)区和有与非人抗体的氨基酸序列基本上相同的氨基酸序列的互补决定区(CDR)的抗体。人源化抗体包含至少一个并且通常为两个可变结构域(Fab、Fab’、F(ab’)2、FabC、Fv)的基本上全部，其中全部或基本上全部CDR区与非人免疫球蛋白（即，供体免疫球蛋白）的CDR区相对应并且全部或基本上全部框架区为人免疫球蛋白共有序列的框架区。在一些实施方案中，人源化抗体还包含免疫球蛋白恒定区(Fc)的至少一部分，通常为人免疫球蛋白恒定区的至少一部分。在一些实施方案中，人源化抗体含有轻链以及至少重链的可变结构域。所述抗体还可包括重链恒定区的CH_1、铰链、CH_2、CH_3和任选地，CH_4区。在一些实施方案中，人源化抗体仅含人源化V_L区。在一些实施方案中，人源化抗体仅含人源化V_H区。在某些实施方案中，人源化抗体含有人源化V_H和V_L区。

[0114] Fynomer：如本文中所用，术语“fynomer”是指源自人Fyn激酶的Src同源(SH3)结构域的一类结合蛋白，其是由63个氨基酸残基组成的人蛋白(D.Grabulovski等J.Biol.Chem.282,3196-3204(2007))。Fynomer可以与抗体相同的亲和力和特异性与靶分子结合。Fynomer可在细菌中以高产量生成。而且，可连接几个Fynomer以产生具有多种结合特异性的蛋白。

[0115] 提高、增加或减少：如本文中所用，术语“提高”、“增加”或“减少”或语法等效词，是指相对于基线测量，例如在本文所述治疗开始之前在相同个体中的测量，或在不存在本

文所述治疗时在对照个体（或多个对照个体）中的测量的值。“对照个体”是受与受治个体相同类型且大约相同严重程度的硬皮病所折磨，与受治个体年龄相仿的个体（以确保受治个体和对照个体中该疾病的阶段是相当的）。

[0116] 试剂盒：如本文中所用，术语“试剂盒”是指用于递送材料的任何递送系统。此类递送系统可包括允许从一个位置到另一个位置储存、运输或递送各种诊断或治疗试剂（例如，在合适的容器中的寡核苷酸、酶等）和/或辅助材料（例如，缓冲液、用于进行测定的书面指导等）的系统。例如，试剂盒包括容纳有关反应试剂和/或辅助材料的一个或多个外壳（例如，盒子）。如本文中所用，术语“分段试剂盒”是指包含各自容纳总试剂盒组分的子部分的两个或更多个独立容器的递送系统。所述容器可一起或单独递送给预期接收者。例如，第一容器可容纳用于测定中的酶，而第二容器容纳寡核苷酸。术语“分段试剂盒”旨在涵盖容纳根据联邦食品、药品和化妆品法案第520(e)章监管的分析物特效试剂(ASR)的试剂盒，但是不限于此。实际上，包含各自容纳总试剂盒组分的子部分的两个或更多个独立容器的任何递送系统均包括在术语“分段试剂盒”内。相反，“组合试剂盒”是指在单个容器中容纳所有组分（例如，在单个盒子中容纳每种所需组分）的递送系统。术语“试剂盒”包括分段和组合试剂盒两种。

[0117] 正常：如本文中所使用，术语“正常”，当用于修饰术语“个体”或“受试者”时是指没有特定疾病或病状并且也不是所述疾病或病状的携带者的个体或一组个体。术语“正常”本文也用于限定从正常或野生型个体或受试者分离的生物标本或样品，例如“正常生物样品”。

[0118] 核酸：如本文中所用，术语“核酸”是指寡核苷酸、核苷酸或多核苷酸及其片段或部分，并且是指可为单链或双链，并且表示有义或反义链的基因组或合成来源的DNA或RNA。

[0119] 核酸分子：术语“核酸分子”和“多核苷酸”在本文中可交换使用。它们是指呈单链或双链形式的脱氧核糖核苷酸或核糖核苷酸聚合物，并且除非另有规定，否则涵盖可按与天然存在的核苷酸类似的方式起作用的天然核苷酸的已知类似物。该术语涵盖具有合成骨架的核酸样结构，以及扩增产物。

[0120] 蛋白：一般而言，“蛋白”为多肽（即，一串通过肽键彼此连接的至少两个氨基酸）。蛋白质可包括除氨基酸外的部分（例如，可为糖蛋白）和/或可以其它方式加工或修饰。本领域中的普通技术人员将意识到“蛋白”可为如同由细胞生成的完整多肽链（有或无信号序列），或可为其功能部分。普通技术人员将进一步意识到蛋白有时可包括一条以上，例如通过一个或多个二硫键连接或通过其它方式缔合的多肽链。

[0121] 样品：如本文中所用，术语“样品”涵盖从生物来源获得的任何样品。术语“生物样品”和“样品”可交换使用。以非限制性实例而言，生物样品可包括皮肤组织、肝脏组织、肾脏组织、肺部组织、脑脊髓液(CSF)、血液、羊水、血清、尿、粪便、表皮样品、皮肤样品、颊部拭子、精液、羊水、培养的细胞、骨髓样品和/或绒毛膜绒毛。任何生物样品的细胞培养物也可用作生物样品。生物样品也可为，例如从任何器官或组织获得的样品（包括活检或尸检标本），可包含细胞（原代细胞或培养的细胞）、通过任何细胞、组织或器官条件化的培养基、组织培养物。在一些实施方案中，适于本发明的生物样品是已经处理而释放或以其它方式使得核酸可用于如本文所述的检测的样品。也可使用固定或冷冻组织。

[0122] 受试者：如本文中所用，术语“受试者”是指人或任何非人动物（例如，小鼠、大鼠、

兔、狗、猫、牛、猪、绵羊、马或灵长类动物）。人包括产前和产后形式。在许多实施方案中，受试者为人类。受试者可为患者，患者是指出现在医疗提供者面前以诊断或治疗疾病的人。术语“受试者”在本文中可与“个体”或“患者”交换使用。受试者可受疾病或病症所折磨或易感疾病或病症，但是可能或可能不展示出所述疾病或病症的症状。

[0123] 患有：“患有”疾病、病症和/或病状（例如，硬皮病）的个体已经诊断出或展示出所述疾病、病症和/或病状的一种或多种症状。

[0124] 易感：“易感”疾病、病症和/或病状的个体尚未诊断出和/或可能未表现出所述疾病、病症和/或病状的症状。在一些实施方案中，易感疾病、病症和/或病状（例如，硬皮病）的个体可通过以下一项或多项表征：(1) 与所述疾病、病症和/或病状的发展相关的基因突变；(2) 与所述疾病、病症和/或病状的发展相关的遗传多态性；(3) 与所述疾病、病症和/或病状相关的蛋白质的表达和/或活性升高和/或降低；(4) 与所述疾病、病症和/或病状的发展相关的习惯和/或生活方式；(5) 所述疾病、病症和/或病状的家族史；(6) 对某些细菌或病毒的反应；(7) 对某些化学品的暴露。在一些实施方案中，易感疾病、病症和/或病状的个体将发展成所述疾病、病症和/或病状。在一些实施方案中，易感疾病、病症和/或病状的个体将不会发展成所述疾病、病症和/或病状。

[0125] 治疗：如本文中所用，术语“治疗（treatment）”（也为“治疗（treat）”或“治疗（treating）”）是指部分或完全减轻、改善、缓解、抑制、延迟特定疾病、病症和/或病状（例如，硬皮病、纤维化或炎症）的一种或多种症状或特征发作，降低其严重程度和/或降低其发病率的治疗性分子的任何施用（例如，双特异性抗CCL2/LOXL抗体或抗CCL2单克隆抗体或其抗原结合片段和抗LOXL2单克隆抗体或其抗原结合片段的同时或依次共同施用）。此类治疗可具有未表现出有关疾病、病症和/或病状的体征的受试者和/或仅表现出所述疾病、病症和/或病状的早期体征的受试者。可选地或另外，此类治疗可具有表现出有关疾病、病症和/或病状的一个或多个确定体征的受试者。

[0126] 发明详述

[0127] 本发明提供了（除了其它以外）抗CCL2和LOXL2的双特异性分子，包括包括但不限于抗体、fynomer、适配体、融合蛋白、蛋白结合结构域（例如，源自受体的蛋白结合结构域）及其用途，尤其是用于治疗硬皮病和相关纤维化和/或炎性疾病、病症和病状的用途。在一些实施方案中，本发明还提供了基于单特异性抗CCL2和抗LOXL2分子（例如，抗体）的组合用于治疗硬皮病和相关纤维化和/或炎性疾病、病症和病状的方法和组合物。

[0128] 本发明部分基于本发明人观察到的独特见解，即，双特异性分子，包括抗体或融合蛋白，允许CCL2的组织特异性靶向，而不浪费血浆中的抗CCL2分子例如抗体，引起对硬皮病的高效治疗。本发明的实施方案包括与CCL2和LOXL2两者结合的双特异性抗体。能够与CCL2和LOXL2结合的双特异性抗体的特别有利之处在于其拥有独特的组织选择性并且具有阻止和清除硬皮病、纤维化和炎症发展的潜力。因为LOXL2对于结缔组织的发育而言是重要的酶，所以抗LOXL2结合活性可用于优先靶向或鉴定具有相对大量的结缔组织的组织。同样地，抗LOXL2结合活性可用于靶向或鉴定具有异常结缔组织形成的组织；例如，正如在硬皮病中所观察到。而且，抗LOXL2抗体提供了协同治疗益处，因为抑制或中和LOXL2减少了受胺氧化酶活性影响的组织，从而阻断了结缔组织形成中的开始步骤。LOXL2抗体的治疗益处和组织特异性可与中和性抗CCL2单克隆抗体的疗效相结合以协同靶向炎症并减

少纤维化形成。这种协同靶向在更为晚期的硬皮病病例的治疗中特别重要，这是因为 LOX 结合使抗 CCL2 抗体被隔绝并且弥补了由于纤维化引起的渗透性降低。

[0129] 在以下章节中详细描述了本发明的各个方面。使用章节并非意在限制本发明。每个章节均可适用于本发明的任何方面。在本申请中，除非另有规定，否则使用“或”意为“和 / 或”。

[0130] 硬皮病

[0131] 硬皮病或系统性硬化通常被视为特征在于（除了其它以外）纤维化或变硬、血管改变和自身抗体的慢性全身性自身免疫疾病。不希望受理论约束，据认为硬皮病是由陷入加强扩增循环中的极度活跃的自身免疫反应引起。例如，硬皮病的组织学特征在于单核细胞的炎性浸润，这转而活化并且与周围成纤维细胞中胶原蛋白合成增加相关。具体而言，活化的巨噬细胞生成 TGF-β 和 PDGF，其活化受影响区域内的成纤维细胞以生成大量胶原蛋白。

[0132] T 细胞似乎也通过活化巨噬细胞和直接释放炎性促纤维生成细胞因子而在疾病过程中起作用。除胶原蛋白外，活化的成纤维细胞似乎也向受影响区域分泌募集释放细胞因子的附加炎性细胞的因子，这样进一步募集释放细胞因子的炎性细胞，从而导致炎症和组织纤维化不受调控。

[0133] 通常，在硬皮病患者的循环和组织中单核细胞 / 巨噬细胞和 T 细胞在数量和活化上均增加。组织积累既是微血管损伤的起因也是影响，这是在硬皮病发病机理中的早期事件之一。微血管损伤特征在于内皮细胞损伤、基底层增殖、血管壁中外周血单核细胞的偶然截留和初始血管周单核细胞浸润。随着炎症级联反应恶化，以纤维化、内脏器官结构错乱、血管稀疏，及因此而缺氧为主。所有这些因素和单核细胞的不断募集促成纤维化的维持。

[0134] 在一些实施方案中，硬皮病也被认为是通常特征在于皮肤和内部器官中细胞外基质蛋白过度积累、血管损伤和免疫异常的结缔组织疾病。

[0135] 据认为所述疾病的许多临床表现牵涉血管重构的失调。硬皮病的最早症状之一为微血管损伤。据认为这种微血管损伤会引起内皮细胞活化增加。据信活化的内皮细胞会表达粘附分子，导致毛细血管渗透性改变，允许炎性细胞迁移通过内皮并截留在血管壁内。据认为免疫活化会促成持续的内皮活化，导致内皮细胞破坏。据信这个过程会促成在硬皮病患者中通常所观察到的血管弹性丧失和变窄。此外，据认为微血管损伤促成真皮内的单核细胞血管周浸润，据认为这会促成成纤维细胞活化并且可具有硬皮病的相关典型症状。

[0136] 通常认为所述疾病的许多临床表现会牵涉成纤维细胞失调。成纤维细胞的主要功能是通过不断分泌细胞外基质的前体维持结缔组织的结构完整性。成纤维细胞为许多组织提供了结构框架（基质），在伤口愈合中起重要作用并且是动物中最常见的结缔组织细胞。成纤维细胞根据其位置和活性因不同外观而在形态上不均匀。

[0137] 存在两种主要的硬皮病形式：局限型系统性硬化 / 硬皮病和弥漫型系统性硬化 / 硬皮病。在局限型皮肤硬皮病中，皮肤的纤维化通常限于邻近肘部的区域。有局限型皮肤硬皮病的患者通常经历血管损害。皮肤和器官纤维化通常在有局限型硬皮病的患者中进展缓慢。有弥漫型硬皮病的患者通常经历比在局限型硬皮病中进展更快的皮肤和器官纤维化和 / 或分布广泛的炎症和 / 或比在局限型硬皮病中所看到的更严重的内部器官累及。

[0138] 通常认为导致肺纤维化的间质性肺病是硬皮病相关死亡的主要原因

(Ludwicka-Bradley,A.等Coagulation and autoimmunity in scleroderma interstitial lung disease.Semin Arthritis Rheum,41(2),212-22,2011)。更多导致硬皮病相关死亡的并发症包括但不限于癌症、心力衰竭、肺动脉高压、肾衰竭和吸收障碍或其任何组合。

[0139] 硬皮病最常通过检查皮肤症状而诊断。诊断的试验包括但不限于肉眼和/或手动检查皮肤、血压检测、胸部x-光、肺部CT、超声心动图、尿检、皮肤活检和血液检测，包括抗核抗体检测、抗拓扑异构酶抗体检测、抗着丝点抗体检测、抗U3抗体检测、抗RNA抗体检测、其它类型的抗体检测、红细胞沉降率和类风湿因子。

[0140] 双特异性抗CCL2和抗LOXL2分子

[0141] 本发明提供了基于施用结合CCL2和LOXL2两者的分子，尤其是双特异性抗CCL2和LOXL2分子用于治疗硬皮病和相关纤维化和/或炎性疾病、病症和病状的方法和组合物。在一些实施方案中，双特异性分子为核酸，例如双特异性核酸适配体。在一些实施方案中，双特异性分子为蛋白，例如双特异性融合蛋白、蛋白适配体和蛋白结合结构域。在一些实施方案中，双特异性分子包含双特异性fynomer。在一些实施方案中，双特异性分子包含双特异性抗体。在一些实施方案中，适于本发明的双特异性抗体包括与LOXL2特异性结合的第一抗原结合位点和与CCL2特异性结合的第二抗原结合位点（参见图1）。

[0142] CCL2

[0143] CCL2是由各种细胞类型生成的趋化因子。也称为单核细胞趋化蛋白-1(MCP-1)。已知CCL2对于免疫系统的许多细胞类型，包括但不限于单核细胞、CD4和CD8记忆T淋巴细胞和NK细胞而言是有效的吸引剂(Carulli,M.等Can CCL2serum levels be used in risk stratification or to monitor treatment response in systemic sclerosis？Ann Rheum Dis,67,105-109,2008,Yamamoto,T.Scleroderma－Pathophysiology.Eur J Dermatol,19(1),14-24)。已经证实CCL2会促进白细胞迁移跨过内皮单层，表明了在促进单核细胞血管周浸润中的作用（同上）。还已证实CCL2会促进成纤维细胞的活化并且上调体外大鼠成纤维细胞中胶原蛋白I型mRNA表达。已经证实在有硬皮病的患者中并且同样在硬皮病动物模型中CCL2水平升高（同上）。具体地，已经证实在硬皮病皮肤中CCL2表达水平增高并且已经证实在硬皮病成纤维细胞中CCL2RNA和蛋白增加（同上）。

[0144] 人CCL2是含有76个氨基酸残基的8.6kDa蛋白，表1中示出了其氨基酸序列。CCL2由各种细胞类型表达，其中包括单核细胞、血管内皮细胞、平滑肌细胞、某些上皮细胞并且结合其受体CCR2。CCL2属于CC趋化因子家族，其含有相邻的两个半胱氨酸残基（在表1中相邻半胱氨酸残基加有下划线）。

[0145] 表1

[0146]

人CCL2蛋白序列(GeneBank：NP_002973)	MKVSAALLCLLLIAATFIPQGLAQPDAINAPVTCCY NFTNRKISVQRLASYRRITSSKCPKEAVIFKTIVAKEI CADPKQKWVQDSMDHLDKQTQTPKT **(SEQ ID NO: 1)**

[0147] CCL2也已从非人来源纯化、表征、克隆并测序并且可重组生成或化学合成。如本文中所用，术语CCL2涵盖在其它物种，略举数例，包括但不限于小鼠、大鼠、灵长类动物、猪、鸡、狗、山羊、绵羊、马、骆驼、美洲驼中天然存在的任何CCL2蛋白，和与人CCL2基本上同

源或同一的任何重组或合成 CCL2。在一些实施方案中，如本文中所用的 CCL2 蛋白具有与 SEQ ID NO:1 至少 50%、55%、60%、65%、70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或更高同源性的序列。在一些实施方案中，如本文中所用的 CCL2 蛋白具有与 SEQ ID NO:1 至少 50%、55%、60%、65%、70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或更高同一性的序列。通常，与人 CCL2 基本上同源或同一的 CCL2 蛋白也保持了人 CCL2 的基本活性。

[0148] 上述任何 CCL2 蛋白均可用于生成和鉴定与 CCL2 特异性结合的单特异性和 / 或双特异性抗体。参见以下抗 CCL2 抗体和双特异性抗 CCL2 和 LOXL2 抗体章节。

[0149] LOXL2

[0150] LOXL2 是铜依赖型胺氧化酶的赖氨酰氧化酶家族的成员。不希望受理论约束，据认为 LOXL2 催化胶原蛋白组分侧链与弹性蛋白组分侧链的共价交联，从而稳定细胞外基质 (ECM) 中的这些蛋白。如表 2 中所示，人 LOXL2 的多肽序列经良好表征。

[0151] 表 2

[0152]

人 LOXL2 蛋白序列 (GeneBank: AAD34343)	MEGYVEVKEGKTWKQICDKHWTAKNSRVVCGMFGFP GERTYNTKVYKMFASRRKQRYWPFSMDCTGTEAHISS CKLGPQVSLDPMKNVTCENGQPAVVSCVPGQVFSPDG PSRFRKAYKPEQPLVRLRGGAYIGEGRVEVLKNGEWGT VCDDKWDLVSASVVCRELGFGSAKEAVTGSRLGQGIG PIHLNEIQCTGNEKSIIDCKFNAESQGCNHEEDAGVRCN TPAMGLQKKLRLNGGRNPYEGRVEVLVERNGSLVWG MVCGQNWGIVEAMVVCRQLGLGFASNAFQETWYWH GDVNSNKVVMSGVKCSGTELSLAHCRHDGEDVACPQ GGVQYGAGVACSETAPDLVLNAEMVQQTTYLEDRPMF MLQCAMEENCLSASAAQTDPTTGYRRLLRFSSQIHNN GQSDFRPKNGRHAWIWHDCHRHYHSMEVFTHYDLLN LNGTKVAEGQKASFCLEDTECEGDIQKNYECANFGDQ GITMGCWDMYRHDIDCQWVDITDVPPGDYLFQVVINP NFEVAESDYSNNIMKCRSRYDGHRIWMYNSHIGGSFSE ETEKKFEHFSGLLNNQLSPPVKKPAWSTPVFRPHHIFHG TSPQQLSLNECHVPSPSPAPTLSRPLQLCLSSGGKGPSH HSWGAAT **(SEQ ID NO: 2)**

[0153] LOXL2 也已从非人来源纯化、表征、克隆并测序并且可重组生成或化学合成。如本文中所用，术语 LOXL2 涵盖在其它物种，略举数例，包括但不限于小鼠、大鼠、灵长类动物、猪、鸡、狗、山羊、绵羊、马、骆驼、美洲驼中天然存在的任何 LOXL2 蛋白，和与人 LOXL2 基本上同源或同一的任何重组或合成 LOXL2。在一些实施方案中，如本文中所用的 LOXL2 蛋白具有与 SEQ ID NO:2 至少 50%、55%、60%、65%、70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或更高同源性的序列。在一些实施方案中，如本文中所用的 LOXL2 蛋白具有与 SEQ ID NO:2 至少 50%、55%、60%、65%、70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或更高同一性的序列。通常，与人 LOXL2 基本上同源或同一的 LOXL2 蛋白也保持了人 LOXL2 的基本活性。

[0154] 上述任何 LOXL2 蛋白均可用于生成和鉴定与 LOXL2 特异性结合的所需单特异性和

/ 或双特异性抗体。参见以下抗 LOXL2 抗体和双特异性抗 CCL2 和 LOXL2 抗体章节。

[0155] 相对于本文鉴定的 CCL2 和 LOXL2 序列的“氨基酸序列同一性百分比（％）”定义为比对序列并且如有必要，为达到最高序列同一性百分比而引入空位后，并且不将任何保守性取代视为序列同一性的一部分，候选序列中与 CCL2 或 LOXL2 序列中的氨基酸残基同一的氨基酸残基的百分比。为了测定氨基酸序列同一性百分比的比对可按在本领域技术范围之内的各种方式实现，例如，使用公开可用的计算机软件如 BLAST、ALIGN 或 Megalign(DNASTAR) 软件。本领域技术人员可确定用于测量比对的适当参数，包括在所比较的序列全长上实现最大比对所需的任何算法。优选地，使用 WU-BLAST-2 软件测定氨基酸序列同一性 (Altschul 等，Methods in Enzymology 266,460-480(1996) ;http://blast.wustl/edu/blast/README.html)。WU-BLAST-2 使用几个搜索参数，其中大多数设为默认值。可调参数设为以下值 :重叠间隔＝ 1，重叠分数＝ 0.125，世界阈值 (T) ＝ 11。HSP 得分 (S) 和 HSP S2 参数为动态值并且由程序本身根据特定序列的组成而确定，然而，最小值可调并且如以上所示设置。

[0156] 抗 CCL2 和抗 LOXL2 单特异性抗体

[0157] 可通过本领域技术人员众所周知的方法使用本文所述的 CCL2 和 LOXL2 蛋白或其片段生成抗体。如本文中所用，抗 CCL2 单特异性抗体包括与 CCL2 的任何表位特异性结合的任何抗体或抗体片段并且抗 LOXL2 单特异性抗体包括与 LOXL2 的任何表位特异性结合的任何抗体或抗体片段。如本文中所用，术语“抗体”旨在包括对指定蛋白或肽或其片段有特异性反应的免疫球蛋白及其片段。例如，术语“抗体”包括完整单克隆抗体、多克隆抗体、单结构域抗体 (例如，鲨鱼单结构域抗体 (例如，IgNAR 或其片段)) 和抗体片段，只要其表现出所需生物活性。合适的抗体还包括但不限于人抗体、灵长类源化抗体、嵌合抗体、双特异性抗体、人源化抗体、偶联抗体 (即，与其它蛋白、放射性标记、细胞毒素偶联或融合的抗体)、小模块免疫药物 (“SMIP™”) 和抗体片段。

[0158] 如本文中所用，术语“抗体片段”包括完整抗体的一部分，如例如抗体的抗原结合或可变区。抗体片段的实例包括 Fab、Fab’、F(ab’)2 和 Fv 片段 ;三链抗体 ;四链抗体 ;线性抗体 ;单链抗体分子。术语“抗体片段”还包括通过像与特异性抗原结合以形成复合物的抗体一样作用的任何合成或基因工程化蛋白。例如，抗体片段包括分离的片段、由重链和轻链可变区组成的“Fv”片段、其中轻链和重链可变区通过肽接头连接的重组单链多肽分子 (“ScFv 蛋白”) 和由模拟高变区的氨基酸残基组成的最小识别单元。

[0159] 可使用本领域众所周知的方法生成单特异性抗体。例如，Harlow 和 Lane，Antibodies:A Laboratory Manual,(1988) 描述了抗体生成的方案。通常，可以在小鼠、大鼠、豚鼠、仓鼠、骆驼、美洲驼、鲨鱼或其它合适的宿主中生成抗体。可选地，可在鸡中产生抗体，生成 IgY 分子 (Schade 等，(1996)ALTEX 13(5):80-85)。在一些实施方案中，适于本发明的抗体为类人灵长类抗体。例如，在狒狒中产生治疗有用的抗体的一般技术可以例如，在 Goldenberg 等，国际专利公布第 WO91/11465(1991) 号中和在 Losman 等，Int.J.Cancer 46:310(1990) 中找到。在一些实施方案中，可使用杂交瘤方法制备单克隆抗体 (Milstein 和 Cuello，(1983)Nature 305(5934):537-40.)。在一些实施方案中，也可通过重组方法制备单克隆抗体 (1979 年，美国专利第 4,166,452 号)。

[0160] 通过使用噬菌体展示，在大肠杆菌 (E.coli) 中工程化和表达抗体片段可以克服

与通过B细胞永生化生成单克隆抗体相关的许多困难。为确保回收高亲和力、单克隆抗体，免疫球蛋白组合文库通常必须含有大的谱尺寸。一种典型策略利用从免疫小鼠的淋巴细胞或脾细胞获得的mRNA以使用逆转录酶合成cDNA。重链和轻链基因通过PCR单独扩增并连接到噬菌体克隆载体中。生成两个不同文库，一个含有重链基因而一个含有轻链基因。从每个文库分离出噬菌体DNA，并且将重链和轻链序列连接在一起并包装以形成组合文库。每个噬菌体含有一对随机的重链和轻链cDNA并且在大肠杆菌感染后指导抗体链在感染细胞中的表达。为鉴定识别目标抗原的抗体，接种噬菌体文库，并且将斑块中存在的抗体分子转移到过滤器。用放射性标记抗原孵育过滤器，然后洗涤以去除过量未结合的配体。放射自显影照片上的放射性斑点鉴定出含有结合抗原的抗体的斑块。用于生成人免疫球蛋白噬菌体文库的克隆和表达载体可例如，从STRATAGENE克隆系统(La Jolla,Calif.)获得。

[0161] 可采用类似策略来获得高亲和力scFv。参见，例如，Vaughn等，Nat. Biotechnol.,14:309314(1996)。可通过使用与所有已知V_H、V_k和V_λ基因家族相对应的PCR引物从非免疫人类供体分离V基因来构建具有较大谱的scFv文库。扩增后，使V_k和V_λ池组合以形成一个池。将这些片段连接到噬菌粒载体中。然后将scFv接头，$(Gly_4, Ser)_3$，连接到噬菌粒中V_L片段的上游。V_H和接头-V_L片段经扩增并组装在JH区上。将所得V_H-接头-V_L片段连接到噬菌粒载体中。可使用如上所述的过滤器，或使用免疫管(Nunc；Maxisorp)淘选噬菌粒文库。通过由免疫兔的淋巴细胞或脾细胞构建免疫球蛋白组合文库并且通过在毕赤酵母(P.pastoris)中表达scFv构建体，可以达到类似结果。参见，例如，Ridder等，Biotechnology,13:255260(1995)。另外，分离适当scFv后，可通过亲和力成熟过程例如CDR3诱变和链改组获得具有较高结合亲和力和较慢解离速率的抗体片段。参见，例如，Jackson等，Br.J.Cancer,78:181188(1998)；Osbourn等，Immunotechnology,2:181196(1996)。

[0162] 抗体片段的另一种形式是编码单一CDR的肽。可通过构建编码目标抗体的CDR的基因获得CDR肽(“最小识别单元”)。例如，通过使用聚合酶链式反应由抗体生成细胞的RNA合成可变区，制备此类基因。参见，例如，Larrick等，Methods:A Companion to Methods in Enzymology 2:106(1991)；Courtenay-Luck，"Genetic Manipulation of Monoclonal Antibodies,"在MONOCLONAL ANTIBODIES:PRODUCTION,ENGINEERING AND CLINICAL APPLICATION中，Ritter等(编)，第166179页(Cambridge University Press 1995)；和Ward等，"Genetic Manipulation and Expression of Antibodies,"在MONOCLONAL ANTIBODIES:PRINCIPLES AND APPLICATIONS中，Birch等(编)，第137185页(Wiley-Liss,Inc.1995)。

[0163] 在一些实施方案中，适于本发明的抗体可包括人源化或人抗体。非人抗体的人源化形式为含有源自非人Ig的最小序列的嵌合Ig、Ig链或片段(例如Fv、Fab、Fab'、F(ab')2或Ab的其它抗原结合子序列)。通常，人源化抗体具有从非人来源引入的一个或多个氨基酸残基。这些非人氨基酸残基常常被称为“外来的”残基，其通常得自“外来的”可变结构域。通过用啮齿动物互补决定区(CDR)或CDR序列取代人抗体的相应序列来实现人源化(Riechmann等，Nature 332(6162):323-7,1988；Verhoeyen等，Science.239(4847):1534-6,1988.)。此类“人源化”抗体为嵌合Ab(1989年，美国专利第4,816,567号)，其中已经用来自于非人物种的相应序列取代了基本上不太完整的人可变

结构域。在一些实施方案中，人源化抗体通常为其中一些 CDR 残基及可能一些 FR 残基经来自于啮齿动物 Ab 中的类似位点的残基取代的人抗体。人源化抗体包括人 Ig（受者抗体），其中来自于受者 CDR 的残基经来自于具有所需特异性、亲和力和能力的非人物种（供者抗体）例如小鼠、大鼠或兔的 CDR 的残基置换。在一些情况下，相应的非人残基置换人 Ig 的 Fv 框架残基。人源化抗体可包含既未在受者抗体也未在外来的 CDR 或框架序列中找到的残基。一般而言，人源化抗体包含至少一个并且通常为两个可变结构域的基本上全部，其中大多数（若非全部）CDR 区与非人 Ig 的 CDR 区相对应并且大多数（若非全部）FR 区为人 Ig 共有序列的 FR 区。人源化抗体还最佳地包含 Ig 恒定区 (Fc) 的至少一部分，通常为人 Ig 的至少一部分 (Riechmann 等，Nature332(6162):323-7,1988 ;Verhoeyen 等，Science.239(4847):1534-6,1988.)。

[0164] 也可使用各种技术，包括噬菌体展示文库 (Hoogenboom 等，Mol Immunol.(1991)28(9):1027-37 ;Marks 等，J Mol Biol.(1991)222(3):581-97) 和制备人单克隆抗体 (Reisfeld 和 Sell,1985,Cancer Surv.4(1):271-90) 生成人抗体。类似地，可采用向其中内源 Ig 基因已经部分或完全灭活的转基因动物引入人 Ig 基因来合成人抗体。攻毒后，观察到人抗体生成，这在所有方面，包括基因重排、组装和抗体谱，都非常类似于在人中观察到的抗体生成 (Fishwild 等，High-avidity human IgG kappa monoclonal antibodies from a novel strain of minilocus transgenic mice,Nat Biotechnol.1996 年 7 月；14(7):845-51 ;Lonberg 等，Antigen-specific human antibodies from mice comprising four distinct genetic modifications,Nature 1994 年 4 月 28 日 ;368(6474):856-9 ；Lonberg 和 Huszar，Human antibodies from transgenic mice,Int.Rev.Immunol.1995 ；13(1):65-93 ;Marks 等，By-passing immunization:building high affinity human antibodies by chain shuffling.Biotechnology(N Y).1992 年 7 月 ;10(7):779-83)。

[0165] 在一些实施方案中，适于本发明的单特异性抗 CCL2 抗体或其片段具有为或高于大 约 500nM、100nM、10nM、1nM、500pM、100pM、50pM、10pM、1pM、500fM、400fM、300fM、200fM、100fM、50fM、10fM、1fM 的结合亲和力。在一些实施方案中，适于本发明的单特异性抗 CCL2 抗体或其片段具有范围介于大约 500nM 和 1fM 之间、介于 500nM 和 10fM 之间、介于 500nM 和 100fM 之间、介于 500nM 和 1pM 之间、介于 10nM 和 1fM 之间、介于 10nM 和 100fM 之间、介于 10nM 和 1pM 之间、介于 1nM 和 1fM 之间、介于 1nM 和 100fM 之间、介于 1nM 和 500fM 之间、介于 1nM 和 1pM 之间、介于 1nM 和 10pM 之间、介于 1nM 和 50pM 之间、介于 1nM 和 100pM 之间、介于 1nM 和 500pM 之间的结合亲和力。

[0166] 在一些实施方案中，适于本发明的单特异性抗 LOXL2 抗体或其片段具有为或高于大约 10nM、1nM、500pM、100pM、50pM、10pM、1pM、500fM、400fM、300fM、200fM、100fM、50fM、10fM、1fM 的结合亲和力。在一些实施方案中，适于本发明的单特异性抗 LOXL2 抗体或其片段具有范围介于大约 10nM 和 1fM 之间、介于 10nM 和 100fM 之间、介于 10nM 和 1pM 之间、介于 1nM 和 1fM 之间、介于 1nM 和 100fM 之间、介于 1nM 和 500fM 之间、介于 1nM 和 1pM 之间、介于 1nM 和 10pM 之间、介于 1nM 和 50pM 之间、介于 1nM 和 100pM 之间、介于 1nM 和 500pM 之间的结合亲和力。

[0167] 双特异性抗 CCL2 和抗 LOXL2 抗体和融合蛋白

[0168] 在一些实施方案中，本发明提供了双特异性抗 CCL2 和抗 LOXL2 抗体和 / 或融合蛋

白。如本文中所用，术语“双特异性抗体或融合蛋白”涵盖含有具不同特异性的至少两个抗原结合位点或抗原结合臂的任何抗体、融合蛋白或其片段。例如，适于本发明的双特异性抗CCL2和抗LOXL2抗体或融合蛋白含有与LOXL2特异性结合的至少第一抗原结合位点或臂和与CCL2特异性结合的至少第二抗原结合位点或臂。

[0169] 双特异性抗体的每一独立抗原结合位点或臂均可对其特异性结合靶标（例如，CCL2或LOXL2）具有所需结合亲和力。在一些实施方案中，抗原结合位点或臂以为或高于大 约 500nM、100nM、10nM、1nM、500pM、100pM、50pM、10pM、1pM、500fM、400fM、300fM、200fM、100fM、50fM、10fM、1fM的结合亲和力与CCL2特异性结合。在一些实施方案中，抗原结合位点或臂以范围介于大约500nM和1fM之间、介于500nM和10fM之间、介于500nM和100fM之间、介于500nM和1pM之间、介于10nM和1fM之间、介于10nM和100fM之间、介于10nM和1pM之间、介于1nM和1fM之间、介于1nM和100fM之间、介于1nM和500fM之间、介于1nM和1pM之间、介于1nM和10pM之间、介于1nM和50pM之间、介于1nM和100pM之间、介于1nM和500pM之间的结合亲和力与CCL2特异性结合。在一些实施方案中，抗原结合位点或臂以为或高于大约10nM、1nM、500pM、100pM、50pM、10pM、1pM、500fM、400fM、300fM、200fM、100fM、50fM、10fM、1fM的结合亲和力与LOXL2特异性结合。在一些实施方案中，抗原结合位点或臂以范围介于大约10nM和1fM之间、介于10nM和100fM之间、介于10nM和1pM之间、介于1nM和1fM之间、介于1nM和100fM之间、介于1nM和500fM之间、介于1nM和1pM之间、介于1nM和10pM之间、介于1nM和50pM之间、介于1nM和100pM之间、介于1nM和500pM之间的结合亲和力与LOXL2特异性结合。本发明涵盖双特异性抗体与具有上述任何结合亲和力的抗CCL2和抗LOXL抗原结合位点或臂的组合。具体而言，双特异性抗体可含有以1pM或更高的结合亲和力与LOXL2特异性结合的第一抗原结合位点或臂和以范围介于500nM和1fM之间的结合亲和力与CCL2结合的第二抗原结合位点或臂。具体而言，双特异性抗体可含有以1pM或更高的结合亲和力与LOXL2特异性结合的第一抗原结合位点或臂和以高于1pM的结合亲和力与CCL2结合的第二抗原结合位点或臂。

[0170] 双特异性抗体的每个抗原结合位点均可独立地为完整抗原结合臂，包括全长重链和全长轻链、Fab片段、单链可变区片段（scFv）或其它形式的抗体片段。在一些实施方案中，可由使用上述技术生成的抗CCL2和LOXL2的单特异性抗体制备所需抗原结合位点或臂，然后缔合此类所需抗CCL2和抗LOXL2抗原结合位点或臂以生成所需双特异性抗体。例如，可从上述单特异性单克隆抗体分离、分出或酶促消化所需抗CCL2或抗LOXL2抗原结合位点或臂。双特异性抗体的抗原结合位点或臂可以呈允许所述两位点或臂缔合，同时保持其抗原结合能力的各种构型排列。

[0171] 合适的双特异性抗体或融合蛋白可以呈各种双特异性抗体形式，包括但不限于四源杂交瘤、化学杂偶联物、使用选定异源二聚化结构域的重组构建体和由两个最小抗原结合位点组成的最小尺寸的重组构建体。一般而言，四源杂交瘤看起来像单克隆抗体，但是具有两条不同的抗原结合臂。其传统生成方式基于两种不同杂交瘤（肿瘤细胞和产抗体的正常细胞之间融合的）细胞的体细胞融合，每种杂交瘤细胞生成独特的单克隆抗体（例如，抗CCL2或抗LOXL2单克隆抗体）。可通过两条不同的抗体重链和轻链的随机配对生成具有所需抗原结合臂（例如，抗CCL2和抗LOXL2）的双特异性抗体。各种优先配对方法可用于减少错配副产物并增加双特异性抗体产量。例如，鼠和大鼠杂交瘤细胞系可融合以表达优先彼

此配对的特定 IgG 亚类的单克隆抗体。另外，两条不同抗体重链的优先配对可通过人 IgG1 的 CH3 结构域中的某些突变，所称的“旋钮成孔 (knobs-into-holes)”策略实现。

[0172] 四源杂交瘤形式通常含有可以与 Fc 受体相互作用的 Fc 区。因此，具有 Fc 部分的双特异性抗体也称为三特异性抗体。在一些实施方案中，四源杂交瘤的 Fc 部分可经酶切去除，产生双特异性 $F(ab')_2$（通过二硫键化学连接的抗体的两条抗原结合臂）。另外，两个抗原结合位点或臂可经硫醚键合或通过所述抗体或片段上的一个或多个官能团，包括胺基、羧基、苯基、硫醇基或羟基连接。

[0173] 在一些实施方案中，可通过两种不同的单克隆抗体或抗体片段与例如，异源双功能交联剂的化学偶联来生成双特异性抗体。例如，两个不同的 Fab’（抗体的单价抗原结合臂）可在其铰链半胱氨酸残基处以定点方式化学交联。适于化学交联或偶联的化学品的实例包括但不限于 N- 琥珀酰亚胺基 -3-(2- 吡啶基二硫代）丙酸酯)(SPDP)、1- 乙基 -3-(3- 二甲氨基丙基）碳二亚胺盐酸盐 (EDC)、5,5’- 二硫代双 -(2- 硝基苯甲酸）(DTNB)、邻亚苯基二马来酰亚胺、碳二亚胺、二异氰酸酯、重氮苯、六亚甲基二胺、二马来酰亚胺、戊二醛、4- 琥珀酰亚胺基 - 氧羰基 -.α.- 甲基 .α.(2- 吡啶基硫代）甲苯 (SMPT)、N- 琥珀酰亚胺基 -S 乙酰基 - 硫代乙酸酯 (SATA) 及其组合。

[0174] 在一些实施方案中，自然形成杂二聚体的某些蛋白结构域用于构建适于大规模表达的杂二聚双特异性抗体。一个实例为可与两个不同 Fab’或单链 (sc)Fv（可变区片段）抗体片段的羧基端融合的转录因子 Fos 和 Jun 的亮氨酸拉链结构域。在一些实施方案中，抗体恒定区结构域 Cκ 和 CH1 可代替 Fos 和 Jun 二聚化结构域用于在细菌例如大肠杆菌中表达双特异性抗体。在某些实施方案中，除了别的以外，两个抗原结合位点或臂可经由 GST（谷胱甘肽 S 转移酶）融合蛋白或其二聚化基序、PDZ 二聚化结构域、FK-506BP（结合蛋白）或其二聚化基序、天然或人工螺旋 - 转角 - 螺旋二聚化结构域（例如，p53)、蛋白 A 或其二聚化结构域、结构域 B 而缔合。在某些实施方案中，两个抗原结合位点或臂可经由与外源性组分的相互作用而缔合。例如，两个抗原结合位点或臂可含有亲和素基序并且二者均与添加的亲和素相互作用。

[0175] 在一些实施方案中，双特异性抗体包括所称的双链抗体和串联单链 Fv 构建体。通常，这些形式的双特异性抗体由两个不同的抗原结合位点与用作接头序列的最小附加蛋白序列构成。每个抗原结合位点使用分别来自于抗体重和轻两条链的最小 V_H 和 V_L 结构域。在双链抗体中，一个抗原结合位点的 V_L 结构域通过短肽接头与另一抗原结合位点的 V_H 结构域连接并且反之亦然。呈串联 scFv 形式的双特异性抗体通常包括通过单一蛋白链上的柔性肽接头连接的两对不同 V_L/V_H。在一些实施方案中，串联 scFv 构建体可以在能够正确折叠四个连续对齐的抗体 V 区的哺乳动物宿主细胞中表达。全功能双特异性串联单链抗体分泌到细胞培养上清液中并且可经由聚组氨酸标签通过亲和色谱法，接着通过尺寸排阻色谱法有效地纯化。合适的肽接头可包括不干扰抗原结合位点或臂的构象的任何序列。在某些实施方案中，合适的肽接头为 1 个、2 个、3 个、4 个、5 个、6 个、7 个、8 个、9 个、10 个或更多个氨基酸长。

[0176] 通过本文描述的各种方法生成的抗体通常含有同种特异性和双特异性分子两种。测定双特异性单克隆的存在的方法在本领域中已知，包括桥联 ELISA 测定法（参见，例如，Suresh 等 (1986)Proc.Natl.Acad.Sci.USA 83,7989-93 ;Koolwijk 等 (1988)Hybridoma

7,217-225；和De Lau等(1989)J.Immunol.149,1840-46)。如果有足够量的相应抗原可用，则可采用双抗原ELISA。

[0177] 上述双特异性抗体制备的特定方法偶尔导致单特异性以及双特异性抗体形成(例如，按照化学偶联的程序)。发生这种情况时，可通过允许区分两种形式的各种程序中的任一种将所需双特异性抗体与单特异性抗体分离。此类程序包括但不限于从制备型、非变性丙烯酰胺凝胶被动洗脱或各种常规色谱技术，例如离子交换、HPLC或嗜硫吸附色谱法(参见，例如，Kreutz等(1998).J.Chromatography 14,161-170)。另外，每一抗原结合位点或臂可经不同标签标记，并且通过双重亲和色谱法将双重标记的双特异性抗体与单一标记的单特异性抗体分离。

[0178] 另外的生成、纯化和表征双特异性抗体的方法在本领域中已知；例如，如美国专利第5,601,819、6,004,555、5,762,930、6,060,285、6,010,902、5,959,083、5,807,706号和美国专利公布第2002/0025317号中所公开，每个专利通过引用并入本文。

[0179] 可使用本领域已知的标准方法，包括本文描述的各种方法进一步修饰所需双特异性抗CCL2和抗LOXL2抗体以生成嵌合、人源化或全人双特异性抗体。

[0180] 硬皮病和相关疾病、病症或病状的治疗

[0181] 本文描述的双特异性和/或单特异性抗CCL2和抗LOXL2分子(例如，抗体、fynomer、适配体、融合蛋白或蛋白结合结构域)可用于有效地治疗患有或易感硬皮病或相关纤维化、炎性疾病、病症或病状的个体。如本文中所用，术语“治疗(treat)”或“治疗(treatment)”是指改善相关疾病、病症或病状的一种或多种症状，预防或延迟一种或多种症状的发作，和/或降低一种或多种症状的严重程度或频率。

[0182] 本发明的各种分子可单独或组合施用。在一些实施方案中，根据本发明的治疗方法牵涉向需要治疗的受试者施用本文描述的双特异性分子。在一些实施方案中，根据本发明的治疗方法牵涉向需要治疗的受试者施用本文描述的抗CCL2和抗LOXL2单特异性抗体或其片段。抗CCL2和抗LOXL2单特异性抗体或其片段可经由相同或不同施用途径，同时或依次施用。

[0183] 在一些实施方案中，本文描述的分子可单独或连同其它治疗剂，例如在治疗纤维化或炎性疾病、病症或病状中有用的治疗剂一起施用。此类治疗剂包括但不限于皮质类固醇、NSAID、免疫抑制药物(例如，氨甲蝶呤和环磷酰胺)、小分子免疫调节剂、干扰素受体抗体、抗纤维化药物(包括D-青霉胺、秋水仙碱、PUVA、松弛素和环孢霉素)及抗TGFβ治疗剂和内皮素受体拮抗剂。

[0184] 在一些实施方案中，可使用常规剂量和递送方法，例如对其它、相当的治疗剂描述的剂量和递送方法施用本文描述的分子。可通过本领域技术人员已知的常规程序确定要施用的剂量。参见，例如，The Pharmacological Basis of Therapeutics，Goodman和Gilman编，Macmillan Publishing Co.，New York。一般而言，有效剂量是足够大而产生所需效果，例如中和CCL2和/或LOXL2和/或阻断CCL2和/或LOXL2与其同源受体的结合的剂量。所述剂量不应如此大以致于引起不良副作用，例如不必要的交叉反应、过敏反应等。要考虑的因素包括所牵涉的特异性抗体/试剂的活性、其代谢稳定性和作用时长、施用模式和时间、药物组合、排泄率及经受治疗的宿主的年龄、体重、总体健康状况、性别、饮食和特定疾病状态的严重程度。

[0185] 本文描述的分子可按治疗有效的任何给药方案施用。在一些实施方案中，抗CCL2/LOXL2双特异性或单特异性抗体每两个月、每月、每三周、每两周、每周、每日或以可变间隔施用一次。

[0186] 可使用任何施用方法包括肠胃外和非肠胃外施用途径施用本文描述的分子。肠胃外途径包括，例如静脉内、动脉内、门静脉内、肌肉内、皮下、腹腔内、脊柱内、鞘内、脑室内、颅内、胸腔内或其它注射途径。非肠胃外途径包括，例如口、鼻、经皮、肺部、直肠、颊部、阴道、眼。也可通过连续输注、局部施用、从植入物（凝胶、膜等）持续释放和/或静脉注射施用。当使用抗CCL2和抗LOXL2抗体的组合时，可经由相同施用途径或经由不同施用途径施用抗CCL2和抗LOXL2抗体。

[0187] 硬皮病

[0188] 在一些实施方案中，本文描述的方法和组合物可用于治疗患有或易感所有形式的硬皮病，包括局限型系统性硬化/硬皮病、弥漫型系统性硬化/硬皮病和其它形式的硬皮病的受试者。局限型系统性硬化/硬皮病通常牵涉主要影响手、臂和面部的皮肤表现。根据以下并发症也称为CREST综合征：钙质沉着症、雷诺氏现象(Raynaud's phenomenon)、食道功能障碍、指端硬化和毛细管扩张。另外，在多达三分之一的患者中可发生肺动脉高压，并且是这种形式的硬皮病最严重的并发症。弥漫型系统性硬化/硬皮病进展迅速并且影响大面积的皮肤和一种或多种内部器官，常常为肾脏、食道、心脏和肺部。其它形式的硬皮病包括没有皮肤变化，但是有系统性表现的系统性无皮肤硬化的硬皮病，及影响皮肤，但是不影响内部器官的两种局部化形式：硬斑病型和带状硬皮病。

[0189] 在一些实施方案中，治疗是指与未治疗的对照或治疗前状态相比，部分或完全减轻、改善、缓解、抑制硬皮病相关的一种或多种症状，延迟其发作、降低其严重程度和/或发病率，所述症状包括但不限于内皮细胞损伤、基底层增殖、血管周单核细胞浸润、纤维化、内脏器官结构错乱、血管稀疏、缺氧、手指、背部和前臂肿胀、四肢感觉寒冷、指部溃疡、甲皱伸长、凹点甲出血、指甲凹陷瘢痕、肺动脉高压、皮肤纤维化、脱发、皮肤紧绷、皮肤变硬、色素沉着、色素减退、皮肤瘙痒、腕管综合征、肌无力、关节疼痛、关节僵硬、肾纤维化、食道纤维化、口腔纤维化、心脏纤维化和肺纤维化、肝脏纤维化、肌肉纤维化、干咳、呼吸短促、呼吸困难、齿槽炎、肺炎、喘息、饭后腹胀、便秘、腹泻、吞咽困难、胃窦血管扩张、食管返流、胃灼热、大便失禁、口腔扁平白斑、附着牙龈粘膜损失、牙龈萎缩、牙周韧带弥漫性扩宽、吞咽困难、口腔无弹性、下颌骨后支、尺骨喙突和髁再吸收、癌症、心力衰竭、肺动脉高压、肾衰竭、吸收障碍或其任何组合。

[0190] 在一些实施方案中，治疗是指部分或完全减轻、改善、缓解、抑制纤维化，延迟其发作、降低其严重程度和/或发病率。如本文中所用，术语“纤维化”是指在器官或组织中形成过量纤维性结缔组织。不希望受特定理论约束，据认为纤维化可由某些成纤维细胞的活化引起。已知不同亚型的成纤维细胞即使在单个组织中也执行不同功能。例如，皮肤上层的乳头状成纤维细胞生成细胶原蛋白束并且具有高增殖速率，而来自于皮肤更深真皮层的网状成纤维细胞生成粗胶原蛋白束和丰富的多能蛋白聚糖(versican)，并且促进快速晶格收缩。成纤维细胞可处于静态或在活化的不同阶段。在正常细胞作用期间，成纤维细胞例如响应于损伤变得活化以利于伤口愈合。活化的成纤维细胞生成更多的细胞外基质组分，包括胶原蛋白和胶原蛋白修饰酶。在有硬皮病的个体中，通常观察到成纤维细胞活化增多，

伴有 ECM 过度生成。通常认为 ECM 的这种过度生成会引起纤维化，器官或组织中形成过量纤维性结缔组织，这是硬皮病的特征。

[0191] 在一些实施方案中，治疗是指部分或完全减轻、改善、缓解、抑制皮肤、肾脏、肝脏、肺部和 / 或食道中的纤维化，延迟其发作、降低其严重程度和 / 或发病率。

[0192] 在一些实施方案中，治疗引起部分或完全减轻、改善、缓解、抑制皮肤纤维化，延迟其发作、降低其严重程度和 / 或发病率。通常，皮肤纤维化与皮肤增厚、变硬或形成瘢痕（例如，瘢痕瘤或烧伤瘢痕等）有关。在一些实施方案中，通过改良的 Rodnan 皮肤得分评估皮肤纤维化。例如，如图 2 所说明，未累及的皮肤给予得分 0 ；轻度增厚给予得分 1 ；中等增厚给予得分 2 ；并且严重增厚给予得分 3。在一些实施方案中，治疗引起改良的 Rodnan 皮肤得分与治疗前状态相比降低超过 10％、超过 15％、超过 20％、超过 25％、超过 30％、超过 35％、超过 40％、超过 45％、超过 50％、超过 55％、超过 60％、超过 65％、超过 70％、超过 75％、超过 80％、超过 85％、超过 90％、超过 95％或更多。在一些实施方案中，治疗引起皮肤纤维化大幅消除。

[0193] 不希望受理论约束，还认为硬皮病患者中成纤维细胞的活化可通过生成细胞因子激活免疫反应而引起。细胞因子的实例包括但不限于 TGF-β 、CCL2、CTGF、ET-1、成纤维细胞生长因子、IL-1、IL-4、IL-6、IL-12、IL-13、IL-17、MCP-1、MCP-3 和 PDGF。细胞因子可由免疫系统的促炎性细胞，例如活化的 T 细胞、单核细胞或巨噬细胞生成或，可选地，细胞因子可由上皮细胞生成。促成成纤维细胞活化的一个因素可为与毛细血管通透性增加相关的真皮中血管周单核细胞浸润。替代性或另外的成纤维细胞活化方式包括与细胞外基质的相互作用和 / 或机械拉伸。因此，在一些实施方案中，根据本发明对硬皮病患者的治疗引起一种或多种促炎性细胞因子，例如本文描述的促炎性细胞因子的生成减少。在一些实施方案中，治疗引起促炎性细胞因子（例如，TGF-β 、CCL2、CTGF、ET-1、成纤维细胞生长因子、IL-1、IL-4、IL-6、IL-12、IL-13、IL-17、MCP-1、MCP-3 和 / 或 PDGF）与治疗前状态相比减少超过 10％、超过 15％、超过 20％、超过 25％、超过 30％、超过 35％、超过 40％、超过 45％、超过 50％、超过 55％、超过 60％、超过 65％、超过 70％、超过 75％、超过 80％、超过 85％、超过 90％、超过 95％或更多。测定细胞因子水平的各种方法在本领域中已知并且可用于实践本发明。

[0194] 在一些实施方案中，治疗引起 CCL2 血清水平降低。在一些实施方案中，治疗引起 CCL2 血清水平与治疗前状态相比降低超过 10％、超过 15％、超过 20％、超过 25％、超过 30％、超过 35％、超过 40％、超过 45％、超过 50％、超过 55％、超过 60％、超过 65％、超过 70％、超过 75％、超过 80％、超过 85％、超过 90％、超过 95％或更多。在一些实施方案中，治疗引起 CCL2 血清水平低于约 800pg/ml、700pg/ml、600pg/ml、500pg/ml、400pg/ml、350pg/ml、300pg/ml、250pg/ml、200pg/ml、150pg/ml 或 100pg/ml。在一些实施方案中，治疗引起 CCL2 血清水平与基本上相同年龄或发育阶段的健康对照的 CCL2 血清水平相当。

[0195] 纤维化疾病、病症或病状

[0196] 除硬皮病外，根据本发明的方法和组合物可用于治疗纤维化疾病、病症或病状，通常包括但不限于多灶性纤维硬化、硬皮病性移植物抗宿主疾病、肾原性系统性纤维化、器官特定性纤维化等。说明性器官特定性纤维化病症包括但不限于肺纤维化、肺动脉高压、囊肿性纤维化、哮喘、慢性阻塞性肺病、肝纤维化、肾纤维化、NASH 等。许多纤维化疾病、病症或

病状在受影响的组织中具有无序和/或夸张的细胞外基质沉积。纤维化可与炎症相关，作为基础疾病的症状出现，和/或由外科手术或伤口愈合过程引起。未加抑制的纤维化可导致基础器官或组织的结构破坏，常常称为瘢痕形成。

[0197] NASH通常是具有很少或没有症状的沉默疾病。患者通常在早期感觉良好并且只有一旦该疾病更为晚期或肝硬化发展才开始有症状－例如疲劳、体重减轻和虚弱。NASH的进展可以需要数年，甚至数十年。该过程可以停止并且，在一些情况下无需特定疗法，甚至可能开始自身逆转。或NASH可缓慢恶化，引起在肝脏中瘢痕形成或纤维化出现并积累。随着纤维化恶化，肝硬化发展，其中肝脏变得严重瘢痕性、变硬并且不能正常作用。并非有NASH的每个患者都发展肝硬化，但是一旦存在严重的瘢痕形成或肝硬化，就很少治疗可以中断该进展。有肝硬化的人经历液体潴留、肌肉萎缩、肠道出血和肝衰竭。肝脏移植是对有肝衰竭的晚期肝硬化的唯一疗法，并且越来越多地在有NASH的人中进行移植。在美国NASH列为丙型肝炎和酒精性肝病后肝硬化的主要原因之一。

[0198] 肾脏（肾）纤维化由肾脏中纤维性结缔组织的过量形成而引起。肾脏纤维化引起重大的发病率和死亡率并且导致对透析或肾脏移植的需要。纤维化可以发生在肾单位（肾的功能单位）的过滤或重吸收组分中。许多因素可促成肾脏瘢痕形成，特别是肾小球过滤的自动调节中牵涉的生理学紊乱。这转而导致积累的细胞外基质置换正常结构。单个细胞生理学上的一系列变化导致生成许多刺激细胞外基质合成和降解之间的平衡改变的肽和非肽纤维蛋白原以利于瘢痕形成。

[0199] 炎性疾病、病症或病状

[0200] 在一些实施方案中，根据本发明的方法和组合物可用于治疗炎性疾病、病症或病状，包括但不限于：全身炎症反应(SIRS)；阿尔茨海默病（和相关病状和症状，包括：慢性神经炎症、神经胶质活化；小神经胶质增加；神经炎斑块形成；和治疗反应）；肌萎缩侧索硬化(ALS)、关节炎（和相关病状和症状，包括但不限于：急性关节炎症、抗原诱导性关节炎、与慢性淋巴细胞性甲状腺炎相关的关节炎、胶原诱导性关节炎、幼年型关节炎；类风湿性关节炎、骨关节炎、预后和链球菌诱导性关节炎、脊柱关节病变、痛风性关节炎）、哮喘（和相关病状和症状，包括：支气管哮喘；慢性阻塞性气道疾病；慢性阻塞性肺病、幼年型哮喘和职业性哮喘）；心血管疾病（和相关病状和症状，包括动脉粥样硬化；自身免疫性心肌炎、慢性心脏缺氧、充血性心力衰竭、冠状动脉疾病、心肌病和心脏细胞功能障碍，包括：主动脉平滑肌细胞活化；心脏细胞凋亡；和心脏细胞功能的免疫调节；糖尿病和相关病状和症状，包括自身免疫性糖尿病、胰岛素依赖性(1型）糖尿病、糖尿病牙周炎、糖尿病性视网膜病变和糖尿病性肾病）；胃肠道炎症（和相关病状和症状，包括乳糜泻病、相关骨质减少、慢性结肠炎、克罗恩氏病、炎性肠病和溃疡性结肠炎）；胃溃疡；肝脏炎症例如病毒和其它类型的肝炎、胆固醇胆结石和肝纤维化、HIV感染（和相关病状和症状，包括退行性反应、神经退行性反应和HIV相关的何杰金氏病(Hodgkin's Disease))、川崎综合征(Kawasaki's Syndrome)(和相关疾病和病状，包括黏膜皮肤淋巴结综合征、颈淋巴结病变、冠状动脉病变、水肿、发烧、白细胞增多、轻度贫血、皮肤剥脱、皮疹、结膜发红、血小板增多；多发性硬化、肾病（和相关疾病和病状，包括糖尿病性肾病、终末期肾病、急慢性肾小球性肾炎、急慢性间质性肾炎、狼疮性肾炎、古德帕斯丘综合征(Goodpasture's syndrome)、血液透析生存和肾缺血再灌注损伤）、神经退行性疾病（和相关疾病和病状，包括急性神

经退化、衰老和神经退行性疾病中 IL-1 的诱导、IL-1 诱导的下丘脑神经元塑性和慢性压力高反应性）、眼病（和相关疾病和病状，包括糖尿病性视网膜病变、格雷夫斯氏眼病 (Graves' opthalmopathy) 和葡萄膜炎、骨质疏松症（和相关疾病和病状，包括齿槽、股骨、桡骨、椎骨或腕骨流失或发生骨折、绝经后骨流失、大量骨折发生率或骨流失率）、中耳炎（成人或小儿）、胰腺炎或胰腺腺泡炎、牙周病（和相关疾病和病状，包括成人、早发型和糖尿病型）；肺病，包括慢性肺部疾病、慢性鼻窦炎、透明膜病、缺氧和 SIDS 中的肺病；冠状动脉或其它血管移植物的再狭窄；风湿包括类风湿性关节炎、风湿性阿绍夫小体 (rheumatic Aschoff bodies)、风湿性疾病和风湿性心肌炎；甲状腺炎包括慢性淋巴细胞性甲状腺炎；尿路感染包括慢性前列腺炎、慢性盆腔疼痛综合征和尿石病。免疫病症，包括自身免疫性疾病，例如斑秃、自身免疫性心肌炎、格雷夫斯氏病、格雷夫斯氏眼病、苔藓型硬化、多发性硬化、银屑病、全身性红斑狼疮、系统性硬化、甲状腺病（例如甲状腺肿和淋巴瘤性甲状腺瘤（桥本氏甲状腺炎 (Hashimoto's thyroiditis)、淋巴细胞性甲状腺肿）、睡眠障碍和慢性疲劳综合征及肥胖（非糖尿病型或糖尿病相关型）。对由细菌、病毒（例如巨细胞病毒、脑炎病毒、爱泼斯坦 - 巴尔二氏病毒 (Epstein-Barr Virus)、人免疫缺陷病毒、流感病毒）或原生动物（例如恶性疟原虫、锥体虫）引起的传染病，例如利什曼病 (Leishmaniasis)、麻风、莱姆病 (Lyme Disease)、莱姆心脏炎 (Lyme Carditis)、疟疾、脑型疟疾、脑膜炎、与疟疾相关的肾小管间质肾炎）的抗性。对创伤的反应，包括脑创伤（包括中风和缺血、脑炎、脑病、癫痫、围产期脑损伤、长期高热惊厥、SIDS 和蛛网膜下出血）、低出生体重（例如大脑麻痹）、肺损伤（急性出血性肺损伤、古德帕斯丘综合征、急性缺血性再灌注）、由职业和环境污染物引起的心肌功能障碍（例如对毒油综合征硅肺病的易感性）、辐射创伤及伤口愈合反应的效率（例如烧伤或由热造成的伤口、慢性伤口、手术伤口和脊柱损伤）。激素调节包括生育 / 生殖力、怀孕可能性、早产发生率、产前和新生期并发症，包括早产低出生体重、大脑麻痹、败血病、甲状腺机能减退、氧依赖、颅畸形、早发性更年期。受试者对移植物的反应（排斥或接受）、急性期反应（例如发热反应）、全身性一般的炎症反应、急性呼吸窘迫反应、急性全身炎症反应、伤口愈合、粘连、免疫炎症反应、神经内分泌反应、热病发展和抗性、急性期反应、应激反应、疾病易感性、反复性运动应激、肘部发炎及疼痛管理和反应。

[0201] 用于患者分层、治疗监测和 / 或优化的生物标志物或指标

[0202] 在一些实施方案中，基于本文描述的抗 CCL2/LOXL2 双特异性或单特异性分子（例如，抗体、fynomer、适配体、融合蛋白或蛋白结合结构域）的方法和组合物可与生物标志物一起用于患者分层、治疗监测和 / 或优化。在一些实施方案中，合适的生物标志物为差异性表达的生物标志物。如本文中所用，术语“差异性表达的生物标志物”是指相对于其在健康或正常受试者（或健康或正常受试者群体）中的表达水平，在受硬皮病折磨的受试者（或受试者群体）中其表达水平不同的生物标志物。该术语也涵盖对于不同疾病亚型（即，局限型皮肤或弥漫型皮肤）而言表达水平不同的生物标志物。该术语还涵盖在所述疾病的不同阶段（例如，轻度或早期硬皮病、重度或晚期硬皮病）表达水平不同的生物标志物。差异性表达包括在生物标志物的时间或细胞表达模式上的定量以及定性差异。如以下更详细地描述，差异性表达的生物标志物，单独或与其它差异性表达的生物标志物组合，用于诊断、分期、治疗、药物开发和相关领域中的多种不同应用。本文公开的差异性表达的生物标志物的表达模式可以描述为硬皮病、硬皮病亚型、硬皮病分期和硬皮病严重程度和 / 或进

展的指纹或印记。其可以用作参考点以比较和表征未知样品和要探索更多信息的样品。术语“表达水平降低”,如本文中所用,是指通过本文描述的一种或多种方法测量,表达降低至少 10%或更多,例如 20%、30%、40%或 50%、60%、70%、80%、90%或更多,或表达降低大于 1 倍、2 倍、3 倍、4 倍、5 倍、10 倍、50 倍、100 倍或更多。术语“表达水平提高”,如本文中所用,是指通过一种或多种方法,例如本文描述的方法测量,表达提高至少 10%或更多,例如 20%、30%、40%或 50%、60%、70%、80%、90%或更多,或表达提高大于 1 倍、2 倍、3 倍、4 倍、5 倍、10 倍、50 倍、100 倍或更多。

[0203] 皮肤基因表达分析

[0204] 鉴定硬皮病患者中的差异性表达的生物标志物的各种方法在本领域中已知并且可用于实践本发明。例如,皮肤基因表达分析可为构造患者亚群、鉴定响应性患者亚群的蛋白生物标志物和指标的有力工具。在一些实施方案中,可通过比较健康个体与有硬皮病的个体的皮肤样品的转录谱来鉴定在有硬皮病的患者中受差异性调节的基因。进一步地,可通过将处于程度发展不同阶段的硬皮病患者包括在内来鉴定与疾病严重程度相关的基因转录产物。如例如由 Milano 等在通过引用整体并入本文的“Molecular Subsets in the Gene Expression Signatures of Scleroderma Skin”(PLOS One,3:7,1-18,2008) 中已经描述,可通过微阵列分析来分析转录谱。例如,可对来自于有弥漫型硬皮病、局限型硬皮病、硬斑病(类似于硬皮病,无内部器官累及的疾病)的患者和健康对照的皮肤样品(例如,前臂和后背样品)进行微阵列分析。为鉴定与硬皮病最高度相关的基因,选择在复制品和样品部位之间最内在一致,而在个体间最具可变性的基因进行进一步分析。基于与硬皮病严重程度相关的基因差异性表达的集群分析可用于选择受硬皮病影响的基因。

[0205] 已经报道硬皮病中差异性表达的示例性基因被聚集为 6 个组。第一组包括在有弥漫型硬皮病的患者亚群中和在有硬斑病的患者中高度表达的免疫球蛋白基因,包括但不限于 CCR2、CCL4 和 IGLL1。第二组包括增殖印记,包括只有在细胞分裂时表达的基因。在这个集群中显示出更高表达的基因包括细胞周期调控基因例如 CKS1B、CDKS2、CDC2、MCM8 和 E2F7。增殖印记的存在与来自于皮肤活检的报告一致,证明弥漫型硬皮病组织的细胞经历更多增殖。第三组包括胶原蛋白和细胞外基质组分,包括但不限于 COL5A2、COL8A1、COL10A1、COL12A1。第四组包括通常与 T 淋巴细胞和巨噬细胞的存在相关的基因,其与第三组表达类似并且包括 T 细胞活化所需的 PTPRC,以及在 T 淋巴细胞表面表达的 CD2 和 CDW52。第五组包括在弥漫型硬皮病中显示出低表达的基因。这些基因在其它活检中显示出更高的表达水平并且其中包括 WIF1、四连接素 (Tetranectin)、IGFBP6 和 IGFBP5。最后一组是在局限型硬皮病和弥漫型硬皮病亚群中较高的异源基因表达集群,包括但不限于 UTS2R、GALR3、PARD6G、PSEN1、PHOX2A、CENTG3、HCN4、KLF16 和 GPR15G。Milano 等在通过引用整体并入本文的“Molecular Subsets in the Gene Expression Signatures of Scleroderma Skin”(PLOS One,3:7,1-18,2008) 中描述了另外的差异性表达的示例性基因。

[0206] 作为替代标志的多基因印记

[0207] 基因的组合可用作生物标志物。例如,Farina 等,在通过引用整体并入本文的“A Four-Gene Biomarker Predicts Skin Disease in Patients with Diffuse Cutaneous Systemic Sclerosis”(Arthritis Rheum.62(2),580-588,2010) 中提供了用于生物标志物鉴定的示例性方法。从已知会在硬皮病中受调节的靶标例如 TGFβ 和干扰素开始,Farina

鉴定了包括基因 CTGF、THS1、COL4 和 PAI1 的四基因生物标志物。发现这四个基因的组合转录与改良的 Rodnan 皮肤得分 (mRSS) 高度相关并且高度预测弥漫型硬皮病。

[0208] mRSS 用作硬皮病的一种临床标志物。通常，如图 2 所示指定 mRRS :未累及的皮肤指定得分 0 ;轻度增厚给予得分 1 ;中等增厚给予得分 2 ;并且严重增厚给予得分 3。通常，可基于 0-3 等级确定患者 17 个皮肤区域处范围在 0-51 的 mRSS 总分。mRSS 可单独或与其它生物标志物组合用作诊断和监测治疗的指标。

[0209] 可使用类似策略鉴定和验证硬皮病的潜在印记生物标志物。具体地，单独地或呈组合来试验鉴定为在硬皮病中受正向或负向调控的基因转录产物以鉴定由与硬皮病临床标志物最高度相关的基因转录产物或基因转录产物的组合组成的生物标志物。除 mRSS 外，可使用其它临床标志物，例如 HAQ - DI、DLCO 或 FVC。

[0210] CCL2 水平

[0211] CCL2 水平，例如 CCL2 血清水平，可用作测定疾病严重程度、器官累及、选择适当疗法、监测疾病进展和患者反应的生物标志物或指标。为测定 CCL2 水平作为生物标志物或指标，测定了处于硬皮病多个阶段的患者和未受影响的个体的血清中的 CCL2 水平。这可通过例如 ELISA 测定血清中的 CCL2 蛋白水平进行，并且与皮肤和其它器官（例如，肺部、肝脏、肾脏、食道）累及相关联。在 Carulli 等 Ann Rheum Dis.67:105-109,2008 中描述了示例性方法。

[0212] 在皮肤（例如来自于活检）和 / 或血清中的 CCL2 水平也可与 mRSS 或其它临床标志物相关联，例如健康评估量表 (HAQ -DI)、肺一氧化碳弥散量 (DLCO) 或用力肺活量 (FVC)。

[0213] 各种生物标志物可单独地或组合使用，或可选地，与临床诊断标志物，例如 mRSS 一起使用，以基于硬皮病的严重程度为患者分层，选择合适的疗法或给药方案，评价疗法的有效性，监测对疗法的反应性，病程预后和测量在受试者中的疾病进展。通常，在此类方法中，将从一个或多个时间点对从受试者获得的生物样品测定的适合生物标志物的水平（例如，如选自本文描述的各种差异性表达基因的生物标志物和其它已知标志物如 CCL2 水平）与从一个或多个其它时间点来自于受试者的水平做比较。例如，可在疗程开始之前或开始时测量生物标志物水平。可在整个疗程的一个或多个时间点测量生物标志物水平并且与治疗之前或来自于疗程早期时间点的水平做比较。合适的治疗的鉴定或选择，确定患者是否对治疗具有积极反应和 / 或治疗的优化可基于生物标志物的评价确定。

[0214] 药物组合物

[0215] 本发明还提供了包含一种或多种所提供的分子（例如，抗体、fynomer、适配体、融合蛋白、蛋白结合结构域）的组合物。在一些实施方案中，本发明提供了至少一种分子和至少一种药学上可接受的赋形剂。此类药物组合物可任选地包含一种或多种附加治疗活性物质和 / 或与一种或多种附加治疗活性物质组合施用。在一些实施方案中，提供的药物组合物用于药品中。在一些实施方案中，提供的药物组合物在硬皮病或与硬皮病相关或关联的负面分支的治疗或预防中用作预防剂（即，疫苗）。在一些实施方案中，提供的药物组合物例如在患有或易感硬皮病的个体中，用于治疗应用。在一些实施方案中，配制药物组合物用于向人施用。

[0216] 例如，此处提供的药物组合物可呈无菌注射形式（例如，适于皮下注射或静脉输注的形式）提供。例如，在一些实施方案中，药物组合物呈适于注射的液体剂型提供。在一

些实施方案中，药物组合物呈粉剂（例如，经冻干和/或灭菌）提供，任选在真空下，其在注射之前用水性稀释剂（例如，水、缓冲液、盐溶液等）复溶。在一些实施方案中，药物组合物在水、氯化钠溶液、醋酸钠溶液、苯甲醇溶液、磷酸盐缓冲盐水等中稀释和/或复溶。在一些实施方案中，粉剂应与水性稀释剂轻轻混合（例如，不振荡）。

[0217] 在一些实施方案中，提供的药物组合物包含一种或多种药学上可接受的赋形剂（例如，防腐剂、惰性稀释剂、分散剂、表面活性剂和/或乳化剂、缓冲剂等）。在一些实施方案中，药物组合物包含一种或多种防腐剂。在一些实施方案中，药物组合物不含防腐剂。

[0218] 在一些实施方案中，药物组合物呈可冷藏和/或冷冻的形式提供。在一些实施方案中，药物组合物呈不可冷藏和/或冷冻的形式提供。在一些实施方案中，复溶溶液和/或液体剂型可在复溶后储存一段时间（例如，2小时、12小时、24小时、2天、5天、7天、10天、2周、一个月、两个月或更长）。在一些实施方案中，抗体组合物的储存比规定时间更长导致分子降解。

[0219] 液体剂型和/或复溶溶液在施用之前可包含微粒物质和/或变色。在一些实施方案中，如果变色或浑浊和/或如果过滤后仍有微粒物质，则不应使用溶液。

[0220] 可通过药理学领域中已知或此后开发的任何方法制备本文描述的药物组合物的组成成分。在一些实施方案中，此类制备方法包括使得活性成分与一种或多种赋形剂和/或一种或多种其它辅助成分缔合，然后，如有必要和/或需要，将产品成形和/或包装成所需单剂量或多剂量单位的步骤。

[0221] 按照本发明的药物组合物可呈散装、作为单一单位剂量和/或作为多个单一单位剂量制备、包装和/或销售。如本文中所用，“单位剂量”是包含预定量的活性成分的药物组合物的个别量。活性成分的量通常等于将要施用给受试者的剂量和/或此类剂量的便利分数，如例如此类剂量的一半或三分之一。

[0222] 按照本发明的药物组合物中活性成分、药学上可接受的赋形剂和/或任何附加成分的相对量可根据受治受试者的特征、大小和/或情况和/或根据施用所述化合物的途径而改变。举例而言，所述组合物可包含介于0.1％和100％(w/w)之间的活性成分。

[0223] 本发明的药物组合物可另外包含药学上可接受的赋形剂，如本文中所用，赋形剂可以是或包含适于所需特定剂型的溶剂、分散介质、稀释剂或其它液体媒介物、分散或悬浮助剂、表面活性剂、等渗剂、增稠剂或乳化剂、防腐剂、固体粘合剂、润滑剂等。Remington的The Science and Practice of Pharmacy，第21版，A.R.Gennaro,(Lippincott,Williams &Wilkins,Baltimore,MD,2006)公开了用于配制药物组合物的各种赋形剂及其已知制备技术。除非任何传统赋形剂介质例如因产生任何不良生物学效应或另外以有害方式与药物组合物的任何其它组分相互作用而与物质或其衍生物不相容，否则考虑赋形剂介质的用途在本发明的范围之内。

实施例

[0224] 将通过以下非限制性实施例进一步说明本发明。提出这些实施例是为了帮助理解本发明，而非旨在以任何方式限制其范围，并且不应解释为以任何方式限制其范围。所述实施例不包括对本领域普通技术人员众所周知的常规方法的详细描述。除非另外指出，否则份为重量份，分子量为平均分子量，温度用摄氏度表示，并且压力为或接近大气压。

[0225] 实施例 1. 双特异性抗 CCL2/LOXL2 抗体的制备

[0226] 这个实施例说明了双特异性抗 CCL2/LOXL2 抗体的制备。如上所述，各种方法可用于生成并选择具有所需特异性和结合亲和力的双特异性抗体。

[0227] 在这个特定实施例中，双特异性抗体由抗 CCL2 的完整抗原结合臂和抗 LOLX2 的完整抗原结合臂组成。具体地，生成人源化 CCL2 特异性单克隆抗体的小鼠细胞系与生成人源化 LOXL2 特异性单克隆抗体的大鼠细胞系融合以产生四源杂交瘤。通过 FACS 分析检验四源杂交瘤细胞上清液与靶细胞的结合。通过蛋白 A 亲和色谱法，接着通过离子交换色谱法从四源杂交瘤细胞培养物中纯化抗体。

[0228] 实施例 2. 剂量范围试验

[0229] 这个实施例说明了为估计用于治疗硬皮病的双特异性抗 CCL2 和抗 LOXL2 抗体的有效剂量范围而设计的剂量反应研究。

[0230] 在这个实施例中使用博莱霉素诱导的硬皮病小鼠模型。通常，通过向背部皮肤重复皮下注射博莱霉素、聚肌苷酸 - 聚胞苷酸和 / 或 LPS 在小鼠中诱导纤维化。具体地，向各 10 只的多组 B6 小鼠皮下植入装有浓度为 10-110 μg 和高达 200 μg 的博莱霉素、浓度为 300 μg 的 LPS、浓度为 100 μg 的聚胞苷酸或单独的 PBS 的渗透泵 (7 天)。在这个小鼠模型中，皮肤中的组织病理学变化非常类似于在硬皮病中看到的组织病理学变化。早期单核细胞积累及 TGF-β 和趋化因子表达上调之后是特征在于粗胶原蛋白束和活化的成纤维细胞积累的真皮纤维化。小鼠也表现出肺和肾纤维化的迹象。

[0231] 浓度逐步升高的双特异性 CCL2/LOXL2 抗体或对照抗体的剂量经由腹腔内注射向小鼠施用。

[0232] 实施例 3. 双特异性抗 CCL2/LOXL2 抗体的体内功效

[0233] 这个实施例说明了为估计对于硬皮病而言用抗 CCL2/LOXL2 抗体治疗对博莱霉素小鼠模型中的炎症和纤维化的影响而设计的研究。

[0234] 将向 B6 小鼠皮下植入装有单独的 PBS 或 10-110 μg 和高达 200 μg 于 PBS 中的博莱霉素的 7 或 28- 天渗透泵。每 2 天，将经由腹腔内注射用如实施例 2 中所测定的适合浓度的抗 CCL2/LOXL2 双特异性抗体或用对照抗体治疗小鼠。

[0235] 在 7 天渗透泵的情况下，在 7 天后，或在 28 天渗透泵的情况下，在 28 天后，收获皮肤和肺组织用于转录和组织学分析。通过 ELISA 测量组织样品中 CCL2 蛋白的水平。对于转录分析，从皮肤组织提取 RNA 并且使用本领域通常已知的技术使分离的 RNA 进行半定量或定量逆转录酶 PCR。使用商购可获得的引物(TaqMan®)测量 TGF β 基因表达水平和促炎性基因，包括但不限于 PAI1、COMP、COL1a1、F4/80、IL-6 和 TNF α 的基因表达水平。对于组织学分析，通过显微镜检查经苏木精和伊红 (H&E) 染色的组织切片分析皮肤纤维化。H&E 染色用于使组织形态可视化的用途在本领域众所周知。使用免疫组织化学通过显微镜检测使用本领域众所周知的技术用单核细胞特异性抗 F4/80 抗体探测的组织切片来量化单核细胞浸润。

[0236] 预计用抗 CCL2/LOXL2 抗体治疗将减少单核细胞和巨噬细胞的浸润，将减少炎症基因表达 (例如，IL-6、TNF α)，并且将降低 TGF β 诱导的标记物基因表达。预料这会导致纤维化普遍降低。

[0237] 实施例 4. 治疗建模

[0238] 这个实施例说明了用于预测组织靶标水平的在各种组织和血浆中的 CCL2 生成和更新模型。说明的模型表示高 CCL2 水平的极端呈现。

[0239] 通常,CCL2 在疾病组织中生成并分泌到血浆中。在健康个体中,皮肤中的 CCL2 合成较低或不可检测。在未受影响和受影响的皮肤中 CCL2 合成随总皮肤的累及率而增加,导致血清 CCL2 水平增加。血清 CCL2 水平还随器官累及率而增加。通常,健康个体具有低于约 100pg/ml 的平均血清 CCL2 水平。具有所称的雷诺氏现象的个体具有略微提高的平均血清 CCL2 水平。患有硬化的患者通常具有约 250pg/ml 的平均血清 CCL2 水平。患有局限型皮肤系统性硬化的患者通常具有约 250pg/ml 的平均血清 CCL2 水平。患有弥漫型皮肤系统性硬化的患者通常具有约 380pg/ml 的平均血清 CCL2 水平。患有局限型皮肤系统性硬化的患者通常具有约 250pg/ml 的平均血清 CCL2 水平。

[0240] CCL2 的分子量为约 8.6kDa,比约 50kDa 的肾小球过滤阈值小得多,导致肾脏快速清除。CCL2 通过活性受体介导的内化作用而内化。CCL2 结合其受体 CCR2 的典型 kd 为约 60pM-2nM。CCR2 主要存在于淋巴源细胞和淋巴管内皮上。考虑到硬皮病在疾病进展早期引起血管通透性增加,这容许 CCL2 和任何治疗性抗体在间质组织和血清之间的基本平衡。因此,基于来自于小鼠和兔的数据,CCL2 的血清半衰期为约 10 分钟。预计在人类中 CCL2 血清半衰期相似。相对可渗透的组织允许 CCL2 从组织到血清(半最大)快速达到平衡,例如在约 2 小时内。在一些情况下,血清 CCL2 水平可达到 1000pg/ml(～ 75pM),全身皮肤累及但无器官累及。图 3 中示出了显示血清和组织 CCL2 平衡的目标曲线,预测了中和 3nM 组织 CCL2 并且与之竞争脱离其受体需要的抗体所需量。

[0241] 静脉注射的单克隆抗体通常结合血浆中的 CCL2 并且在其到达患病组织之前形成复合物,导致单克隆抗体浪费。双特异性 mAb 允许我们用“自由”抗 CCL2 臂将 mAb 隔绝在患病组织中以与组织 CCL2 结合,这样提供了 CCL2 的组织特异性靶向(参见图 4)。我们可以为 CCL2 设计亲和力,使其不与血清 CCL2 结合但是结合组织 CCL2。此外,我们还可以通过增加剂量以 60pM 亲和力竞争 CCR2。因此,这种方法允许我们优先抑制与血浆 CCL2 相对的组织 CCL2,导致对硬皮病的高效治疗。

[0242] 图 5 中基于以下假设示出了来自于为双特异性结合蛋白建模的一组初步结果:抗 LOXL2 臂具有 1pM 或更佳的 kd;抗 CCL2 臂具有范围介于 500pM 和 1nM 之间的 kd;并且结合 LOXL2 的 mAb 未内化或降解。

[0243] 实施例 5:临床设计

[0244] 基于动物治疗的成功,在健康个体和具有不同阶段的硬皮病的个体中设计了表 3-7 中详述的抗 CCL2/LOXL2 双特异性抗体 I-III 期剂量范围和单剂量研究以评价抗 CCL2/LOXL2 的安全性、耐受性、功效和药代动力学。

[0245] 人临床试验 1 的主要目的包括测定在健康个体中施用的抗 CCL2/LOXL2 抗体的 4 个剂量水平的安全性。次要目的包括评价在健康个体中施用的抗 CCL2/LOXL2 抗体的 4 个不同剂量水平的药代动力学。表 3 中示出了这种临床试验的详细方案概要。

[0246] 表 3:人临床试验 1

[0247]

阶段	1 期

[0248]

试验次数	1 次
患者群体	健康志愿者
试验设计和终点	单剂量、剂量递增 主要目的：安全性 次要目的：PK
受试者数量	4 个剂量组 各组 n=4 总共 16 名受试者
试验时长(FPI 到 LPV)	0.5 年 服药~6 周 ~15 周跟踪 PK
注释	1 期单一单位

[0249] 人临床试验 2 的主要目的包括测定在具有硬皮病早期症状的个体中施用的抗 CCL2/LOXL2 抗体的 4 个剂量水平的安全性。次要目的包括 (1) 测定在具有硬皮病早期症状的个体中施用的抗 CCL2/LOXL2 抗体的 4 个不同剂量水平的药代动力学，(2) 通过在连续皮肤活检中测定基因表达来测定具有硬皮病早期症状的个体对抗 CCL2/LOXL2 抗体的 4 个不同剂量水平的药效动力学 (PD) 反应及 (3) 正如通过改良的 Rodnan 皮肤得分 (mRSS) 所测量，测定具有硬皮病早期症状的个体对抗 CCL2/LOXL2 抗体的 4 个不同剂量水平的临床反应。表 4 中示出了这种临床试验的详细方案概要。

[0250] 表 4 :人临床试验 2

[0251]

阶段	1/2 期
试验次数	1 次
患者群体	早期(自非雷诺氏现象(RP)症状发作以来<2 年)弥漫型 SSc mRSS≥15
试验设计和终点	多剂量递增 双盲安慰剂对照 治疗持续时间：6 个月 4 个剂量水平 主要目的：安全性 次要目的：PK PD 反应(连续皮肤活检基因表达–基线、4 周、6 个月) 临床反应(mRSS)
受试者数量	4 个剂量组 各组 n=10 (8 名给予活性剂/2 名给予安慰剂)

[0252]

	总共 40 名受试者
试验时长(FPI 到 LPV)	1.5 年
注释	1 年内多达 8 个部位恢复

[0253] 人临床试验 3 的主要目的包括正如通过改良的 Rodnan 皮肤得分 (mRSS) 所测量，

测定在具有硬皮病早期症状的个体中施用的抗 CCL2/LOXL2 抗体的单个剂量水平的功效。次要目的包括 (1) 正如通过健康评估量表 - 功能障碍指数 (HAQ -DI) 所测量，测定在具有硬皮病早期症状的个体中施用的抗 CCL2/LOXL2 抗体的单个剂量水平的功效及 (2) 正如通过器官特异性评估所所测量，测定在具有硬皮病早期症状的个体中施用的抗 CCL2/LOXL2 抗体的单个剂量水平的功效。表 5 中示出了这种临床试验的详细方案概要。

[0254] 表 5 :人临床试验 3

[0255]

阶段	2 期
试验次数	1 次
患者群体	早期(自非雷诺氏现象(RP)症状发作以来<2 年)弥漫型 SSc mRSS≥15
试验设计和终点	1 个剂量水平 双盲安慰剂对照平行组 治疗持续时间 6 个月 公开标签扩展 主要目的：mRSS 次要目的：HAQ DI、器官特异性评估
受试者数量	2:1 随机化 总共 120 名受试者
试验时长(FPI 到 LPV)	1.5 年
注释	1 年内多达 20 个部位恢复

[0256] 人临床试验 4 的主要目的包括正如通过用力肺活量 (FVC) 所测量，测定在具有局限型或弥漫型硬皮病和肺部疾病的个体中施用的抗 CCL2/LOXL2 抗体的单个剂量水平相对于口服环磷酰胺的功效。次要目的包括 (1) 正如通过 HAQ - DI 所测量，测定在具有局限型或弥漫型硬皮病和肺部疾病的个体中施用的抗 CCL2/LOXL2 抗体的单个剂量水平相对于口服环磷酰胺的功效，(2) 正如通过 mRSS 所测量，测定在具有局限型或弥漫型硬皮病和肺部疾病的个体中施用的抗 CCL2/LOXL2 抗体的单个剂量水平相对于口服环磷酰胺的功效，及 (3) 正如通过肺一氧化碳弥散量 (DLCO) 所测量，测定在具有局限型或弥漫型硬皮病和肺部疾病的个体中施用的抗 CCL2/LOXL2 抗体的单个剂量水平相对于口服环磷酰胺的功效。表 6 中示出了这种临床试验的详细方案概要。

[0257] 表 6 :人临床试验 4

[0258]

阶段	2 期
试验次数	1 次
患者群体	局限型或弥漫型 SSc 和肺部疾病： 通过 HRCT 测定的活动性齿槽炎 自非 RP 症状发作以来<7 年 预测 FVC<85%>45%
试验设计和终点	1 个剂量水平 双盲对照平行组 对比剂：SoC(口服环磷酰胺) 治疗持续时间 12 个月 公开标签扩展 主要目的：FVC 次要目的：DLCO、HAQ DI、mRSS
受试者数量	2:1 随机化 总共 120 名受试者
试验时长(FPI 到 LPV)	1.5 年
注释	6 个月内多达 10 个部位恢复

[0259] 人临床试验 5 的目的包括 (1) 正如通过用力肺活量 (FVC) 所测量，测定在具有硬皮病早期症状和 / 或局限型或弥漫型硬皮病和肺部疾病的个体中施用的抗 CCL2/LOXL2 抗体的单个剂量水平相对于口服环磷酰胺的功效，(2) 正如通过 HAQ - DI 所测量，测定在具有硬皮病早期症状和 / 或局限型或弥漫型硬皮病和肺部疾病的个体中施用的抗 CCL2/LOXL2 抗体的单个剂量水平相对于口服环磷酰胺的功效，(3) 正如通过 mRSS 所测量，测定在具有硬皮病早期症状和 / 或局限型或弥漫型硬皮病和肺部疾病的个体中施用的抗 CCL2/LOXL2 抗体的单个剂量水平相对于口服环磷酰胺的功效，及 (4) 正如通过 DLCO 所测量，测定在具有硬皮病早期症状和 / 或局限型或弥漫型硬皮病和肺部疾病的个体中施用的抗 CCL2/LOXL2 抗体的单个剂量水平相对于口服环磷酰胺的功效。

[0260] 表 7 :人临床试验 5

[0261]

阶段	3 期
试验次数	各 1 次
试验设计和终点	单个剂量水平，根据 2 期结果在早期 dSSc 或 SSc 肺部疾病的一者或二者中与 SoC 双盲逐一比较 与 2 期中相同的终点
受试者数量	各 120 名患者
试验时长(FPI 到 LPV)	2.0 年 0.5 至 1 年招募期
注释	治疗持续时间 12 个月

[0262] 预计用抗 CCL2/LOXL2 抗体治疗的表现出硬皮病早期症状的患者会展示出正如通过 mRSS 和 HAQ -DI 所测量的明显症状改善。预计用抗 CCL2/LOXL2 抗体治疗的具有局限型或弥漫型硬皮病和肺部疾病的患者会展示出正如通过 mRSS、HAQ - DI 和 FVC 测量的明显症状改善。预计正如通过 mRSS、HAQ - DI 和 FVC 所测量，抗 CCL2/LOXL2 抗体在具有硬皮病早

期症状或具有局限型或弥漫型硬皮病和肺部疾病的患者的治疗中比环磷酰胺更有效。

[0263] 实施例 6 :在博莱霉素诱导的纤维化中抗 CCL2 和抗 LOXL2 组合疗法的体内功效

[0264] 这个实施例描述了在硬皮病动物模型中用单特异性抗 CCL2 和抗 LOXL2 抗体的组合治疗经两周时程对炎症和纤维化的影响。进行在纤维化鼠类模型中使用抗 CCL2 或抗 LOXL2 抗体的单一疗法和组合疗法（抗 CCL2 和抗 LOXL2 抗体二者）的评价。使用慢性博莱霉素 14- 天皮下微量渗透泵并以皮肤和肺部纤维化作为药物功效的结果。如下所示，用两种抗体的组合疗法在皮肤和肺部纤维化中展示出显著效果。

[0265] 简单地说，使用博莱霉素 14- 天泵鼠类 SSc- 皮肤和肺部纤维化模型测试药物的功效。多组 (n ＝ 5 ;8-10 周）雌性 C57BL/6 小鼠经由渗透泵同时暴露于博莱霉素 (90U/Kg) 或 PBS(n ＝ 3 只小鼠)7 天，在第 14 天收获皮肤和肺部。从泵插入当天开始，暴露于博莱霉素的小鼠经腹腔用抗 CCL2（剂量 2mg/Kg/2×/ 周）、抗 LOXL2（剂量 15mg/Kg/2×/ 周）或 IgG- 对照（剂量 17mg/Kg/2×/ 周）每周处理两次，直至第 14 天。表 8 中列出了处理组。

[0266] 表 8

[0267]

处理组	描述
PBS	仅有 PBS 的微量渗透泵
IgG	有博莱霉素、IgG/IP/2×/周的微量渗透泵
抗 CCL2	有博莱霉素、抗 CCL2/IP/2×/周的微量渗透泵
抗 LOXL2	有博莱霉素、抗 LOXL2/IP/2×/周的微量渗透泵
两者	有博莱霉素、抗 CCL2 和抗 LOXL2/IP/2×/周的微量渗透泵

[0268] 皮肤纤维化的结果测量

[0269] 为测定在真皮纤维化中的药物功效，通过苏木精 - 伊红 (H&E) 和胶原沉积，通过曼森氏三色染色法 (Masson’s trichrome staining) 分析皮肤。另外，在临床上评估溃疡的存在并且使用 Olympus DP70 相机和 OLYMPUS® Micro Suite Basic 软件通过来自于每只小鼠的 4 个不同皮肤切片中表皮 - 真皮接合处和真皮 - 皮下脂肪接合处之间的最大距离来测量皮肤厚度。由不知情的调查员分析组织切片。

[0270] 肺部纤维化的结果测量

[0271] 为测定在肺部纤维化中的药物功效，分析组织学和基因表达。吹入的肺部在福尔马林中固定，石蜡包埋并且经 H&E、曼森氏三色染色法和精氨酸酶 -1 染色。曼森氏染色后盲法分析所有组的 Aschroft 得分 (Ashcroft 等，1988，J.Clin.Pathol.41:467-470)。在每张载玻片至少 4 个切片中也从 0 到 4 为精氨酸酶 -1 染色盲法评分。每只小鼠中 Aschroft 和精氨酸酶 -1 得分的平均数用作最终得分。

[0272] 统计分析

[0273] 通过单因素 ANOVA 和 Bonferroni 多重比较事后检验分析基因表达比较、特定染色的组织学分析和肺部得分。通过学生 T 检验分析两组比较。微阵列分析按照低于 10％的标准错误发现率 (FDR)，将处理样品与对照做比较。P 值小于或等于 0.05 视为在统计上显著。

[0274] 结果

[0275] 皮肤纤维化

[0276] 植入微量渗透泵的外科手术在小鼠中总体耐受良好。PBS 处理组中的一只小鼠在 18 小时后死亡,这可能归因于麻醉。图 6 示出了在每个处理组中观察到的皮肤溃疡的百分比。图 7 示出了为每个处理组测量的皮肤组织样品的厚度。

[0277] 如图 6 所示,没有经 PBS 处理的小鼠发展皮肤溃疡。相反,暴露于博莱霉素并且经 IgG(对照)或抗 CCL2 处理的所有小鼠均发展皮肤溃疡,而仅两只暴露于博莱霉素并且经抗 LOXL2 处理的小鼠发展皮肤溃疡。在组合处理组中,没有小鼠发展皮肤溃疡。

[0278] 如图 7 所示,与对照 (IgG) 相比在单一和组合疗法 (抗 CCL2、抗 LOX2、两者) 组中皮肤厚度受强烈抑制。表 9 中列出了每个组的平均倍数变化。

[0279] 表 9

[0280]

处理组	平均倍数变化 皮肤厚度
PBS	1.07
IgG	1.64
抗 CCL2	1.30
抗 LOXL2	1.34
两者	1.21 ($p<0.001$)

[0281] 肺部纤维化

[0282] 使用 Ashcroft 法 (如上所述 ;图 8) 为肺部组织样品评分。盲法分析全部 5 个处理组的肺部中的 Ashcroft 得分 (表 8)。表 10 中列出了每个处理组 Ashcroft 得分的平均数。对于所有处理组而言使用 ANOVA 分析,仅组合处理组 (两者) 与对照 (IgG ;$p<0.01$) 相比在统计上降低。比较对照 (IgG) 和抗 CCL2 组的学生 T 检验不显著,而对照与抗 LOXL2 处理组相比显示出倾向 ($p = 0.07$)。对照与组合处理组相比降低 ($p<0.01$)。

[0283] 表 10

[0284]

处理组	平均 Aschroft 得分
PBS	1.5
IgG	5.76
抗 CCL2	4.87
抗 LOXL2	3.38
两者	2.64 ($p<0.01$)

[0285] 精氨酸酶 -1 肺部染色

[0286] 对于每个处理组而言对肺部组织样品进行巨噬细胞活化的特定标志物,精氨酸酶 1(Arg1) 的染色 (图 9)。发明人先前已经观察到博莱霉素慢性模型基于 CD163 染色在肺部展示出强烈的巨噬细胞活化,这在 CCL2 缺陷小鼠中几乎消除。因此,因为已知进入肺部的细胞流入量峰值在博莱霉素模型的第 14 天发生,所以进行对来自于处理后所有博莱霉素暴露组的肺部细胞中 $CD163^{+}$表达的分析。暴露于博莱霉素后确认了在肺部中 $CD163^{+}$的强烈表达。

[0287] 盲法量化了在每只小鼠肺部的至少 4 个切片中的精氨酸酶 -1 表达。表 11 中列出了每个处理组的平均精氨酸酶 -1 得分。精氨酸酶 -1 染色与 Aschroft 得分强相关（图 10）。图 11 示出了经三色法染色的肺部组织样品的组织切片。图 12 和 13 示出了经精氨酸酶 1(Arg1) 染色的肺部组织样品的组织切片。

[0288] 表 10

[0289]

处理组	平均 Arg1 得分
PBS	0
IgG	2.82
抗 CCL2	3.32
抗 LOXL2	3.05
两者	2.01 (p=0.06)

[0290] 如这个实施例中所示，IgG 和抗 CCL2 处理组中的所有小鼠含皮肤溃疡。然而，抗 LOXL2 处理组的 5 只小鼠中仅 2 只含皮肤溃疡。有趣的是，接受抗 CCL2 和抗 LOXL2 抗体两者的处理组在收获的皮肤组织中未显示出任何溃疡（参见图 6）。方差分析 (ANOVA) 确认对于单一处理（单独的抗 CCL2 或抗 LOXL2) 和组合处理（抗 CCL2 和抗 LOXL2 一起）而言皮肤厚度的减小与 IgG 处理组相比显著（参见图 7）。因此，通过用抗 CCL2 和抗 LOXL2 抗体的组合疗法治疗纤维化疾病在经博莱霉素处理的小鼠中有效终止皮肤溃疡的形成，并且有效减少了皮肤组织的增厚。

[0291] 进一步地，ANOVA 还确认用抗 CCL2 和抗 LOXL2 抗体的组合疗法与 IgG 处理组 (Aschroft 得分为约 6) 相比显著地降低了肺部组织样品中的纤维化程度 (Aschroft 得分为约 2)（参见图 8）。用抗 CCL2 和抗 LOXL2 抗体的组合疗法在全部 4 个处理组的肺部中展示出最低的巨噬细胞活化水平 (Arg1 染色，参见图 9)。

[0292] 总的来说，这些数据证明在博莱霉素 14- 天鼠类模型中用单独的抗 CCL2、抗 LOXL2 或组合治疗显示皮肤和 / 或肺部纤维化减轻，当用抗 LOXL2 抗体或抗 CCL2 和抗 LOXL2 抗体的组合治疗时在皮肤和肺部中观察到最强效果。因此，抗 CCL2 和抗 LOXL2 抗体可组合施用以有效治疗和 / 或改善纤维化或相关炎性疾病（例如，硬皮病）、病症或病状的一种或多种症状。

[0293] 等效方案和范围

[0294] 本领域的技术人员使用不超出常规的实验将认识到，或能够确定本文描述的本发明的特定实施方案的许多等效方案。本发明的范围并非旨在限于以上说明书，而是如所附权利要求中所阐述。

[0295] 在权利要求中，除非指出相反或另外从上下文显而易见，否则冠词如“一”、“一种（个）”和“所述（该）”可意指一个或一个以上。因此，例如，提到“一种抗体”包括多种此类抗体，并且提到“所述细胞”包括提到本领域中技术人员已知的一种或多种细胞，等等。除非指出相反或另外从上下文显而易见，否则在一组的一个或多个成员之间包括“或”的权利要求或描述，在该组的一个、一个以上或所有成员存在、用于给定产物或工艺中或另外与给定产物或工艺相关时，被视为满足。本发明包括其中确切地该组的一个成员存在、用于给定产物或工艺中或另外与给定产物或工艺相关的实施方案。本发明包括其中该组的一个以上

或所有成员存在、用于给定产物或工艺中或另外与给定产物或工艺相关的实施方案。此外，应理解本发明涵盖其中将来自于所列权利要求中的一项或多项的一个或多个限制、要素、从句、描述性术语等引入另一权利要求中的所有变型、组合和排列。例如，从属于另一权利要求的任何权利要求均可修改为包括在从属于相同基本权利要求的任何其它权利要求中发现的一个或多个限制。此外，当权利要求叙述一种组合物时，除非另外指出或除非本领域中的普通技术人员显而易见将产生矛盾或不一致性，否则应理解包括将所述组合物用于本文公开的任一目的的方法，并且包括根据本文公开的制备方法或本领域中已知的其它方法的任一种制备所述组合物的方法。

[0296] 当要素呈列表，例如呈马库西群组(Markush group)形式呈现时，应理解也公开了所述要素的每个子群，并且可以从所述群组中去除任何要素。应理解，通常，在本发明或本发明的方面被称为包含特定要素、特征等时，本发明或本发明的方面的某些实施方案由或基本上由此类要素、特征等组成。例如，为了简单起见在本文的文字中尚未具体阐述那些实施方案。应当指出的是术语“包含”旨在为开放性并且容许包含附加要素或步骤。

[0297] 给定范围时，将端点包括在内。此外，应理解除非另外指出或本领域中的普通技术人员另外从上下文显而易见并且理解，否则表示为范围的值可以在本发明的不同实施方案中采用规定范围内的任何特定值或子范围，除非上下文另外明确指出，否则达到所述范围下限单位的十分之一。

[0298] 另外，应理解落入现有技术范围内的本发明的任何特定实施方案可明确地从权利要求的任一项或多项排除。因为将此类实施方案视为本领域中的普通技术人员已知，所以即使本文未明确阐述排除也可以将其排除。本发明的组合物的任何特定实施方案（例如，任何HCV基因型/亚型、任何HCV抗体、任何表位、任何药物组合物、任何施用方法、任何治疗应用等）可以出于任何原因，无论是否与现有技术的存在有关，都可从任何一项或多项权利要求中排除。

[0299] 以上和正文通篇所论述的出版物仅针对它们在本申请提交日之前的公开内容而提供。不得将本文任何内容解释为承认发明人由于先前公开而无权先于此类公开。

[0300] 其它实施方案

[0301] 本领域中的普通技术人员将易于意识到前述内容仅代表本发明的某些优选实施方案。如以下权利要求中所述，在不背离本发明的精神或范围的前提下可对上述程序和组合物做各种变化和修改。

[0001]

序列表

<110> 夏尔人类遗传性治疗公司

<120> 用于治疗硬皮病的抗 CCL2 和抗 LOXL2 组合疗法

<130> 2006685-0569

<150> 61/826692
<151> 2013-05-23

<160> 2

<170> PatentIn 版本 3.5

<210> 1
<211> 99
<212> PRT
<213> 智人

<400> 1

Met Lys Val Ser Ala Ala Leu Leu Cys Leu Leu Leu Ile Ala Ala Thr
1 5 10 15

Phe Ile Pro Gln Gly Leu Ala Gln Pro Asp Ala Ile Asn Ala Pro Val
20 25 30

Thr Cys Cys Tyr Asn Phe Thr Asn Arg Lys Ile Ser Val Gln Arg Leu
35 40 45

Ala Ser Tyr Arg Arg Ile Thr Ser Ser Lys Cys Pro Lys Glu Ala Val
50 55 60

Ile Phe Lys Thr Ile Val Ala Lys Glu Ile Cys Ala Asp Pro Lys Gln
65 70 75 80

Lys Trp Val Gln Asp Ser Met Asp His Leu Asp Lys Gln Thr Gln Thr
85 90 95

[0002]

Pro Lys Thr

<210> 2
<211> 638
<212> PRT
<213> 智人

<400> 2

Met Glu Gly Tyr Val Glu Val Lys Glu Gly Lys Thr Trp Lys Gln Ile
1 5 10 15

Cys Asp Lys His Trp Thr Ala Lys Asn Ser Arg Val Val Cys Gly Met
20 25 30

Phe Gly Phe Pro Gly Glu Arg Thr Tyr Asn Thr Lys Val Tyr Lys Met
35 40 45

Phe Ala Ser Arg Arg Lys Gln Arg Tyr Trp Pro Phe Ser Met Asp Cys
50 55 60

Thr Gly Thr Glu Ala His Ile Ser Ser Cys Lys Leu Gly Pro Gln Val
65 70 75 80

Ser Leu Asp Pro Met Lys Asn Val Thr Cys Glu Asn Gly Gln Pro Ala
85 90 95

Val Val Ser Cys Val Pro Gly Gln Val Phe Ser Pro Asp Gly Pro Ser
100 105 110

Arg Phe Arg Lys Ala Tyr Lys Pro Glu Gln Pro Leu Val Arg Leu Arg
115 120 125

[0003]

Gly Gly Ala Tyr Ile Gly Glu Gly Arg Val Glu Val Leu Lys Asn Gly
130 135 140

Glu Trp Gly Thr Val Cys Asp Asp Lys Trp Asp Leu Val Ser Ala Ser
145 150 155 160

Val Val Cys Arg Glu Leu Gly Phe Gly Ser Ala Lys Glu Ala Val Thr
165 170 175

Gly Ser Arg Leu Gly Gln Gly Ile Gly Pro Ile His Leu Asn Glu Ile
180 185 190

Gln Cys Thr Gly Asn Glu Lys Ser Ile Ile Asp Cys Lys Phe Asn Ala
195 200 205

Glu Ser Gln Gly Cys Asn His Glu Glu Asp Ala Gly Val Arg Cys Asn
210 215 220

Thr Pro Ala Met Gly Leu Gln Lys Lys Leu Arg Leu Asn Gly Gly Arg
225 230 235 240

Asn Pro Tyr Glu Gly Arg Val Glu Val Leu Val Glu Arg Asn Gly Ser
245 250 255

Leu Val Trp Gly Met Val Cys Gly Gln Asn Trp Gly Ile Val Glu Ala
260 265 270

Met Val Val Cys Arg Gln Leu Gly Leu Gly Phe Ala Ser Asn Ala Phe
275 280 285

Gln Glu Thr Trp Tyr Trp His Gly Asp Val Asn Ser Asn Lys Val Val
290 295 300

[0004]

Met Ser Gly Val Lys Cys Ser Gly Thr Glu Leu Ser Leu Ala His Cys
305 310 315 320

Arg His Asp Gly Glu Asp Val Ala Cys Pro Gln Gly Gly Val Gln Tyr
325 330 335

Gly Ala Gly Val Ala Cys Ser Glu Thr Ala Pro Asp Leu Val Leu Asn
340 345 350

Ala Glu Met Val Gln Gln Thr Thr Tyr Leu Glu Asp Arg Pro Met Phe
355 360 365

Met Leu Gln Cys Ala Met Glu Glu Asn Cys Leu Ser Ala Ser Ala Ala
370 375 380

Gln Thr Asp Pro Thr Thr Gly Tyr Arg Arg Leu Leu Arg Phe Ser Ser
385 390 395 400

Gln Ile His Asn Asn Gly Gln Ser Asp Phe Arg Pro Lys Asn Gly Arg
405 410 415

His Ala Trp Ile Trp His Asp Cys His Arg His Tyr His Ser Met Glu
420 425 430

Val Phe Thr His Tyr Asp Leu Leu Asn Leu Asn Gly Thr Lys Val Ala
435 440 445

Glu Gly Gln Lys Ala Ser Phe Cys Leu Glu Asp Thr Glu Cys Glu Gly
450 455 460

Asp Ile Gln Lys Asn Tyr Glu Cys Ala Asn Phe Gly Asp Gln Gly Ile
465 470 475 480

[0005]

Thr Met Gly Cys Trp Asp Met Tyr Arg His Asp Ile Asp Cys Gln Trp
485 490 495

Val Asp Ile Thr Asp Val Pro Pro Gly Asp Tyr Leu Phe Gln Val Val
500 505 510

Ile Asn Pro Asn Phe Glu Val Ala Glu Ser Asp Tyr Ser Asn Asn Ile
515 520 525

Met Lys Cys Arg Ser Arg Tyr Asp Gly His Arg Ile Trp Met Tyr Asn
530 535 540

Ser His Ile Gly Gly Ser Phe Ser Glu Glu Thr Glu Lys Lys Phe Glu
545 550 555 560

His Phe Ser Gly Leu Leu Asn Asn Gln Leu Ser Pro Pro Val Lys Lys
565 570 575

Pro Ala Trp Ser Thr Pro Val Phe Arg Pro His His Ile Phe His Gly
580 585 590

Thr Ser Pro Gln Gln Leu Ser Leu Asn Glu Cys His Val Pro Ser Pro
595 600 605

Ser Pro Ala Pro Thr Leu Ser Arg Pro Leu Gln Leu Cys Leu Ser Ser
610 615 620

Gly Gly Lys Gly Pro Ser His His Ser Trp Gly Ala Ala Thr
625 630 635

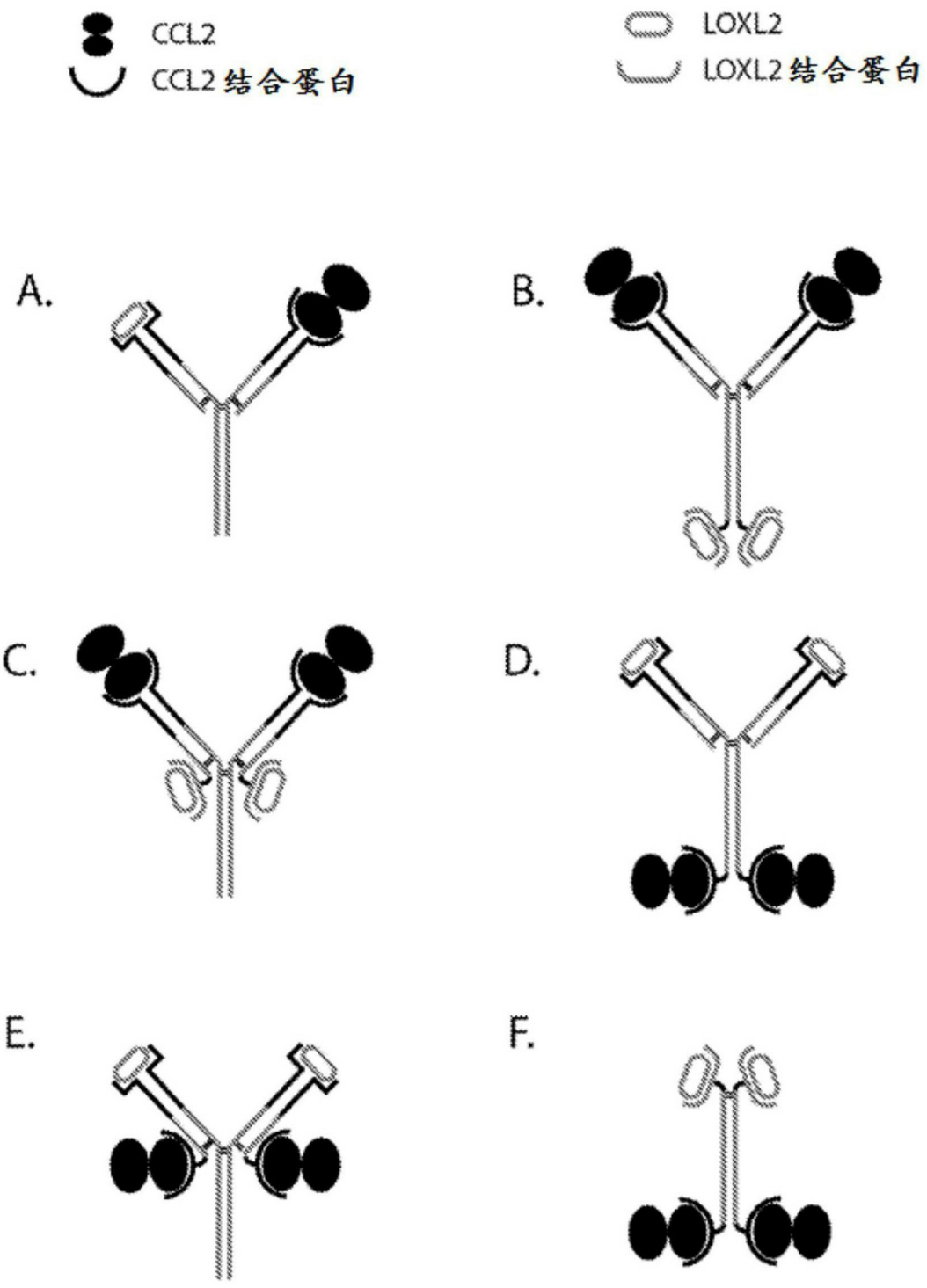


图 1

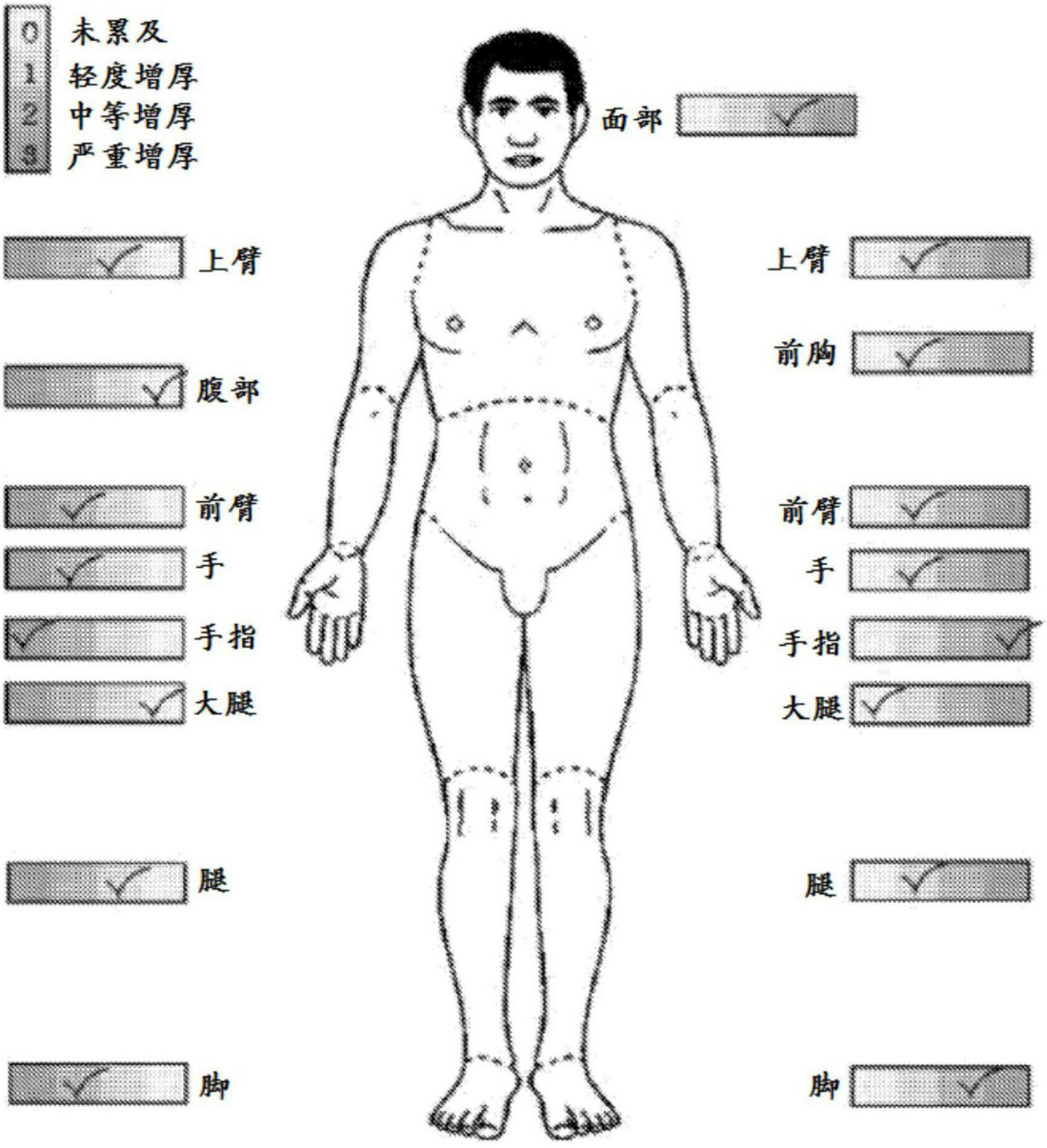


图 2

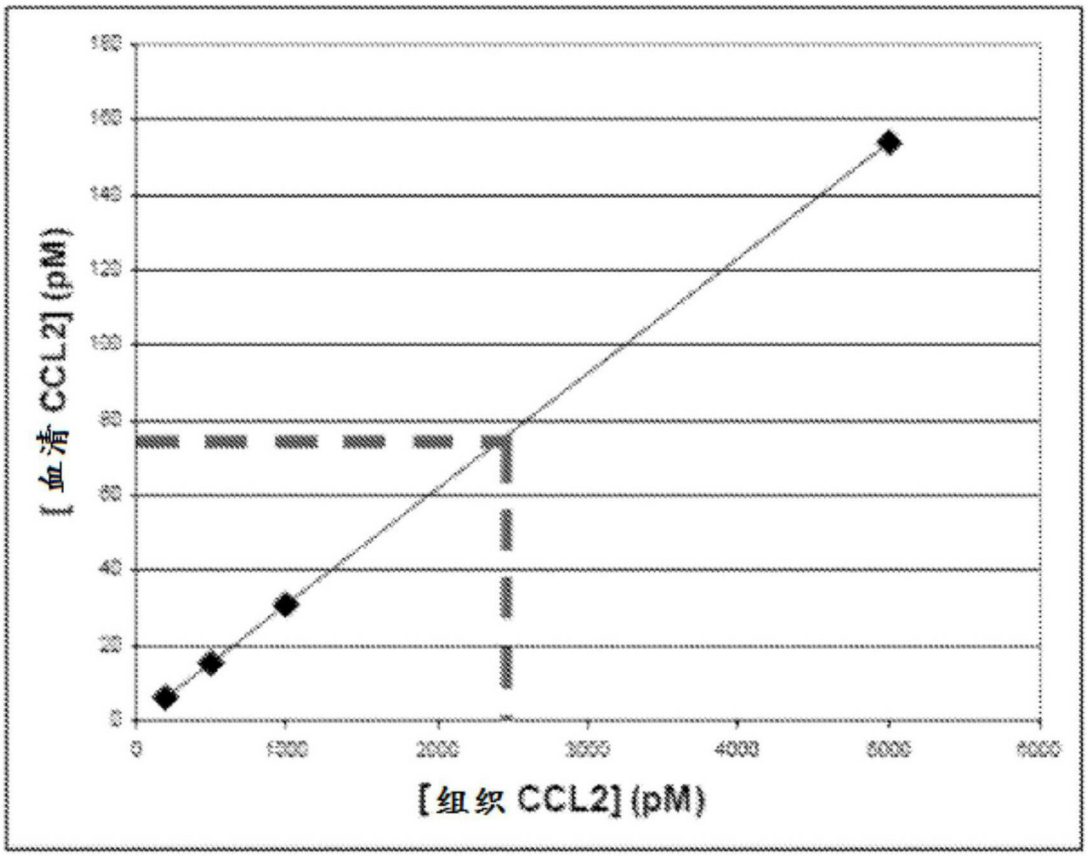

[血清 CCL2] (pM)
[组织 CCL2] (pM)
0
2000
4000

图 3

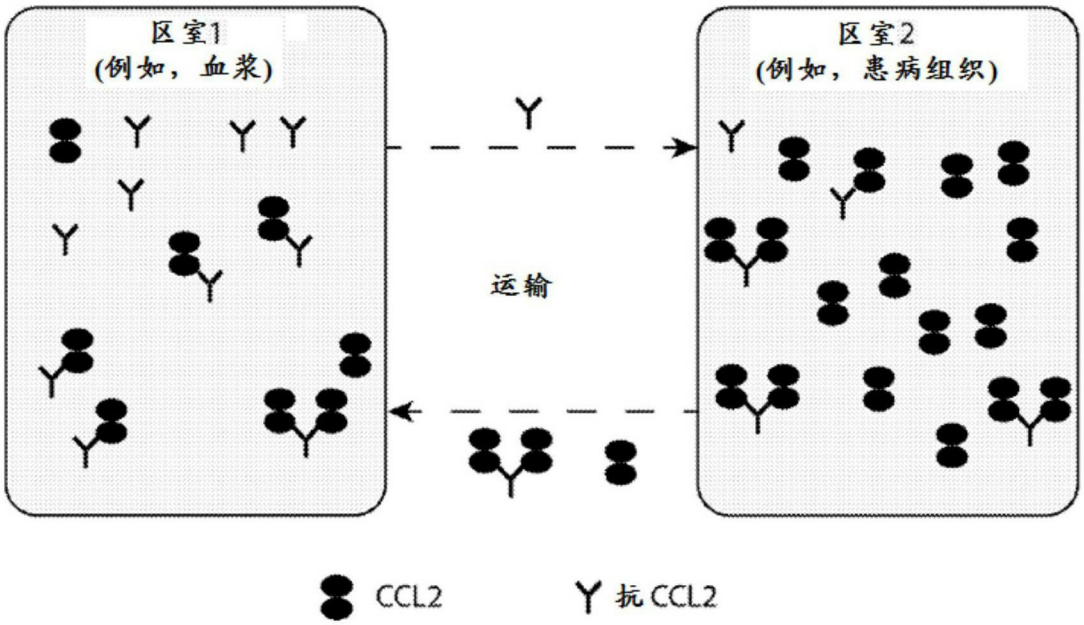

区室1
(例如，血浆)
区室2
(例如，患病组织)
运输
CCL2
抗 CCL2

图 4

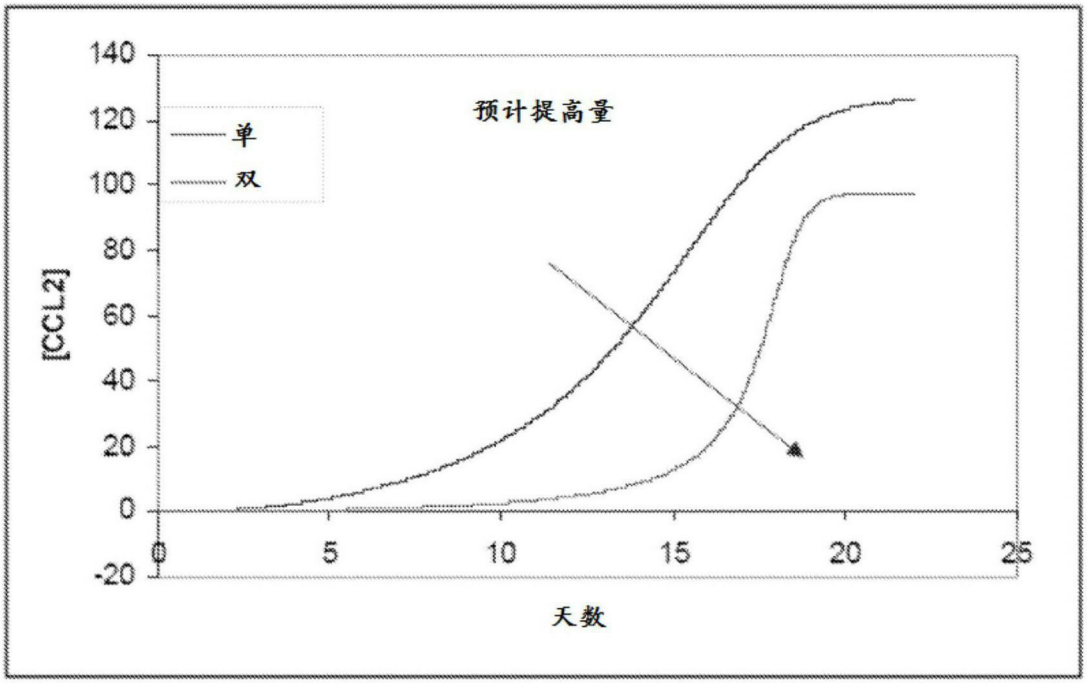

140
120
100
80
60
40
20
0
-20
[CCL2]
单
双
预计提高量
0
5
10
15
20
25
天数

图 5

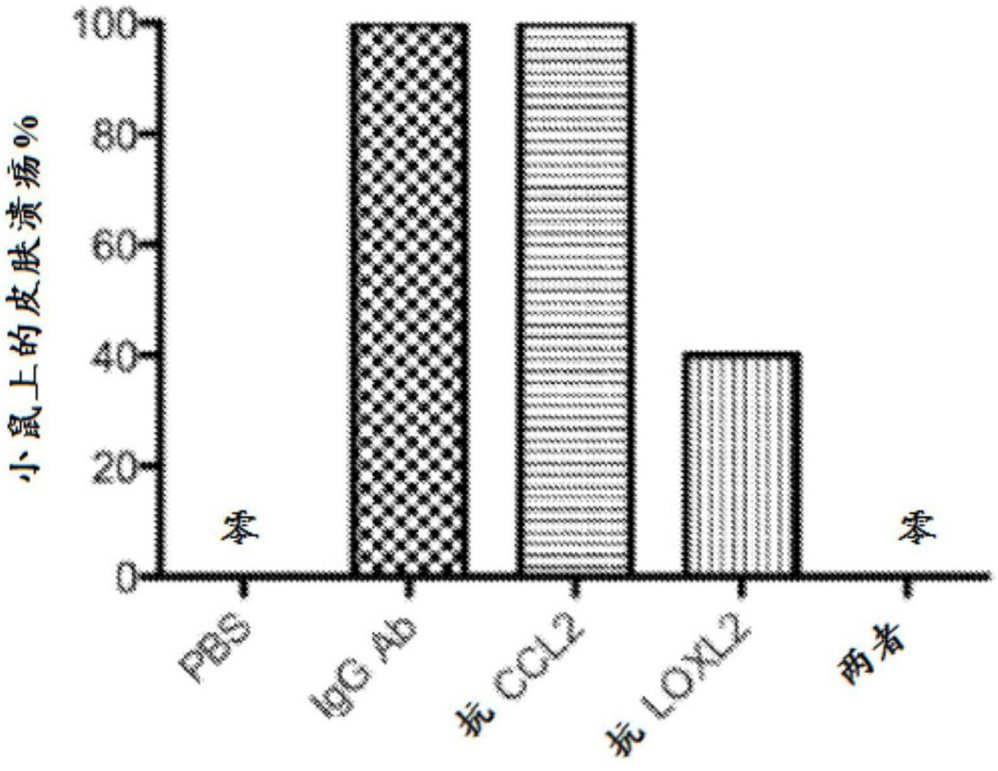
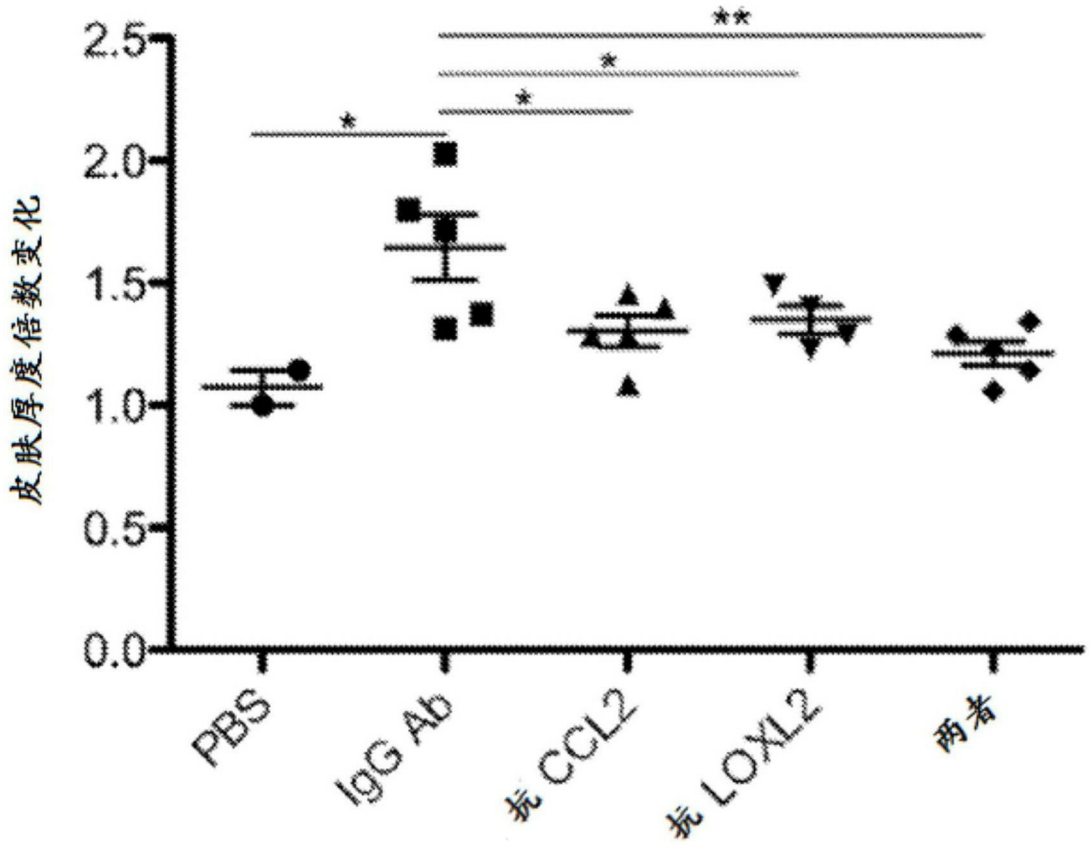

100
80
60
40
20
0
小鼠上的皮肤溃疡%
零
零
PBS
IgG Ab
抗 CCL2
抗 LOXL2
两者

图 6



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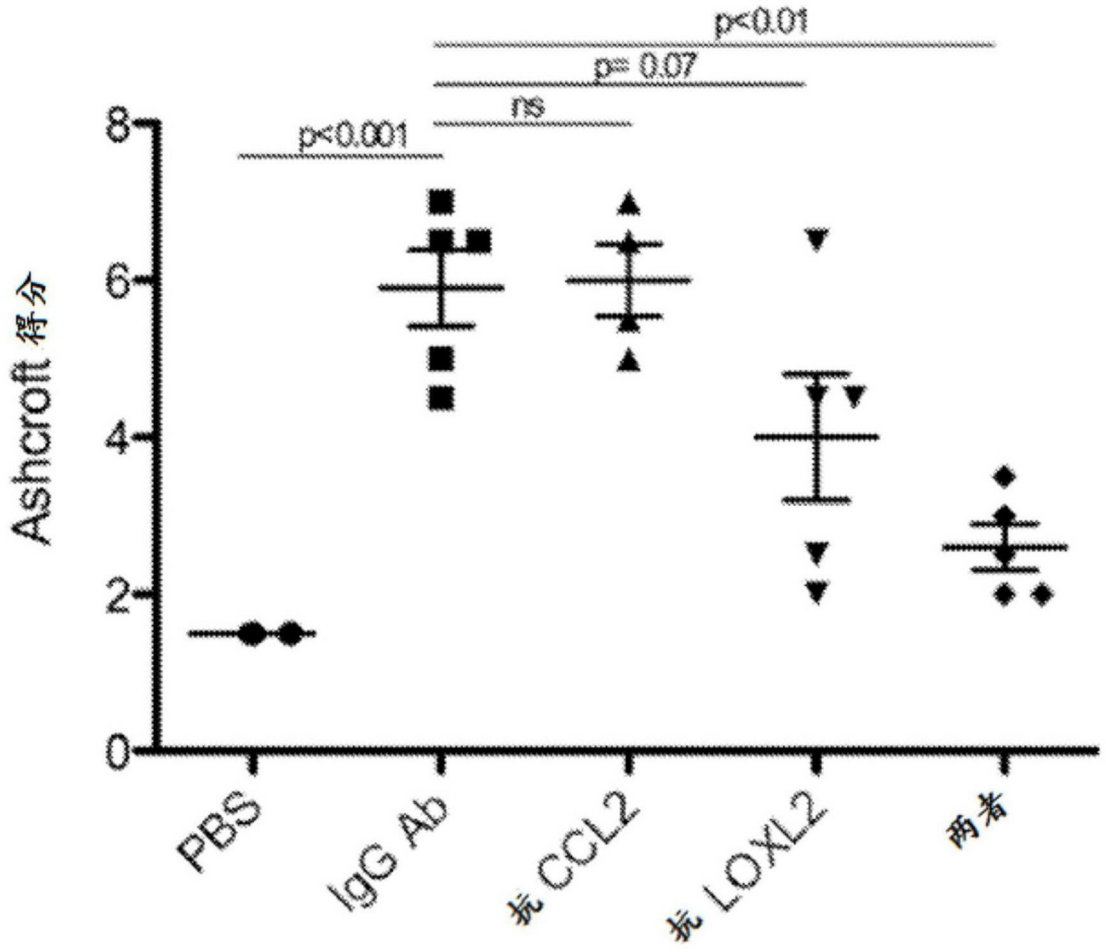
p<0.01
p= 0.07
ns
p<0.001
Ashcroft 得分
8
6
4
2
0
PBS
IgG Ab
抗 CCL2
抗 LOXL2
两者

图 8

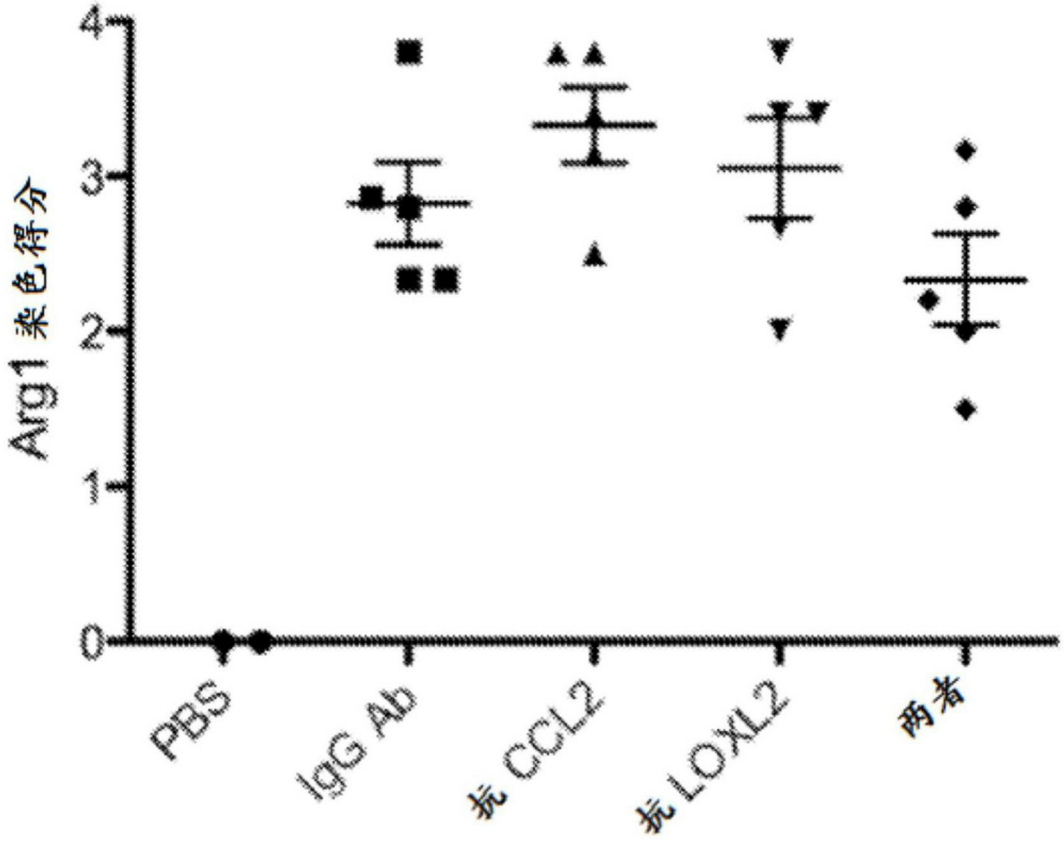

图 9

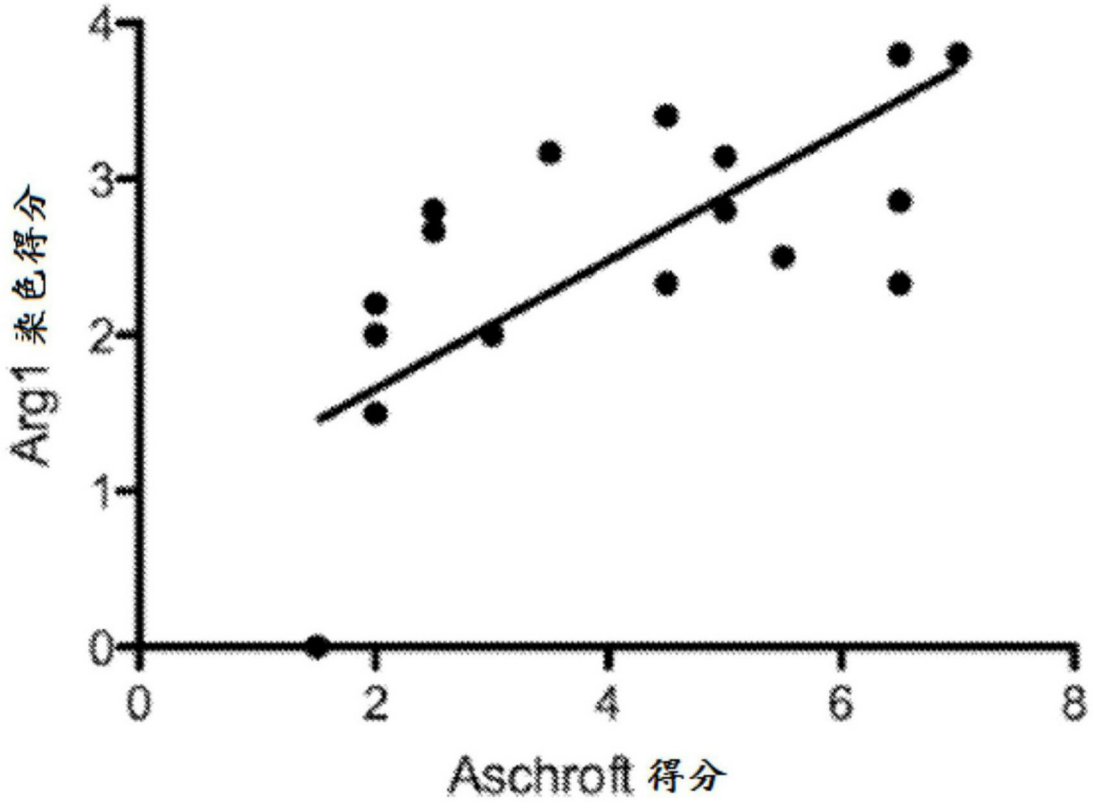

图 10

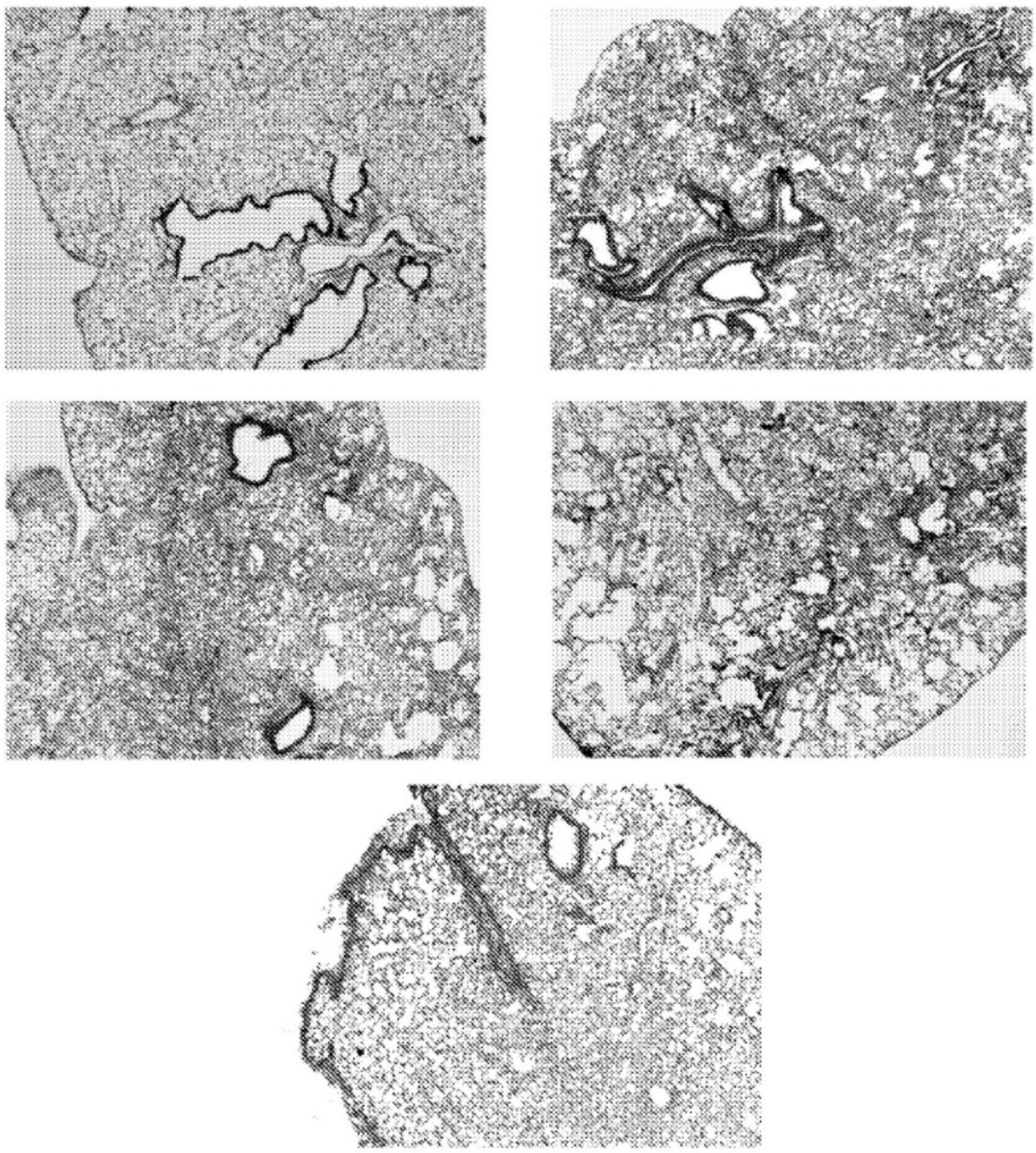

图 11

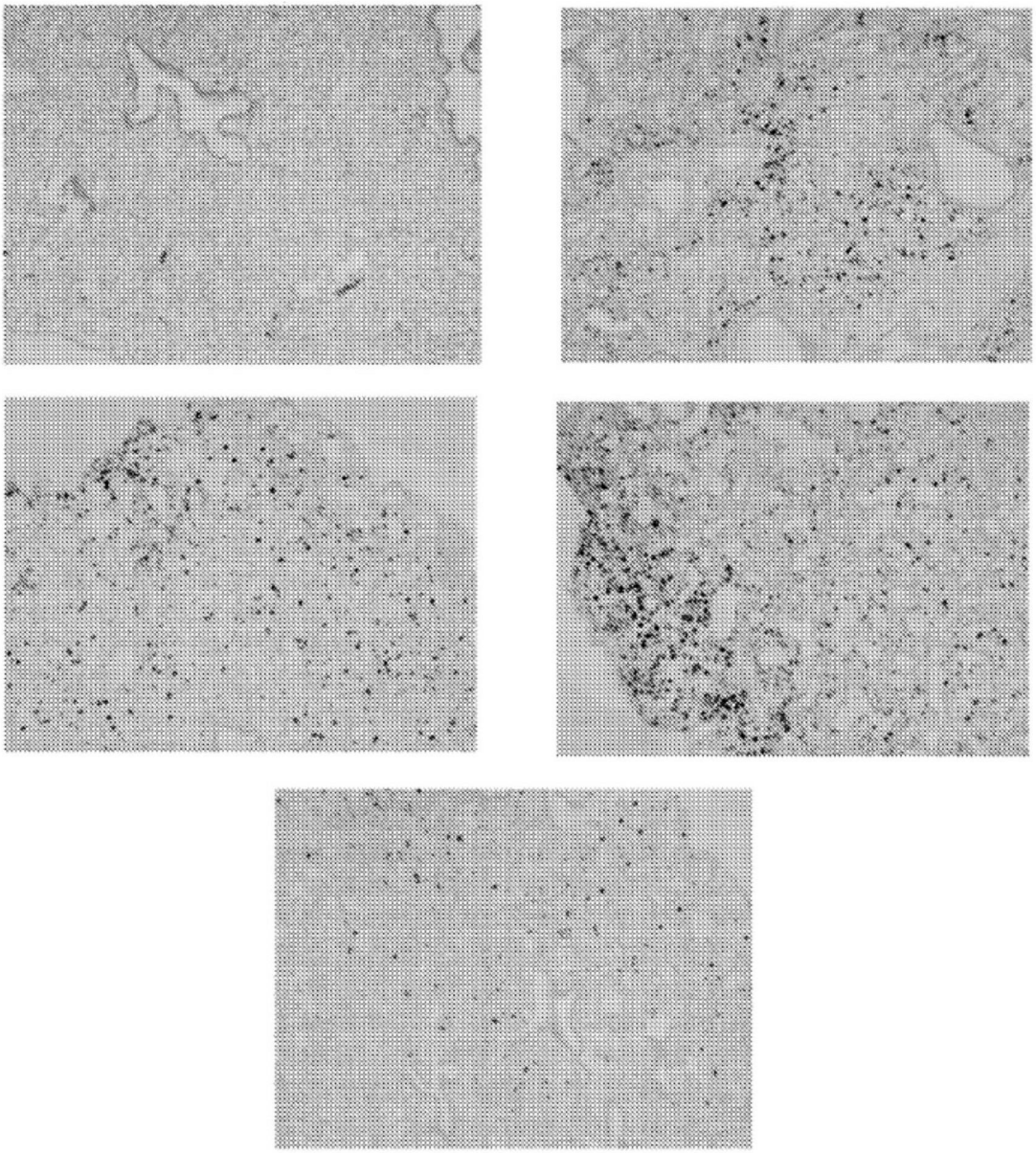

图 12

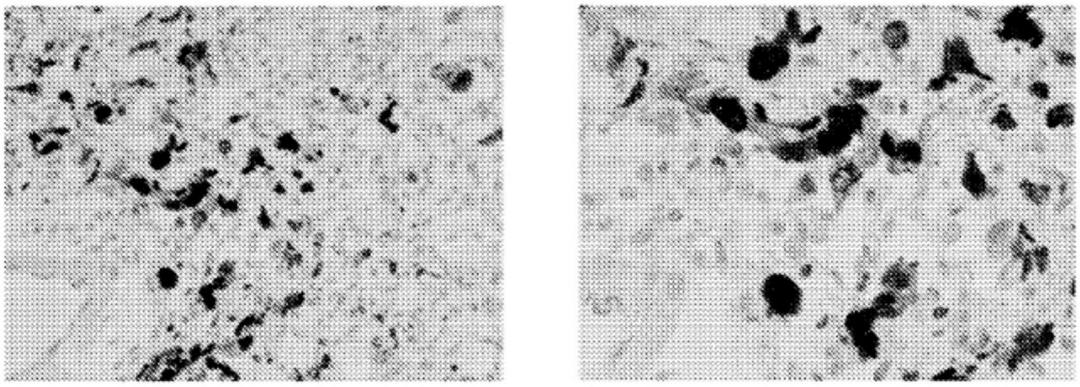

图 13