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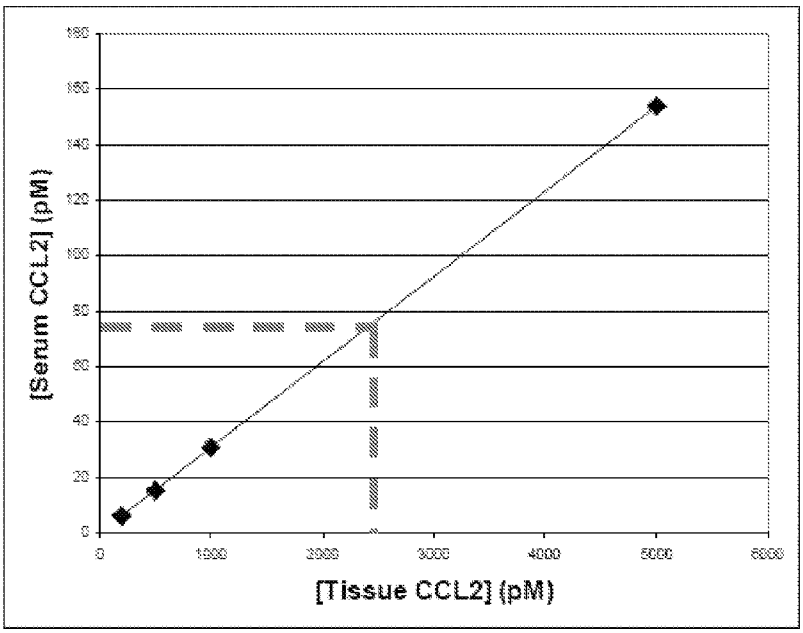
(54) Title: ANTI-CCL2 ANTIBODIES FOR TREATMENT OF SCLERODERMA



(57) Abstract: The present invention provides, among other things, improved anti-CCL2 antibodies characterized with high affinity, potency, tissue selectivity and/or epitope specificity, 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 provides methods and compositions for treatment of scleroderma and related fibrotic and/or inflammatory diseases, disorders and conditions based on an anti-CCL2 antibody having an affinity of 10^{-12} M or greater.

WO 2013/177264 A1

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ANTI-CCL2 ANTIBODIES 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. 61/650,149, filed May 22, 2012, 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 "2006685-0330_Sequences_ST25" on May 22, 2013. The .txt file was generated on May 14, 2013 and is 2 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 improved methods and compositions for effective treatment of scleroderma, in particular, based on improved antibodies or binding proteins that can specifically bind to C-C chemokine ligand-2 ("CCL2") with high affinity, potency, and/or epitope diversity to achieve robust biodistribution and/or tissue-specificity. 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 ineffective targeting of CCL2 in diseased tissues. To solve this problem, the present inventors contemplate the use of anti-CCL2 antibodies with high affinity administered in an amount sufficient to overcome the high levels of serum CCL2 leading to effective targeting of CCL2 in desired diseased tissues. In particular, the inventors contemplate "best in class" anti-CCL2 monoclonal antibody characterized with high binding affinity, tissue selectivity, epitope specificity and/or long half-life. Such inventive antibodies, once administered *in vivo*, result in desired biodistribution and bioavailability such that they binds and blocks CCL2 signaling in target tissues reducing infiltration, inflammation and fibrosis, among other symptoms or features of scleroderma.

[0007] Thus, in one aspect, the present invention provides methods of treating scleroderma comprising administering to an individual who is suffering from or susceptible to scleroderma an effective amount of anti-CCL2 antibody, or fragment thereof, such that at least one symptom or feature of scleroderma in a target tissue is reduced in intensity, severity, or frequency, or has delayed onset.

[0008] In some embodiments, the at least one symptom or feature of scleroderma is selected from 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.

[0009] 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.

[0010] 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.

[0011] In some embodiments, the anti-CCL2 antibody, or fragment thereof, 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.

[0012] In some embodiments, the anti-CCL2 antibody, or fragment thereof, is administered orally.

[0013] In some embodiments, anti-CCL2 antibody, or fragment thereof, is administered bimonthly, monthly, triweekly, biweekly, weekly, daily, or at variable intervals.

[0014] In another aspect, the present invention provides use of an anti-CCL2 antibody, or fragment thereof, 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 anti-CCL2 antibody, or fragment thereof, such that at least one symptom or feature of scleroderma in a target tissue is reduced in intensity, severity, or frequency, or has delayed onset.

[0015] In some embodiments, the present invention provides use of an anti-CCL2 antibody, or fragment thereof in the manufacture of a medicament for treating scleroderma as described herein, wherein the anti-CCL2 antibody, or fragment thereof, is characterized by binding affinity of stronger and/or greater than 10^{-12} M (e.g., greater than 0.5 X 10^{-12} M, 10^{-13} M, 0.5 X 10^{-13} M, 10^{-14} M, 0.5 X 10^{-14} M, or 10^{-15} M).

[0016] In some embodiments, an anti-CCL2 antibody, or fragment thereof, according to the invention is selected from the group consisting of intact IgG, $F(ab')_2$, $F(ab)_2$, Fab', Fab, scFvs, diabodies, triabodies and tetrabodies.

[0017] In some embodiments, the anti-CCL2 antibody, or fragment thereof, is a monoclonal antibody, optionally the anti-CCL2 antibody, or fragment thereof is a humanized monoclonal antibody, optionally the anti-CCL2 antibody, or fragment thereof is a human antibody.

[0018] 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, having a binding affinity of stronger and/or greater than 10^{-12} M (e.g., greater than 0.5 X 10^{-12} M, 10^{-13} M, 0.5 X 10^{-13} M, 10^{-14} M, 0.5 X 10^{-14} M, or 10^{-15} M).

[0019] In some embodiments, the anti-CCL2 antibody, or fragment thereof, is administered at a therapeutically effective dose and an administration interval such that the anti-CCL2 antibody, or fragment thereof, is distributed to one or more target tissues 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 anti-CCL2 antibody, or fragment thereof, is administered at a therapeutically effective dose and an administration interval such that the anti-CCL2 antibody, or fragment thereof, is distributed to lung and/or heart.

[0020] In some embodiments, the administration interval is selected from bimonthly, monthly, triweekly, biweekly, weekly, daily, or at variable intervals.

[0021] In yet 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, at a therapeutically effective dose and an administration interval such that the anti-CCL2 antibody, or fragment thereof, is distributed to lung and/or heart. In some embodiments, the anti-CCL2 antibody, or fragment thereof, is further distributed to skin, kidney, and/or liver.

[0022] In still another aspect, the present invention provides methods as disclosed in various embodiments above, 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.

[0023] In some embodiments, the anti-CCL2 antibody, or fragment thereof, is a monoclonal antibody. In some embodiments, the anti-CCL2 antibody, or fragment thereof, is a humanized monoclonal antibody. In some embodiments, the anti-CCL2 antibody, or fragment thereof, is a human antibody.

[0024] Among other things, the present invention provides anti-CCL2 antibodies with high affinity. In some embodiments, the present invention provides an anti-CCL2 antibody, or

fragment thereof, having a binding affinity of stronger and/or greater than 10^{-12} M (e.g., greater than 0.5 X 10^{-12} M, 10^{-13} M, 0.5 X 10^{-13} M, 10^{-14} M, 0.5 X 10^{-14} M, or 10^{-15} M).

[0025] In some embodiments, an anti-CCL2 antibody, or fragment thereof, according to the invention is selected from the group consisting of intact IgG, $F(ab')_2$, $F(ab)_2$, Fab', Fab, scFvs, diabodies, triabodies and tetrabodies.

[0026] In some embodiments, the anti-CCL2 antibody, or fragment thereof, is a monoclonal antibody.

[0027] In some embodiments, the anti-CCL2 antibody, or fragment thereof, is a humanized monoclonal antibody.

[0028] In some embodiments, the anti-CCL2 antibody, or fragment thereof, is a human antibody.

[0029] In another aspect, the present invention provides an anti-CCL2 antibody, or fragment thereof, as described herein for use in a method of treating scleroderma comprising a step of administering the anti-CCL2 antibody, or fragment thereof, to a subject, wherein the anti-CCL2 antibody, or fragment thereof, is characterized by a binding affinity of stronger and/or greater than 10^{-12} M (e.g., greater than 0.5 X 10^{-12} M, 10^{-13} M, 0.5 X 10^{-13} M, 10^{-14} M, 0.5 X 10^{-14} M, or 10^{-15} M).

[0030] In some embodiments, an anti-CCL2 antibody, or fragment thereof, according to the invention is selected from the group consisting of intact IgG, $F(ab')_2$, $F(ab)_2$, Fab', Fab, scFvs, diabodies, triabodies and tetrabodies.

[0031] In some embodiments, the anti-CCL2 antibody, or fragment thereof, is a monoclonal antibody, optionally the anti-CCL2 antibody, or fragment thereof is a humanized monoclonal antibody, optionally the anti-CCL2 antibody, or fragment thereof is a human antibody.

[0032] In yet another aspect, the present invention provides various compositions and kits containing an anti-CCL2 antibody described herein.

[0033] 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 DRAWINGS

[0034] The drawings are for illustration purposes only not for limitation.

[0035] **Figure 1** illustrates an exemplary diagram depicting the Modified Rodnan Skin Score. Locations on the body where skin fibrosis is assessed are indicated.

[0036] **Figure 2** depicts an exemplary graph plotting serum and tissue concentration of CCL2 following equilibration.

[0037] **Figure 3** illustrates an exemplary diagram depicting CCL2 targeting in plasma and in diseased tissue.

DEFINITIONS

[0038] 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.

[0039] *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.

[0040] *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).

[0041] *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-CH_1 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.

[0042] *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).

[0043] *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 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. In some embodiments, the term Binding Agent as used herein can also include antibody. See the definition of Antibody.

[0044] *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.).

[0045] *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.

[0046] *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.

[0047] *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.

[0048] *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.

[0049] *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.

[0050] *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.

[0051] *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.

[0052] *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.

[0053] *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).

[0054] *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 delivery systems comprising two or more separate containers that each contains 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 contains 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.

[0055] *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."

[0056] *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.

[0057] *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.

[0058] *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.

[0059] *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.

[0060] *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.

[0061] *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.

[0062] *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.

[0063] *Treatment*: As used herein, the term "*treatment*" (also "treat" or "treating") refers to any administration of a therapeutic protein (e.g., administration of an anti-CCL2 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

[0064] The present invention provides, among other things, improved anti-CCL2 antibodies characterized with high affinity, potency, tissue selectivity and/or epitope specificity, 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 provides methods and compositions for treatment of scleroderma and related fibrotic and/or inflammatory diseases, disorders and conditions based on an anti-CCL2 antibody having an affinity of 10^{-12} M or greater.

[0065] The present invention is, in part, based on the unique insights observed by the present inventors, that is, high affinity anti-CCL2 antibodies, particularly when administered in high doses, allow effective inhibition of CCL2 in affected tissues despite high levels of CCL2 in plasma. Embodiments of the invention include anti-CCL2 antibodies having an affinity of 10^{-12} M or greater. Antibodies of such high affinity are particularly advantageous. Because of high circulating levels of CCL2 in plasma of patients with scleroderma, a large fraction of any anti-CCL2 antibody administered is likely to be sequestered by circulating CCL2. Without wishing to be bound by theory, a high affinity anti-CCL2 antibody can effectively neutralize CCL2 in affected tissue, in addition to neutralizing circulating CCL2, partly due to its ability to effectively compete off the receptor, CCR2, which has a binding affinity of 60 pM to CCL2. Thus, a high affinity anti-CCL2 antibody (e.g., an anti-CCL2 antibody with a binding affinity stranger than 60 pM) can effectively sequester CCL2 in diseased tissue preventing the binding between CCL2 and its receptor CCR2. As a result, less amount of high affinity anti-CCL2 antibody in diseased tissue may be required to achieve desired therapeutic effects.

[0066] 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

[0067] 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.

[0068] 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.

[0069] 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 cascade worsens, 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

[0070] 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.

[0071] 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 many of the associated hallmark symptoms of scleroderma. As fibrosis increases, permeability decreases. As a result, it becomes more difficult for antibodies

to penetrate diseased tissues. Therefore, the affinity of anti-CCL2 antibodies becomes particularly important to keep antibodies localized.

[0072] 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.

[0073] 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.

[0074] 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.

[0075] 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, anti-topoisomerase antibody testing, anti-centromere antibody testing, anti-U3 antibody testing, anti-RNA antibody testing, other types of antibody testing, erythrocyte sedimentation rate, and rheumatoid factor.

Anti-CCL2 Antibodies

[0076] The present invention provides methods and compositions for treating scleroderma, and related fibrotic and/or inflammatory diseases, disorders and conditions, based on administration of anti-CCL2 antibodies, in particular, high affinity anti-CCL2 antibodies.

CCL2

[0077] 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.*).

[0078] 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)**

[0079] 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. "Percent (%) amino acid sequence identity" with respect to the CCL2 sequence 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 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.

[0080] Any of the above described CCL2 proteins can be used to generate and identify mono-specific antibodies that specifically bind to CCL2. See the Anti-CCL2 Antibodies section below.

Anti-CCL2 Antibodies

[0081] CCL2 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 antibodies include any antibodies or fragments of antibodies that bind specifically to any epitopes of CCL2. 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.

[0082] 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.

[0083] Anti-CCL2 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).

[0084] 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.).

[0085] 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, Vκ and Vλ gene families. Following amplification, the Vκ and Vλ 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 J_H 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).

[0086] 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).

[0087] 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 (e.g, see U.S. Pat. Nos. 4,816,567; 5,693,762; and 5,225,539), 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.).

[0088] 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). In some embodiments, human anti-CCL2 antibodies are made by immunization of non-human animals engineered to make human antibodies in response to antigen challenge; e.g., immunization with human CCL2 (e.g., see U.S. Pat. Nos. 5,569,825; 6,150,584; and 6,596,541).

[0089] The use of high affinity anti-CCL2 antibodies to treat scleroderma is important. As described above, the binding affinity between CCL2 and the CCR2 receptor is high (i.e., 60 pM), and there is a high level of circulating CCL2 in plasma. Thus, majority of anti-CCL2 antibodies are likely to be sequestered in plasma once administered and only a small fraction may be localized to diseased target tissues. Therefore, anti-CCL2 antibodies are unlikely to be effective at competing CCL2 off of the receptor and inhibiting signaling in target tissue unless they also have a high binding affinity for CCL2. Furthermore, as sclerodema progresses, fibrosis increases and permeability of vasculature and access to target tissue decreases. The use of high affinity anti-CCL2 antibodies ensures that the antibodies retained at the target tissues are still capable of binding CCL2 and preventing interaction with its receptor.

[0090] Thus, in some embodiments, an anti-CCL2 antibody or fragment thereof suitable for the present invention has a binding affinity of or greater than approximately 500 nM, 100 nM, 10 nM, 1 nM, 500 pM, 100 pM, 50 pM, 10 pM, 1 pM, 500 fM, 400 fM, 300 fM, 200 fM, 100 fM, 50 fM, 10 fM, 1 fM. In some embodiments, an anti-CCL2 antibody or fragment thereof suitable for the present invention has a binding affinity ranging between approximately 500 nM and 1 fM, between 500 nM and 10 fM, between 500 nM and 100 fM, between 500 nM and 1 pM, between 10 nM and 1 fM, between 10 nM and 100 fM, between 10 nM and 1 pM, between 1 nM and 1 fM, between 1 nM and 100 fM, between 1 nM and 500 fM, between 1 nM and 1 pM, between 1 nM and 10 pM, between 1 nM and 50 pM, between 1 nM and 100 pM, between 1 nM and 500 pM.

Biodistribution and bioavailability

[0091] In various embodiments, once administered *in vivo*, an anti-CCL2 antibody according to the present invention may be delivered to various target tissues. Exemplary desired target tissues include, but are not limited, skin, blood vessels, lung, heart, kidney, gastrointestinal tract (including liver), esophagus, musculoskeletal system and combinations thereof.

[0092] In various embodiments, once administered *in vivo*, an anti-CCL2 antibody according to the present invention may achieve therapeutically or clinically effective levels or activities in various targets tissues described herein. As used herein, a therapeutically or clinically effective level or activity is a level or activity sufficient to confer a therapeutic effect in a target tissue. The therapeutic effect may be objective (i.e., measurable by some test or marker) or subjective (i.e., subject gives an indication of or feels an effect). For example, a therapeutically or clinically effective level or activity may be a protein level or activity that is sufficient to ameliorate symptoms associated with scleroderma or related diseases, disorders or conditions in the target tissue (e.g., CCL2 level). In some embodiments, an anti-CCL2 antibody described herein delivered according to the present invention may reduce CCL2 levels by at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% in the target tissue as compared to an untreated control or the pre-treatment state.

[0093] In some embodiments, an anti-CCL2 antibody described herein delivered according to the present invention may reduce the CCL2 serum level to less than about 1000 pg/ml, 900 pg/ml, 800 pg/ml, 700 pg/ml, 600 pg/ml, 500 pg/ml, 400 pg/ml, 300 pg/ml, 250 pg/ml, 200 pg/ml, 180 pg/ml, 160 pg/ml, 140 pg/ml, 120 pg/ml, 100 pg/ml, or less.

[0094] In general, once administered *in vivo*, an anti-CCL2 antibody according to the present invention have sufficiently long half time in serum and/or target tissues (e.g., skin, blood vessels, lung, heart, kidney, gastrointestinal tract (including liver), esophagus, or musculoskeletal system). In some embodiments, an anti-CCL2 antibody according to the present invention may have a half-life of at least approximately 30 minutes, 45 minutes, 60 minutes, 90 minutes, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 8 hours, 9 hours, 10 hours, 12 hours, 16 hours, 18 hours, 20 hours, 25 hours, 30 hours, 35 hours, 40 hours, up to 3 days, up to 7 days, up to 14 days, up to 21 days or up to a month. In some embodiments, an anti-CCL2 antibody according to the present invention may retain detectable level or activity in serum and/or target tissues after 12 hours, 24 hours, 30 hours, 36 hours, 42 hours, 48 hours, 54 hours, 60 hours, 66 hours, 72 hours, 78 hours, 84 hours, 90 hours, 96 hours, 102 hours, or a

week following administration. Detectable level or activity may be determined using various methods known in the art.

[0095] In certain embodiments, an anti-CCL2 antibody described herein achieves a concentration of at least 20 μg/ml, at least 15 μg/ml, at least 10 μg/ml, at least 7.5 μg/ml, at least 5 μg/ml, at least 2.5 μg/ml, at least 1.0 μg/ml or at least 0.5 μg/ml in the serum or targeted tissues following administration (e.g., intravenous) to such subject (e.g., one week, 3 days, 48 hours, 36 hours, 24 hours, 18 hours, 12 hours, 8 hours, 6 hours, 4 hours, 3 hours, 2 hours, 1 hour, 30 minutes, or less following administration (e.g., *i.v.*) to the subject).

Treatment of Scleroderma and Related Diseases, Disorders or Conditions

[0096] Anti-CCL2 antibodies 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.

[0097] Various antibodies of the invention may be administered alone or in combination with other antibodies or therapeutic agents. In some embodiments, antibodies 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-TGFβ treatments, and endothelin receptor antagonists.

[0098] In some embodiments, antibodies 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 blocking the binding of CCL2 to its cognate receptor. 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.

[0099] Antibodies described herein can be administered in any dosing regimen that is therapeutically effective. In some embodiments, anti-CCL2 antibodies are administered at bimonthly, monthly, triweekly, biweekly, weekly, daily, or at variable intervals.

[00100] Antibodies 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.

Scleroderma

[00101] 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.

[00102] 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.

[00103] 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, which is a characteristic of scleroderma.

[00104] 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, gastrointestinal tract (including liver) , blood vessels, gastrointestinal tract, musculoskeletal system, lung, and/or esophagus.

[00105] 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 Figure 1 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 than 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.

[00106] 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 than 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.

[00107] 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 than 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

[00108] In addition to Sclerodera, 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.

[00109] 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.

[00110] 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

[00111] 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), ophtlialmopathies (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

[00112] In some embodiments, methods and compositions based on anti-CCL2 antibodies 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 scleroderma). 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

[00113] 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.

[00114] 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-lymphocytes 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 surrogator markers

[00115] 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.

[00116] mRSS is used as one clinical marker of scleroderma. Typically, mRSS is assigned as shown in Figure 1: 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..

[00117] 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 Health Assessment Questionnaire (HAQ - DI), Diffusing capacity of the lung for carbon monoxide (DLCO), or Forced Vital Capacity (FVC).

CCL2 levels

[00118] 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.

[00119] 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 HAQ - DI, DLCO, or FVC.

[00120] 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

[00121] The present invention also provides compositions comprising one or more provided antibodies. In some embodiments the present invention provides at least one antibody 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.

[00122] 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).

[00123] 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.

[00124] 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 antibody degradation.

[00125] 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.

[00126] 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.

[00127] 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.

[00128] 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.

[00129] 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

Example 1. Preparation of High Affinity Anti-CCL2 Antibodies

[00130] This example illustrates preparation of high affinity anti-CCL2 antibodies. As described above, various methods are available to generate and select antibodies with desired specificities and binding affinities.

[00131] In this particular example, the anti-CCL2 antibody is composed of a complete human antibody comprising two full-length antigen binding arms. Transgenic mice expressing human antibody genes are initially immunized with purified human recombinant CCL2 in

complete Freund's adjuvant via subcutaneous injection. Following the initial immunization, each of the mice receives an additional subcutaneous injection once a week for three weeks. Splenocytes are harvested from mice with high antibody titres, as determined by ELISA, and fused to a mouse myeloma cell line as follows. Single cell suspensions of splenocytes from immunized mice are fused to one-fourth the number of non-secreting mouse myeloma cells with 50% PEG. Cells are plated at approximately 1×10^5/well in flat bottom microtiter plates, followed by a one week incubation. Individual wells are then screened by ELISA for human anti-CCL2 monoclonal IgG antibodies. Once extensive hybridoma growth occurs, the antibody-secreting hybridomas are replated, screened again and, if still positive for human IgG, anti-CCL2 monoclonal antibodies are subcloned at least twice by limiting dilution.

[00132] Alternatively, antibodies may be isolated directly from DNA encoding the V_H and V_L domains of single antigen positive B cells from immunized transgenic mice (as described above) employing flow cytometry. Briefly, the human CCL2 immunized transgenic mice is terminated and splenocytes are harvested. Red blood cells are removed by lysis followed by pelleting the harvested splenocytes. Resuspended splenocytes are first incubated with a cocktail of human IgG, FITC-anti-mFc, and biotinylated human CCL2 for 1 hour. The stained cells are washed twice with PBS, then stained with a cocktail of human and rat IgG, APC-anti-mIgM, and SA-PE for one hour. The stained cells are washed once with PBS and then analyzed by flow cytometry on a MOFLO™ XDP (Beckman Coulter, Inc.). Each IgG positive, IgM negative, and antigen positive B cell is sorted and plated into a separate well on a 96-well plate. RT-PCR of antibody genes from these B cells is performed according to a method described by Wang et al. (2000, J. Immunol. Methods 244:217-225). The heavy chain and light chain PCR products are cloned into vectors containing a human heavy chain constant region (e.g., IgG_1) and a human light chain constant region (e.g., Cκ), respectively. Purified recombinant plasmids having a heavy chain variable region sequence and plasmids having a light chain variable region sequence from the same B cell are then combined and transfected into a host cell line (e.g., a CHO cell line).

[00133] In addition to classic mouse immunization, other antibody screening methods based on camelids or phage display can also be used. High affinity antibodies are selected using standard receptor binding assays. Antibodies with affinity greater than 10^{-12}M are purified.

Example 2. Dose range testing

[00134] This example illustrates a dose response study designed to evaluate effective dose ranges of anti-CCL2 antibody for treatment of scleroderma.

[00135] 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 acid and/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.

[00136] Dose(s) of an anti-CCL2 antibody or a control antibody of escalating concentrations are administered into the mice via intraperitoneal injection.

Example 3. *In vivo* efficacy of anti-CCL2 antibody

[00137] This example illustrates a study designed to evaluate the effect of treatment with anti-CCL2 antibodies on inflammation and fibrosis in the bleomycin mouse model for scleroderma.

[00138] 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 antibody at suitable concentrations, as determined in example 2, or with a control antibody.

[00139] 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®) (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.

[00140] It is anticipated that treatment with anti-CCL2 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

[00141] This example illustrates a model of CCL2 production and turnover in various tissues and plasma to predict tissue target levels.

[00142] 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 have 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.

[00143] The molecular weight of CCL2 is about 8.6 kDa, 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 60 pM- 2 nM. 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 (~ 75 pM) with whole skin involvement but without organ involvement. A target profile showing serum and tissue CCL2 equilibration is shown in Figure 2, which predicts the desired amount of antibodies need to neutralize 3nM of tissue CCL2 and competes it off its receptor. The illustrated model represents an extreme presentation of high CCL2 levels.

[00144] Currently available monoclonal antibodies injected intravenously typically are not effective because they bind CCL2 in plasma and forms a complex before they reach diseased tissues. See Figure 3. By providing anti-CCL2 that is high-affinity, we can provide sufficient anti-CCL2 antibody to bind CCL2 in tissue and compete with the 60 pM affinity for CCR2.

Example 5. Clinical design

[00145] Based upon the success of animal treatments, Phase I-III dose ranging and single dose studies of anti-CCL2 antibody detailed in Tables 2-6 are designed in healthy individuals and individuals with different stages of scleroderma to evaluate the safety, tolerability, efficacy, and pharmacokinetics of anti-CCL2 therapy.

[00146] A primary objective of Human Clinical Trial 1 includes determining the safety of 4 dose levels of anti-CCL2 antibody administered in healthy individuals. Secondary objectives include evaluating the pharmacokinetics of 4 different dose levels of anti-CCL2 antibody administered in healthy individuals. A detailed protocol synopsis of this clinical trial is shown in Table 2.

Table 2: 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

[00147] A primary objective of Human Clinical Trial 2 includes determining the safety of 4 dose levels of anti-CCL2 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 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 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 antibody as

measured by the Modified Rodnan Skin Score (mRSS). A detailed protocol synopsis of this clinical trial is shown in Table 3.

Table 3: 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

[00148] A primary objective of Human Clinical Trial 3 includes determining the efficacy of a single dose level of anti-CCL2 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 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 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 4.

Table 4: 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

[00149] A primary objective of Human Clinical Trial 4 includes determining the efficacy relative to oral cyclophosphamide of a single dose level of anti-CCL2 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 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 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 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 5.

Table 5: 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

[00150] Objective of Human Clinical Trial 5 include (1) determining the efficacy relative to oral cyclophosphamide of a single dose level of anti-CCL2 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 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 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 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 6.

Table 6: 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

[00151] Patients exhibiting early symptoms of scleroderma treated with anti-CCL2 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 antibody are expected to demonstrate significant improvement of symptoms as measured by the mRSS, HAQ - DI, and FVC. Anti-CCL2 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.

Equivalents and Scope

[00152] 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.

[00153] 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.

[00154] 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.

[00155] 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.

[00156] 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.

[00157] 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

[00158] 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 method of treating scleroderma comprising

administering to an individual who is suffering from or susceptible to scleroderma an effective amount of anti-CCL2 antibody, or fragment thereof, such that at least one symptom or feature of scleroderma in a target tissue is reduced in intensity, severity, or frequency, or has delayed onset.

2. The method of claim 1, wherein the at least one symptom or feature of scleroderma is selected from 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.

3. The method of claim 1 or 2, 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.

4. The method of any one of the preceding claims, wherein the target tissue is lung.

5. The method of any one of claims 1-3, wherein the target tissue is heart.

6. The method of any one of the preceding claims, wherein the individual is suffering from or susceptible to limited cutaneous scleroderma.

7. The method of any one of the preceding claims, wherein the individual is suffering from or susceptible to diffuse cutaneous scleroderma.

8. The method of any one of the preceding claims, wherein the anti-CCL2 antibody, or fragment thereof, is administered parenterally.

9. The method of claim 8, wherein the parenteral administration is selected from intravenous, intradermal, inhalation, transdermal (topical), subcutaneous, and/or transmucosal administration.

10. The method of claim 9, wherein the parenteral administration is intravenous administration.

11. The method of any one of claims 1-7, wherein the anti-CCL2 antibody, or fragment thereof, is administered orally.

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

13. 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, having a binding affinity of greater than 10^{-12} M.

14. The method of claim 13, wherein the anti-CCL2 antibody, or fragment thereof, is administered at a therapeutically effective dose and an administration interval such that the anti-CCL2 antibody, or fragment thereof, is distributed to one or more target tissues selected from the group consisting of skin, blood vessels, lung, heart, kidney, gastrointestinal tract (including liver), musculoskeletal system and combinations thereof.

15. The method of claim 13, wherein the anti-CCL2 antibody, or fragment thereof, is administered at a therapeutically effective dose and an administration interval such that the anti-CCL2 antibody, or fragment thereof, is distributed to lung and/or heart.

16. The method of claim 15, wherein the administration interval is selected from bimonthly, monthly, triweekly, biweekly, weekly, daily, or at variable intervals.

17. 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, at a therapeutically effective dose and an administration interval such that the anti-CCL2 antibody, or fragment thereof, is distributed to lung and/or heart.

18. The method of claim 17, wherein the anti-CCL2 antibody, or fragment thereof, is further distributed to skin, kidney, and/or liver.

19. The method of any one of the preceding claims, 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.

20. The method of claim 19, wherein the anti-CCL2 antibody, or fragment thereof, is a monoclonal antibody.

21. The method of claim 20, wherein the anti-CCL2 antibody, or fragment thereof, is a humanized monoclonal antibody.

22. The method of claim 20, wherein the anti-CCL2 antibody, or fragment thereof, is a human antibody.

23. An anti-CCL2 antibody, or fragment thereof, having a binding affinity of greater than 10^{-12} M.

24. The anti-CCL2 antibody of claim 23, wherein the anti-CCL2 antibody, or fragment thereof, has a binding affinity of greater than 10^{-13} M.

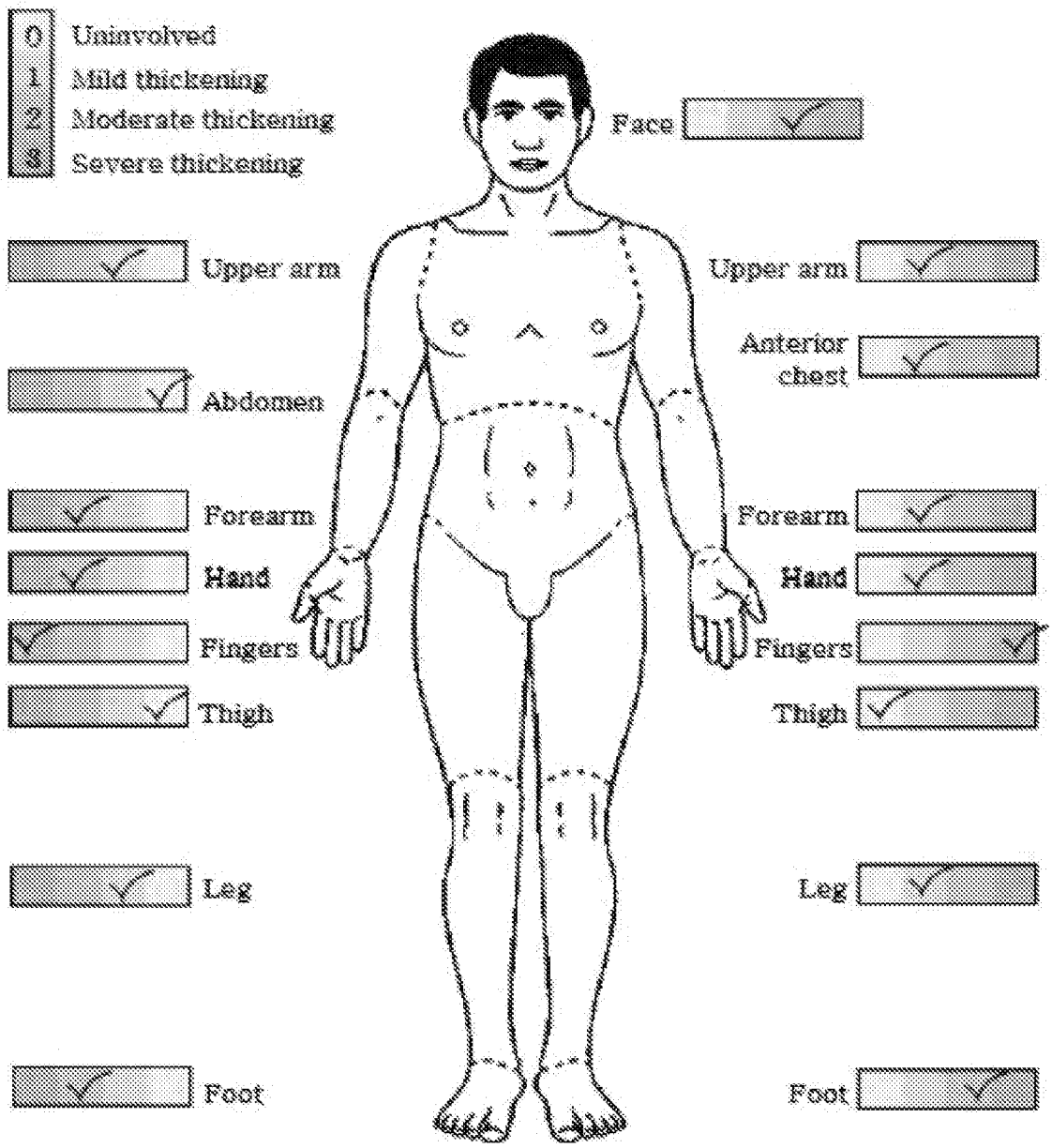
25. The anti-CCL2 antibody of claim 23 or 24, wherein the anti-CCL2 antibody, or fragment thereof, is selected from the group consisting of intact IgG, F(ab')2, F(ab)2, Fab', Fab, scFvs, diabodies, triabodies and tetrabodies.

26. The anti-CCL2 antibody of any one of claims 23-25, wherein the anti-CCL2 antibody, or fragment thereof, is a monoclonal antibody.

27. The anti-CCL2 antibody of claim 26, wherein the anti-CCL2 antibody, or fragment thereof, is a humanized monoclonal antibody.

28. The anti-CCL2 antibody of claim 26, wherein the anti-CCL2 antibody, or fragment thereof, is a human antibody.

29. A kit comprising an anti-CCL2 antibody, or fragment thereof, according to any one of claims 23-28.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1 Mild thickening
2 Moderate thickening
3 Severe thickening
Face
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Abdomen
Forearm
Hand
Fingers
Thigh
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Foot
Upper arm
Anterior chest
Forearm
Hand
Fingers
Thigh
Leg
Foot

Fig. 1

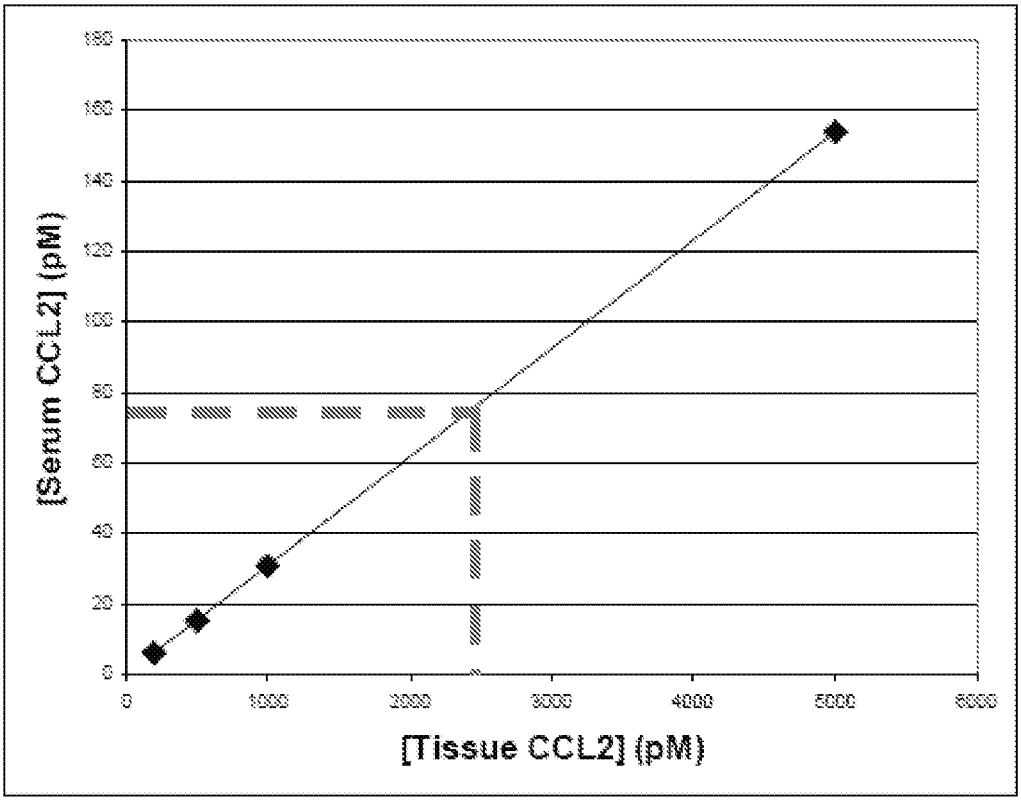
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Fig. 2

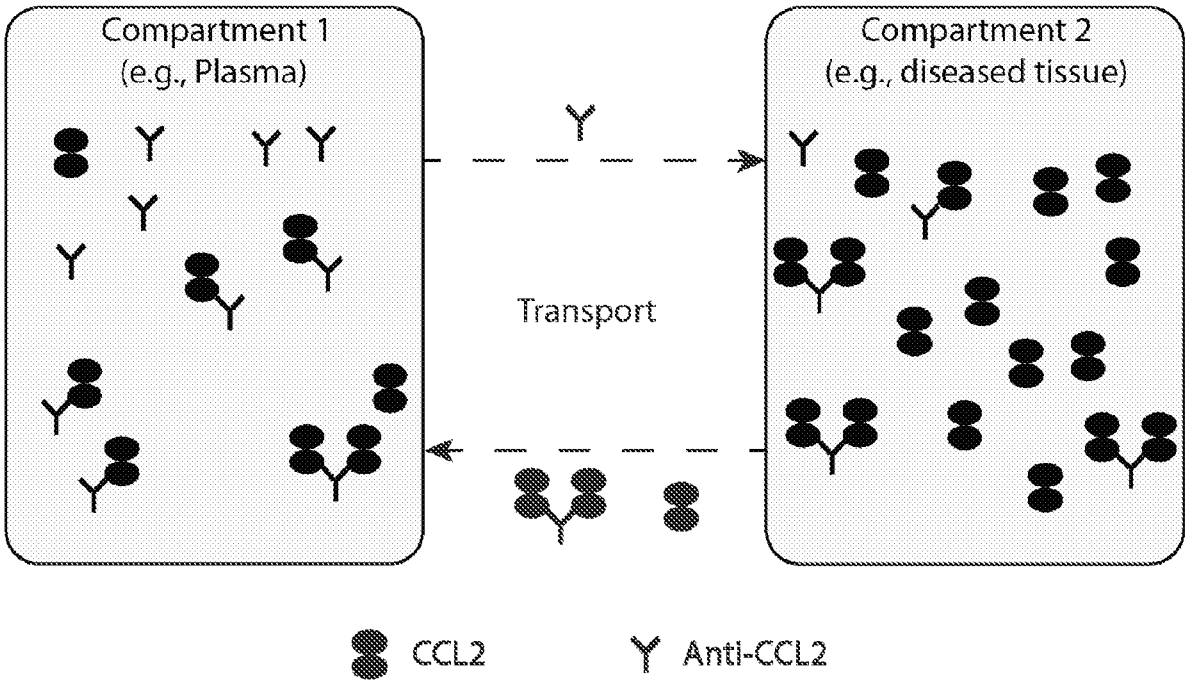
Compartment 1
(e.g., Plasma)
Compartment 2
(e.g., diseased tissue)
Transport
CCL2
Anti-CCL2

Fig. 3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US13/42196

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 39/395; C07K 16/00; C12P 21/08 (2013.01)
USPC - 424/130.1; 530/387.1, 388.23
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)
IPC(8): A61K 39/00, 39/395, 51/10, 49/00; C07K 16/00, 14/00; C12P 21/08; C12Q 1/68 (2013.01)
USPC: 424/133.1, 143.1, 130.1, 141.1, 142.1, 145.1, 1.49, 9.1; 9.6; 530/387.1, 387.3, 388.1, 388.23; 435/6

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)
MicroPatent (US-G, US-A, EP-A, EP-B, WO, JP-bib, DE-C,B, DE-A, DE-T, DE-U, GB-A, FR-A); Google; Google Scholar; DialogPRO; PubMed; Search Terms: 'CCL2,' antibody, 'binding affinity,' 'sub-picomolar,' scleroderma

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2008/0076120 A1 (DONALDSON, DD et al.) March 27, 2008; paragraphs [0036], [0042], [0048], [0053], [0060], [0065], [0197], [0259]-[0261]; Claim 1	1, 2, 3/1, 3/2
X	US 8114964 B2 (DAS, A et al.) February 14, 2012; abstract; column 3, lines 38-56; column 5, lines 22-27; column 27, lines 27-34; column 33, lines 30-36; column 39, lines 1-5; column 40, lines 11-15, lines 53-55; column 42, lines 24-30	13-18, 23, 24, 25/23, 25/24
A	US 2011/0110852 A1 (MILLER, KL et al.) May 12, 2011; entire document	1, 2, 3/1, 3/2, 13-18, 23, 24, 25/23, 25/24

☐ Further documents are listed in the continuation of Box C. ☐

* 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
26 September 2013 (26.09.2013)	02 OCT 2013
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US13/42196

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, the international search was carried out on the basis of a sequence listing filed or furnished:

   a. (means)

   ☐ on paper

   ☒ in electronic form

   b. (time)

   ☒ in the international application as filed

   ☐ together with the international application in electronic form

   ☐ subsequently to this Authority for the purposes of search

2. ☐ In addition, in the case that more than one version or copy of a sequence listing 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/US13/42196

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 4-12, 19-22, 26-29
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

(19)中华人民共和国国家知识产权局

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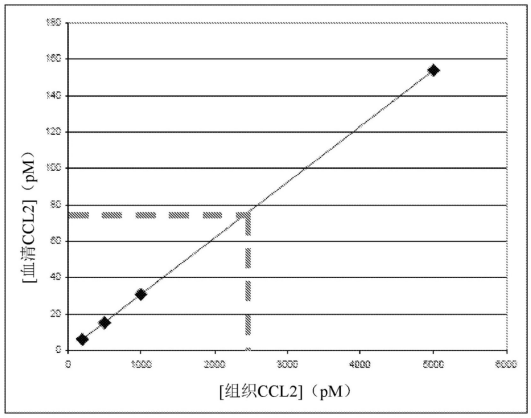
权利要求书2页 说明书29页
序列表1页 附图2页

(54)发明名称

用于治疗硬皮病的抗 CCL2 抗体

(57)摘要

本发明尤其提供特征在于高亲和力、效力、组织选择性和/或表位特异性的经改良抗 CCL2 抗体,和其尤其用于治疗硬皮病和相关纤维化和/或发炎性疾病、病症和病况的用途。在一些实施例中,本发明提供基于亲和力为 10^{-12}M 或更大的抗 CCL2 抗体治疗硬皮病和相关纤维化和/或发炎性疾病、病症和病况的方法和组合物。



CN 104487091 A

1. 一种治疗硬皮病的方法，其包含

向罹患或易患硬皮病的个体投与有效量的抗 CCL2 抗体或其片段，使得目标组织中硬皮病的至少一种症状或特征的强度、严重度或频率降低或具有延迟的发作。

2. 根据权利要求 1 所述的方法，其中所述硬皮病的至少一种症状或特征是选自内皮细胞损伤、基底膜层增生、血管周围单核细胞浸润、纤维化、内脏器官架构紊乱、血管稀疏、缺氧以及其组合。

3. 根据权利要求 1 或 2 所述的方法，其中所述目标组织是选自由以下组成的群组：皮肤、血管、肺、心、肾、胃肠道（包括肝）、肌肉骨胳系统以及其组合。

4. 根据前述权利要求中任一权利要求所述的方法，其中所述目标组织是肺。

5. 根据权利要求 1 到 3 中任一权利要求所述的方法，其中所述目标组织是心。

6. 根据前述权利要求中任一权利要求所述的方法，其中所述个体罹患或易患局限性皮肤硬皮病。

7. 根据前述权利要求中任一权利要求所述的方法，其中所述个体罹患或易患弥漫性皮肤硬皮病。

8. 根据前述权利要求中任一权利要求所述的方法，其中所述抗 CCL2 抗体或其片段是肠胃外投与的。

9. 根据权利要求 8 所述的方法，其中所述肠胃外投与是选自静脉内、皮内、吸入、经皮（表面）、皮下和 / 或经粘膜投与。

10. 根据权利要求 9 所述的方法，其中所述肠胃外投与是静脉内投与。

11. 根据权利要求 1 到 7 中任一权利要求所述的方法，其中所述抗 CCL2 抗体或其片段是口服投与的。

12. 根据前述权利要求中任一权利要求所述的方法，其中所述抗 CCL2 抗体或其片段是两月一次、每月一次、三周一次、两周一次、每周一次、每天一次或以可变的时间间隔投与的。

13. 一种治疗硬皮病的方法，其包含

向罹患或易患硬皮病的个体投与结合亲和力大于 10^{-12}M 的抗 CCL2 抗体或其片段。

14. 根据权利要求 13 所述的方法，其中所述抗 CCL2 抗体或其片段以治疗有效剂量和一定的投与时间间隔投与，使得所述抗 CCL2 抗体或其片段分布到一或多种选自由以下组成的群组的目标组织：皮肤、血管、肺、心、肾、胃肠道（包括肝）、肌肉骨胳系统以及其组合。

15. 根据权利要求 13 所述的方法，其中所述抗 CCL2 抗体或其片段以治疗有效剂量和一定的投与时间间隔投与，使得所述抗 CCL2 抗体或其片段分布到肺和 / 或心。

16. 根据权利要求 15 所述的方法，其中所述投与时间间隔是选自两月一次、每月一次、三周一次、两周一次、每周一次、每天一次或可变的时间间隔。

17. 一种治疗硬皮病的方法，其包含

以治疗有效剂量和一定的投与时间间隔向罹患或易患硬皮病的个体投与抗 CCL2 抗体或其片段，使得所述抗 CCL2 抗体或其片段分布到肺和 / 或心。

18. 根据权利要求 17 所述的方法，其中所述抗 CCL2 抗体或其片段进一步分布到皮肤、肾和 / 或肝。

19. 根据前述权利要求中任一权利要求所述的方法，其中所述抗 CCL2 抗体或其片段是

选自由以下组成的群组：完整 IgG、$F(ab')_2$、$F(ab)_2$、Fab'、Fab、scFvs、双功能抗体、三功能抗体和四功能抗体。

20. 根据权利要求 19 所述的方法，其中所述抗 CCL2 抗体或其片段是单克隆抗体。

21. 根据权利要求 20 所述的方法，其中所述抗 CCL2 抗体或其片段是人类化单克隆抗体。

22. 根据权利要求 20 所述的方法，其中所述抗 CCL2 抗体或其片段是人类抗体。

23. 一种抗 CCL2 抗体或其片段，其具有大于 10^{-12}M 的结合亲和力。

24. 根据权利要求 23 所述的抗 CCL2 抗体，其中所述抗 CCL2 抗体或其片段具有大于 10^{-13}M 的结合亲和力。

25. 根据权利要求 23 或 24 所述的抗 CCL2 抗体，其中所述抗 CCL2 抗体或其片段是选自由以下组成的群组：完整 IgG、F(ab')2、F(ab)2、Fab'、Fab、scFvs、双功能抗体、三功能抗体和四功能抗体。

26. 根据权利要求 23 到 25 中任一权利要求所述的抗 CCL2 抗体，其中所述抗 CCL2 抗体或其片段是单克隆抗体。

27. 根据权利要求 26 所述的抗 CCL2 抗体，其中所述抗 CCL2 抗体或其片段是人类化单克隆抗体。

28. 根据权利要求 26 所述的抗 CCL2 抗体，其中所述抗 CCL2 抗体或其片段是人类抗体。

29. 一种试剂盒，其包含根据权利要求 23 到 28 中任一权利要求所述的抗 CCL2 抗体或其片段。

用于治疗硬皮病的抗 CCL2 抗体

[0001] 相关申请案的交叉引用

[0002] 本申请案依据 35 USC § 119(e) 要求 2012 年 5 月 22 日提交的美国临时专利申请案第 61/650,149 号的权益，所述申请案特此以全文引用的方式并入本文中。

[0003] 序列表

[0004] 本说明书提及 2013 年 5 月 22 日以电子形式作为命名为“2006685-0330_Sequences_ST25”的 ASCII.txt 文档提交的序列表。所述 .txt 文档是在 2013 年 5 月 14 日生成的且大小是 2KB。

技术领域

[0005] 无

背景技术

[0006] 全身性硬化症（硬皮病）是临床上异质性结缔组织病症，引起皮肤的硬化和绷紧。其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。涉及肺、心、肾和胃肠道的基于主要器官的并发症可促进死亡率和发病率。发病机制是未知的。

[0007] 与硬皮病最常有关的特征是纤维化（胶原蛋白在皮肤和器官中积聚）。胶原蛋白的积聚促成病症的症状，包括脱发、皮肤硬化和绷紧、皮肤变色、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）。硬皮病可归类成两个主要子组：局限性皮肤硬皮病和弥漫性皮肤硬皮病。在局限性皮肤硬皮病中，纤维化主要限制于手、臂和面部。弥漫性皮肤硬皮病是一种快速进行性病症，其影响大面积的皮肤并损害一或多个内脏。患有局限性皮肤硬皮病的患者与患有弥漫性皮肤硬皮病的患者相比具有相对更好的长期预后。广泛的全身性硬皮病可损伤心、肾、肺或胃肠道，这可引起死亡。肺纤维化是患有硬皮病的患者死亡的最常见原因。

[0008] 因此，硬皮病是极度致衰弱的疾病，有着潜在的致命后果。在美国约有 50,000 名患者。女性患者与男性患者的比率是约 4:1。当前治疗方法仅基于对在整个疾病过程产生的并发症的对症治疗和管理（例如皮质类固醇、NSAID 和免疫抑制药物（如甲氨蝶呤和环磷氮介））。没有疗法展示出会逆转或停止疾病进展。因此，对于硬皮病的有效疗法存在高度未满足的医学需求。

发明内容

[0009] 本发明具体是基于经改良抗体或结合蛋白提供用于有效治疗硬皮病的经改良方法和组合物，所述经改良抗体或结合蛋白可以高亲和力、效力和 / 或表位多样性特异性地结合于 C-C 趋化因子配体 -2(“CCL2”)，从而实现稳固的生物分布和 / 或组织特异性。已知 CCL2 是硬皮病的经验证目标。数项研究已展示，硬皮病成纤维细胞呈现出 CCL2mRNA 和蛋白的组成型表达增加。在硬皮病皮肤切片中，CCL2 的表达在成纤维细胞、角质细胞和单核细胞中被检测到，而其在正常皮肤中是不可检测的（加林多 (Galindo) 等人，关节炎与风湿病

(Arthritis Rheum.)2001年6月;44(6):1382-6;迪斯特勒(Distler)等人,关节炎与风湿病2001年11月;44(11):2665-78;利奥伊德(Lioyd)等人,实验医学(Exp Med.)1997年4月7日;185(7):1371-80;山本(Yamamoto)等人,皮肤病科学杂志(J Dermatol Sci.)2001年6月;26(2):133-9;登顿(Denton)等人;免疫学趋势(Trends Immunol.)2005年11月;26(11):596-602.2005年9月15日电子出版)。然而,在本发明之前,尚未发展出基于抗CCL2抗体的针对硬皮病的有效疗法。本发明人观察到,血浆中的高水平CCL2螯合静脉内注射的抗CCL2抗体,引起对患病组织中CCL2的低效靶向。为了解决这一问题,本发明人想到使用具有高亲和力的抗CCL2抗体,以足以胜过高水平血清CCL2的量投与,引起对所需患病组织中CCL2的有效靶向。具体来说,本发明人想到"同类之最的"抗CCL2单克隆抗体,其特征在于高结合亲和力、组织选择性、表位特异性和/或长半衰期。所述本发明抗体一旦被体内投与就会引起所需生物分布和生物可用性,使得其结合并阻断目标组织中的CCL2信号传导,除了硬皮病的其它症状或特征以外还减少浸润、炎症和纤维化。

[0010] 因此,在一个方面中,本发明提供治疗硬皮病的方法,其包含向罹患或易患硬皮病的个体投与有效量的抗CCL2抗体或其片段,使得目标组织中硬皮病的至少一种症状或特征的强度、严重度或频率降低或具有延迟的发作。

[0011] 在一些实施例中,硬皮病的至少一种症状或特征是选自内皮细胞损伤、基底膜层增生、血管周围单核细胞浸润、纤维化、内脏器官架构紊乱、血管稀疏、缺氧和其组合。

[0012] 在一些实施例中,目标组织是选自由以下组成的群组:皮肤、血管、肺、心、肾、胃肠道(包括肝)、肌肉骨胳系统以及其组合。在一些实施例中,目标组织是肺。在一些实施例中,目标组织是心。

[0013] 在一些实施例中,个体罹患或易患局限性皮肤硬皮病。在一些实施例中,个体罹患或易患弥漫性皮肤硬皮病。

[0014] 在一些实施例中,抗CCL2抗体或其片段是肠胃外投与的。在一些实施例中,肠胃外投与是选自静脉内、皮内、吸入、经皮(表面)、皮下和/或经粘膜投与。在一些实施例中,肠胃外投与是静脉内投与。

[0015] 在一些实施例中,抗CCL2抗体或其片段是口服投与的。

[0016] 在一些实施例中,抗CCL2抗体或其片段是两月一次、每月一次、三周一次、两周一次、每周一次、每天一次或以可变的时间间隔投与的。

[0017] 在另一方面中,本发明提供如本文中所述的抗CCL2抗体或其片段的用途,其用于制造供治疗硬皮病用的药物,其中治疗包含向罹患或易患硬皮病的个体投与有效量的抗CCL2抗体或其片段,使得目标组织中硬皮病的至少一种症状或特征的强度、严重度或频率降低或具有延迟的发作。

[0018] 在一些实施例中,本发明提供抗CCL2抗体或其片段的用途,其用于制造如本文中所述的供治疗硬皮病用的药物,其中抗CCL2抗体或其片段的特征在于结合亲和力强于和/或大于10^{-12}M(例如大于0.5×10^{-12}M、10^{-13}M、0.5×10^{-13}M、10^{-14}M、0.5×10^{-14}M或10^{-15}M)。

[0019] 在一些实施例中,根据本发明的抗CCL2抗体或其片段是选自由以下组成的群组:完整IgG、$F(ab')_2$、$F(ab)_2$、Fab'、Fab、scFvs、双功能抗体、三功能抗体和四功能抗体。

[0020] 在一些实施例中,抗CCL2抗体或其片段是单克隆抗体,任选地,抗CCL2抗体或其片段是人类化单克隆抗体,任选地,抗CCL2抗体或其片段是人类抗体。

[0021] 在另一方面中，本发明提供治疗硬皮病的方法，其包含向罹患或易患硬皮病的个体投与抗 CCL2 抗体或其片段，所述抗 CCL2 抗体或其片段的结合亲和力强于和 / 或大于 10^{-12}M（例如大于 0.5×10^{-12}M、10^{-13}M、0.5×10^{-13}M、10^{-14}M、0.5×10^{-14}M 或 10^{-15}M）。

[0022] 在一些实施例中，抗 CCL2 抗体或其片段以治疗有效剂量和一定的投与时间间隔投与，使得抗 CCL2 抗体或其片段分布到一或多种选自由以下组成的群组的目标组织：皮肤、血管、肺、心、肾、胃肠道（包括肝）、肌肉骨胳系统以及其组合。在一些实施例中，抗 CCL2 抗体或其片段以治疗有效剂量和一定的投与时间间隔投与，使得抗 CCL2 抗体或其片段分布到肺和 / 或心。

[0023] 在一些实施例中，投与时间间隔是选自两月一次、每月一次、三周一次、两周一次、每周一次、每天一次或可变的时间间隔。

[0024] 在另一方面中，本发明提供治疗硬皮病的方法，其包含以治疗有效剂量和一定的投与时间间隔向罹患或易患硬皮病的个体投与抗 CCL2 抗体或其片段，使得抗 CCL2 抗体或其片段分布到肺和 / 或心。在一些实施例中，抗 CCL2 抗体或其片段进一步分布到皮肤、肾和 / 或肝。

[0025] 在另一方面中，本发明提供如以上各种实施例中公开的方法，其中抗 CCL2 抗体或其片段是选自由以下组成的群组：完整 IgG、$F(ab')_2$、$F(ab)_2$、Fab'、Fab、scFvs、双功能抗体、三功能抗体和四功能抗体。

[0026] 在一些实施例中，抗 CCL2 抗体或其片段是单克隆抗体。在一些实施例中，抗 CCL2 抗体或其片段是人类化单克隆抗体。在一些实施例中，抗 CCL2 抗体或其片段是人类抗体。

[0027] 本发明尤其提供具有高亲和力的抗 CCL2 抗体。在一些实施例中，本发明提供抗 CCL2 抗体或其片段，其结合亲和力强于和 / 或大于 10^{-12}M（例如大于 0.5×10^{-12}M、10^{-13}M、0.5×10^{-13}M、10^{-14}M、0.5×10^{-14}M 或 10^{-15}M）。

[0028] 在一些实施例中，根据本发明的抗 CCL2 抗体或其片段是选自由以下组成的群组：完整 IgG、$F(ab')_2$、$F(ab)_2$、Fab'、Fab、scFvs、双功能抗体、三功能抗体和四功能抗体。

[0029] 在一些实施例中，抗 CCL2 抗体或其片段是单克隆抗体。

[0030] 在一些实施例中，抗 CCL2 抗体或其片段是人类化单克隆抗体。

[0031] 在一些实施例中，抗 CCL2 抗体或其片段是人类抗体。

[0032] 在另一方面中，本发明提供如本文中所述用于治疗硬皮病的方法的抗 CCL2 抗体或其片段，所述方法包含向受试者投与抗 CCL2 抗体或其片段的步骤，其中抗 CCL2 抗体或其片段的特征在于结合亲和力强于和 / 或大于 10^{-12}M（例如大于 0.5×10^{-12}M、10^{-13}M、0.5×10^{-13}M、10^{-14}M、0.5×10^{-14}M 或 10^{-15}M）。

[0033] 在一些实施例中，根据本发明的抗 CCL2 抗体或其片段是选自由以下组成的群组：完整 IgG、$F(ab')_2$、$F(ab)_2$、Fab'、Fab、scFvs、双功能抗体、三功能抗体和四功能抗体。

[0034] 在一些实施例中，抗 CCL2 抗体或其片段是单克隆抗体，任选地，抗 CCL2 抗体或其片段是人类化单克隆抗体，任选地，抗 CCL2 抗体或其片段是人类抗体。

[0035] 在另一方面中，本发明提供含有本文中所述的抗 CCL2 抗体的各种组合物和试剂盒。

[0036] 本发明的其它特征、目标和优点将在随后的具体实施方式、图式和权利要求书显而易知。然而，应理解，具体实施方式、图式和权利要求书在指示本发明的实施例时仅以说

明方式给出且无限制性。在本发明的范围内的各种变化和修改对于所属领域的技术人员将变得显而易见。

附图说明

[0037] 图式是仅出于说明的目的而非限制。

[0038] 图 1 示出了描绘经修改的罗德南皮肤分数的示例性图式。指示了身体上评估皮肤纤维化的位置。

[0039] 图 2 描绘了绘制在平衡之后 CCL2 的血清和组织浓度的示例性图式。

[0040] 图 3 示出了描绘血浆和患病组织中 CCL2 靶向的示例性图式。

[0041] 定义

[0042] 为了使本发明更易于理解，首先对某些术语进行定义。以下术语和其它术语的其它定义阐述在整个说明书中。

[0043] 亲和力：如本领域中已知，“亲和力”是具体配体结合于（例如非共价缔合于）其搭配物的紧密性和 / 或其从其搭配物解离的速率或频率的量度。如本领域中已知，多种技术中的任一者均可用于测定亲和力。在许多实施例中，亲和力代表特异性结合的量度。

[0044] 亲和力成熟的（或亲和力成熟的抗体）：如本文中所用，其是指在其一或多个 CDR 中具有一或多个变化的抗体，所述一或多个变化引起抗体与不具有那些变化的亲本抗体相比对抗原的亲和力提高。在一些实施例中，亲和力成熟的抗体对目标抗原将具有纳摩尔或甚至皮摩尔的亲和力。亲和力成熟的抗体可通过所属领域中已知的多种程序中的任一者产生。马克斯 (Marks) 等人生物技术 (BioTechnology)10:779-783(1992) 描述通过 V_H 和 V_L 域改组产生的亲和力成熟。CDR 和 / 或框架残基的随机诱变由以下各者描述：巴巴斯 (Barbas) 等人美国国家科学院院刊 (Proc Nat.Acad.Sci,USA)91:3809-3813(1994)；希尔 (Schier) 等人基因 (Gene)169:147-155(1995)；耶尔顿 (Yelton) 等人免疫学杂志 (J.Immunol.)155:1994-2004(1995)；杰克逊 (Jackson) 等人，免疫学杂志 154(7):3310-9(1995)；以及霍金斯 (Hawkins) 等人，分子生物学杂志 (J.Mol.Biol.)226:889-896(1992)。

[0045] 抗体：如本文中所用，术语“抗体”是指由一或多个实质上由免疫球蛋白基因或免疫球蛋白基因片段编码的多肽组成的多肽。所识别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。轻链通常归类为 κ 或 λ。重链通常归类为 γ、μ、α、δ 或 ε，其又分别定义免疫球蛋白类别 IgG、IgM、IgA、IgD 和 IgE。典型免疫球蛋白（抗体）结构单元已知包含四聚体。每一四聚体由两对相同的多肽链组成，每一对具有一个“轻”链（约 25kD）和一个“重”链（约 50-70kD）。每一链的 N 端界定具有约 100 到 110 个或更多个主要负责抗原识别的氨基酸的可变区。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。抗体对具体抗原可具有特异性。抗体或其抗原可为分析物或结合搭配物。抗体作为完整免疫球蛋白或作为通过使用各种肽酶消化而产生的多个明确表征的片段存在。因此，举例来说，胃蛋白酶在铰链区中的二硫键下方消化抗体以产生 $F(ab)'_2$（一种自身为由双硫键连接到 V_H-CH_1 的轻链的 Fab 的二聚体）。可在温和条件下还原 $F(ab)'_2$ 以破坏铰链区中的双硫键联进而将 $(Fab')_2$ 二聚体转化成 Fab’ 单体。Fab’ 单体基本上是具有部分铰链区的 Fab（参见基础免疫学 (Fundamental Immunology),W.E. 保

罗 (W.E.Paul) 编，纽约的瑞文出版社 (Raven Press,N.Y.)(1993)，关于其它抗体片段的较详细描述）。虽然各种抗体片段根据完整抗体的消化定义，但所属领域的技术人员将理解所述 Fab' 片段可以化学方式或使用重组 DNA 方法从头合成。因此，如本文中所用的术语“抗体”还包括通过修饰全抗体产生的抗体片段或通过使用重组 DNA 方法从头合成的抗体片段。在一些实施例中，抗体是单链抗体，如单链 Fv(scFv) 抗体，其中可变重链和可变轻链（直接或通过肽连接子）连接在一起以形成连续多肽。单链 Fv(“scFv”) 多肽是共价连接的 $V_H::V_L$ 杂二聚体，其可从包括直接连接或由肽编码连接子连接的 V_H 和 V_L 编码序列的核酸表达。（参见例如休斯顿 (Huston) 等人 (1988) 美国国家科学院院刊，85:5879-5883，其全部内容以引用的方式并入本文中。）多种结构存在用于将来自抗体 V 区的天然聚集但化学上间隔开的轻链和重链多肽链转化成 scFv 分子，所述 scFv 分子将折叠成实质上类似于抗原结合位点结构的三维结构。参见例如美国专利第 5,091,513 号和第 5,132,405 号和第 4,956,778 号。

[0046] 大约：如本文中所用，术语“大约”或“约”在应用于所关注的一或多个值时是指与所述参考值类似的值。在某些实施例中，除非另行说明或另外从上下文显而易见，否则术语“大约”或“约”是指落入所述参考值的任一方向（大于或小于）25％、20％、19％、18％、17％、16％、15％、14％、13％、12％、11％、10％、9％、8％、7％、6％、5％、4％、3％、2％、1％或更低内的一系列值（除了其中所述数目将超过 100％的可能值）。

[0047] 结合剂：如本文中所用，术语“结合剂”包括结合抗原或目标蛋白或肽的任何天然存在、合成或基因工程改造的药剂，如蛋白质。“结合剂”也称为“结合蛋白”。结合剂可来源于天然存在的抗体或以合成方式经工程改造。结合蛋白或结合剂可类似于抗体通过结合于特定抗原以形成复合物并引发生物反应（例如激动或拮抗具体生物活性）来起作用。结合剂或结合蛋白可包括经分离片段、由抗体的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由肽连接子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位。如本文中所用的术语结合剂还可包括通过修饰全抗体产生的抗体片段或通过使用重组 DNA 方法从头合成的抗体片段。在一些实施例中，抗体是单链抗体，如单链 Fv(scFv) 抗体，其中可变重链和可变轻链（直接或通过肽连接子）连接在一起以形成连续多肽。单链 Fv(“scFv”) 多肽是共价连接的 $V_H::V_L$ 杂二聚体，其可从包括直接连接或由肽编码连接子连接的 V_H 和 V_L 编码序列的核酸表达。（参见例如休斯顿 (Huston) 等人 (1988) 美国国家科学院院刊，85:5879-5883，其全部内容以引用的方式并入本文中。）多种结构存在用于将来自抗体 V 区的天然聚集但化学上间隔开的轻链和重链多肽链转化成 scFv 分子，所述 scFv 分子将折叠成实质上类似于抗原结合位点结构的三维结构。参见例如美国专利第 5,091,513 号和第 5,132,405 号和第 4,956,778 号。在一些实施例中，如本文中所用的术语结合剂还可包括抗体。参见抗体的定义。

[0048] CDR：如本文中所用，其是指抗体可变区内的互补决定区。在重链和轻链的每一可变区中有三个 CDR，对于每一可变区，其被指定为 CDR1、CDR2 和 CDR3。“CDR 的组”或“CDR 组”是指在能够结合抗原的单一可变区中存在的三个或六个 CDR 群组或能够结合抗原的同源重链和轻链可变区的 CDR。CDR 的界限已取决于系统以不同方式定义，在所述系统中数种是所属领域中已知的（例如卡巴特 (Kabat)、科西亚 (Chothia) 等）。

[0049] 化合物和药剂：术语“化合物”和“药剂”在本文中可互换使用。其是指任何天然

存在或非天然存在（即合成或重组）的分子，如生物大分子（例如核酸、多肽或蛋白质）、有机或无机分子、或由生物物质（如细菌、植物、真菌或动物（尤其是哺乳动物，包括人类）细胞或组织）制成的提取物。化合物可为单一分子或至少两种分子的混合物或复合物。

[0050] 对照：如本文中所用，术语“对照”具有其作为结果与之相比的标准的本领域理解的含义。通常，对照用于通过隔离变量以便得出关于所述变量的结论来加强实验的完整性。在一些实施例中，对照是与测试反应或分析同时执行以提供比较物的反应或分析。在一个实验中，施用“测试”（即，所测试的变量）。在第二实验中，“对照”，不施用所测试的变量。在一些实施例中，对照是历史对照（即先前执行的测试或分析，或先前已知的量或结果）。在一些实施例中，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。

[0051] 给药方案：“给药方案”（或“治疗方案”）在所述术语在本文中使用时是通常间隔开时间段向受试者个别投与的一组单位剂量（通常是一个以上）。在一些实施例中，给定治疗剂具有推荐的给药方案，其可能涉及一或多个剂量。在一些实施例中，给药方案包含多个剂量，其中的每一者彼此间隔开相同长度的时间段；在一些实施例中，给药方案包含多个剂量以及间隔开个别剂量的至少两个不同时间段。

[0052] 诊断：如本文中所用，术语“诊断”是指旨在判定个体是否罹患疾病（disease 或 ailment）的过程。在本发明的情形下，“硬皮病的诊断”是指旨在以下各项中的一者或多者的过程：判定个体是否罹患硬皮病，鉴别硬皮病亚型（即弥漫性或局限性皮肤硬皮病）以及确定疾病的严重度。

[0053] 有效量：如本文中所用，术语“有效量”是指足以满足预期目的的化合物或药剂的量。在本发明的情形下，目的可为例如：调节硬皮病的病因或症状；和 / 或延缓或预防硬皮病的发作；和 / 或减缓或终止硬皮病症状的进展、加重或恶化；和 / 或缓解与硬皮病有关的一或多个症状；和 / 或为硬皮病症状带来改善，和 / 或治愈硬皮病。

[0054] 框架或框架区：如本文中所用，其是指减去 CDR 的可变区的序列。因为 CDR 序列可通过不同系统测定，所以同样地框架序列经历相对应地不同解读。六个 CDR 将重链和轻链上的框架区划分成各链上的四个子区 (FR1、FR2、FR3 和 FR4)，其中 CDR1 位于 FR1 与 FR2 之间，CDR2 位于 FR2 与 FR3 之间，并且 CDR3 位于 FR3 与 FR4 之间。在不将具体子区指定为 FR1、FR2、FR3 或 FR4 的情况下，如通过其它提及的框架区代表单个天然存在免疫球蛋白链的可变区内的组合的 FR。如本文中所用，FR 代表四个子区中的一者，FR1 例如代表最接近于可变区的氨基末端并且相对于 CDR1 的 5’ 的第一框架区，并且 FRs 代表构成框架区的子区中的两者或更多者。

[0055] 人类抗体：如本文中所用，其打算包括具有从人类免疫球蛋白序列生成（或装配）的可变区和恒定区的抗体。在一些实施例中，抗体（或抗体组分）可视为“人类”，尽管其氨基酸序列例如在一或多个 CDR 并且尤其是 CDR3 中包括不由人类生殖系免疫球蛋白序列（例如包括序列变异体，例如可能（原先）已通过体外随机或定点诱变或通过体内体细胞突变引入的序列变异体）编码的残基或元件。

[0056] 人类化：如本领域中已知，术语“人类化”常用于指氨基酸序列包括来自在非人类物种（例如小鼠）中产生的参考抗体的 V_H 和 V_L 区序列而且在那些序列中包括打算使其更“人类样”（即，与人类生殖系可变序列更类似）的相对于参考抗体的修饰的抗体（或抗体组

分）。在一些实施例中，“人类化”抗体（或抗体组分）是免疫特异性地结合到所关注抗原的抗体，并且其具有具备实质上如人类抗体的氨基酸序列般的氨基酸序列的框架 (FR) 区以及具备实质上如非人类抗体的氨基酸序列般的氨基酸序列的互补决定区 (CDR)。人类化抗体包含实质上所有的至少一个并且通常是两个可变域 (Fab、Fab'、$F(ab')_2$、FabC、Fv)，其中所有或实质上所有的 CDR 区对应于非人类免疫球蛋白（即供体免疫球蛋白）的 CDR 区，并且所有或实质上所有框架区是人类免疫球蛋白共同序列的框架区。在一些实施例中，人类化抗体还包含免疫球蛋白恒定区 (Fc) 的至少一部分，通常是人类免疫球蛋白恒定区的至少一部分。在一些实施例中，人类化抗体含有轻链以及至少重链可变域两者。抗体还可包括重链恒定区的 CH_1、铰链、CH_2、CH_3 以及任选地 CH_4 区。在一些实施例中，人类化抗体仅含有人类化 V_L 区。在一些实施例中，人类化抗体仅含有人类化 V_H 区。在一些某些实施例中，人类化抗体含有人类化 V_H 和 V_L 区。

[0057] 提高、增大或减小：如本文中所用，术语“提高”、“增大”或“减小”或语法上的等效说法指示相对于基线测量值的值，所述基线测量值如在相同个体中在开始本文中所述的治疗之前的测量值或在一个对照个体（或多个对照个体）中在不存在本文中所述的治疗的情况下的测量值。“对照个体”是与所治疗的个体罹患相同类型和大致相同严重度的硬皮病的个体，其与所治疗的个体年龄大致相同（从而确保所治疗的个体与对照个体的疾病阶段是可比的）。

[0058] 试剂盒：如本文中所用，术语“试剂盒”是指用于传递物质的任何传递系统。所述传递系统可包括允许从一个地点到另一个地点储存、运输或传递各种诊断性或治疗性试剂（例如在适当容器中的寡核苷酸、酶等）和 / 或支持物质（例如缓冲液，用于执行分析的书面说明书等）的系统。举例来说，试剂盒包括一或多个含有相关反应试剂和 / 或支持物质的外壳（例如盒子）。术语“分部式试剂盒”是指包含两个或更多个分开的容器的传递系统，所述容器各自含有全部试剂盒组分的亚部分。可一起或分开地将所述容器传递给预期的接受者。举例来说，第一个容器可含有用于分析的酶，而第二个容器含有寡核苷酸。术语“分部式试剂盒”打算涵盖含有在联邦食品、药品和化妆品法案 (Federal Food, Drug, and Cosmetic Act) 的条款 520(e) 的管制之下的分析物特异性试剂 (Analyte Specific Reagents, ASR's) 的试剂盒，但并不局限于此。事实上，在术语“分部式试剂盒”中包括任何包含两个或更多个分开的容器的传递系统，所述容器各自含有全部试剂盒组分的亚部分。相反地，“组合式试剂盒”是指在单个容器中（例如在容纳每一所需组分的单个盒子中）含有所有组分的传递系统。术语“试剂盒”包括分部式和组合式试剂盒两者。

[0059] 正常：如本文中所用，术语“正常”在用于修饰术语“个体”或“受试者”时是指并未患有具体疾病或病况并且也不是所述疾病或病况的携带者的个体或个体群组。术语“正常”在本文中也用于形容从正常或野生型个体或受试者中分离的生物样本或样品，例如“正常生物样品”。

[0060] 核酸：如本文中所用的术语“核酸”是指寡核苷酸、核苷酸或多核苷酸以及其片段或部分，并且是指可为单链或双链的基因组或合成起点的 DNA 或 RNA，并且代表正义或反义链。

[0061] 核酸分子：术语“核酸分子”和“多核苷酸”在本文中可互换使用。其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，并且除非另行说明，否则其涵盖可以

与天然存在的核苷酸类似的方式起作用的已知的天然核苷酸类似物。所述术语涵盖具有合成主链的核酸样结构以及扩增产物。

[0062] 蛋白质：一般来说，“蛋白质”是多肽（即，由肽键彼此连接的至少两个氨基酸的串）。蛋白质可包括除氨基酸之外的部分（例如可为糖蛋白）和/或可以其它方式加工或修饰。所属领域的技术人员将理解“蛋白质”可为如由细胞产生的完整多肽链（具有或不具有信号序列），或可为其功能性部分。一般技术者将进一步理解蛋白质有时可包括例如由一或多个二硫键连接或通过其它方式缔合的一个以上多肽链。

[0063] 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”可互换使用。生物样品可（借助于非限制性实例）包括皮肤组织、肝组织、肾组织、肺组织、脑脊髓液、(CSF)、血液、羊水、血清、尿液、粪便、表皮样品、皮肤样品、颊部抹拭、精子、羊膜液、经培养细胞、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。生物样品还可为例如从任何器官或组织获得的样品（包括活检或尸检样本），可包含细胞（不论是原代细胞还是经培养细胞）、由任何细胞、组织或器官调节的培养基、组织培养物。在一些实施例中，适于本发明的生物样品是已经加工从而释放或以其它方式使核酸可供用于如本文中所述的检测的样品。也可使用固定或冷冻组织。

[0064] 受试者：如本文中所用，术语“受试者”是指人类或任何非人类动物（例如小鼠、大鼠、兔、狗、猫、牛、猪、绵羊、马或灵长类动物）。人类包括出生前和出生后形式。在许多实施例中，受试者是人类。受试者可为患者，其是指呈现给医疗服务提供者以便诊断或治疗疾病的人类。术语“受试者”在本文中与“个体”或“患者”可互换使用。受试者可罹患或易患疾病或病症但可能呈现或可能不呈现所述疾病或病症的症状。

[0065] 罹患：“罹患”疾病、病症和/或病况（例如硬皮病）的个体已诊断有所述疾病、病症和/或病况或展示所述疾病、病症和/或病况的一或多种症状。

[0066] 易患：“易患”疾病、病症和/或病况的个体尚未诊断有所述疾病、病症和/或病况和/或可能不展现所述疾病、病症和/或病况的症状。在一些实施例中，易患疾病、病症和/或病况（例如硬皮病）的个体的特征可在于以下中的一或多者：(1) 与所述疾病、病症和/或病况发展有关的基因突变；(2) 与所述疾病、病症和/或病况发展有关的基因多态性；(3) 增加和/或减少与所述疾病、病症和/或病况有关的蛋白的表达和/或活性；(4) 与所述疾病、病症和/或病况发展有关的习惯和/或生活方式；(5) 所述疾病、病症和/或病况的家族病史；(6) 对某些细菌或病毒的反应；(7) 暴露于某些化学物质。在一些实施例中，易患疾病、病症和/或病况的个体将发展所述疾病、病症和/或病况。在一些实施例中，易患疾病、病症和/或病况的个体将不发展所述疾病、病症和/或病况。

[0067] 治疗：如本文中所用，术语“治疗 (treatment)”（还有“治疗 (treat)”或“治疗 (treating)”）是指治疗性蛋白的任何投与（例如抗 CCL2 单克隆抗体或其抗原结合片段的投与）部分或完全地将具体疾病、病症和/或病况（例如硬皮病、纤维化或炎症）的一或多种症状或特征缓解、改善、减轻、抑制、延缓发作、降低严重度和/或降低发病率。所述治疗可为对并未展现相关疾病、病症和/或病况的征象的受试者的治疗和/或对仅展现疾病、病症和/或病况的早期征象的受试者的治疗。可替代地或另外，所述治疗可为对展现相关疾病、病症和/或病况的一或多种确立征象的受试者的治疗。

具体实施方式

[0068] 本发明尤其提供特征在于高亲和力、效力、组织选择性和/或表位特异性的经改良抗 CCL2 抗体，和其尤其用于治疗硬皮病和相关纤维化和/或发炎性疾病、病症和病况的用途。在一些实施例中，本发明提供基于亲和力为 10^{-12}M 或更大的抗 CCL2 抗体治疗硬皮病和相关纤维化和/或发炎性疾病、病症和病况的方法和组合物。

[0069] 本发明在某种程度上基于由本发明人观察到的独到见解，即，高亲和力抗 CCL2 抗体尤其在以高剂量投与时允许不管血浆中的高水平 CCL2 而有效抑制受影响组织中的 CCL2。本发明的实施例包括亲和力为 10^{-12}M 或更大的抗 CCL2 抗体。所述高亲和力的抗体是尤其有利的。由于硬皮病患者血浆中 CCL2 的循环水平较高，故所投与的任何抗 CCL2 抗体的较大部分很可能被循环 CCL2 所螯合。在不希望受理论束缚的情况下，高亲和力抗 CCL2 抗体除了中和循环 CCL2 之外还可有效中和受影响组织中的 CCL2，这部分归因于其有效地竞争离开受体 CCR2（其对 CCL2 的结合亲和力为 60pM）的能力。因此，高亲和力抗 CCL2 抗体（例如结合亲和力强于 60pM 的抗 CCL2 抗体）可有效螯合患病组织中的 CCL2，防止 CCL2 与其受体 CCR2 之间的结合。因此，患病组织中可能需要较少量的高亲和力抗 CCL2 抗体来实现所需治疗作用。

[0070] 本发明的各种方面详细地描述于以下章节中。章节的使用不意欲限制本发明。每一章节可应用于本发明的任何方面。在本申请案中，除非另外说明，否则“或”的使用意指“和/或”。

[0071] 硬皮病

[0072] 硬皮病或全身性硬化症通常被视为慢性全身性自身免疫疾病，其特征尤其在于纤维化或硬化、血管变化和自身抗体。在不希望受理论束缚的情况下，据认为硬皮病是由强化扩增环中截留的活动过度的自身免疫反应引起的。举例来说，硬皮病的组织学特征在于单核细胞的炎性浸润，其又活化周围成纤维细胞中的胶原蛋白合成并与胶原蛋白合成增加有关。具体来说，活化的巨噬细胞产生 TGF-β 和 PDGF，其活化受影响区域中的成纤维细胞以产生较高量的胶原蛋白。

[0073] T 细胞似乎也通过活化巨噬细胞和炎性前纤维发生细胞因子的直接释放而在疾病过程中起一定作用。除了胶原蛋白之外，活化的成纤维细胞似乎分泌会将其它炎性细胞募集到受影响区域的因子，所述炎性细胞释放细胞因子，所述细胞因子进一步募集细胞因子释放炎性细胞，由此引起未经调节的炎症和组织纤维化。

[0074] 通常，单核细胞/巨噬细胞和 T 细胞在硬皮病患者的循环和组织中的数目和活化均增加。组织积聚既是微血管损伤的原因也是其作用，微血管损伤是硬皮病发病机制中的早期事件之一。微血管损伤的特征在于内皮细胞损伤、基底膜层增生、外周血液单核细胞在血管壁中的偶发性滞留以及初始血管周围单核细胞浸润。随着发炎级联反应恶化，纤维化、内脏器官架构紊乱、血管稀疏以及因此产生的缺氧占主导地位。所有这些因素和单核细胞的持续募集促成纤维化的维持。

[0075] 在一些实施例中，硬皮病也被视为结缔组织疾病，其特征通常在于胞外基质蛋白在皮肤和内脏中的过度积聚、血管损伤以及免疫异常。

[0076] 所述疾病的许多临床表现被认为涉及血管重构的误调节。硬皮病的最早症状之一是微血管损伤。这种微血管损伤被认为引起内皮细胞活化增加。认为活化的内皮细胞会表

达粘附分子，引起毛细管渗透性改变，允许炎性细胞通过内皮迁移并滞留在血管壁中。免疫活化被认为促进内皮活化维持，这引起内皮细胞的分解。认为这一过程会促进在硬皮病患者中通常观察到的血管弹性损失和变窄。此外，认为微血管损伤促成单核细胞在真皮中的血管周围浸润，这被认为会促进成纤维细胞活化和许多硬皮病的相关标志性症状。随着纤维化增加，渗透性降低。因此，抗体渗透患病组织变得更困难。因此，抗 CCL2 抗体的亲和力对于保持抗体局部化来说变得尤其重要。

[0077] 所述疾病的许多临床表现被大体上认为涉及成纤维细胞的误调节。成纤维细胞的主要功能是通过连续分泌胞外基质前体来维持结缔组织的结构完整性。成纤维细胞为许多组织提供结构框架（基质），在创伤愈合中起到重要作用并且是动物中最常见的结缔组织细胞。成纤维细胞在形态上是异质的，取决于其位置和活性具有多样的外观。

[0078] 硬皮病有两种主要形式：局限性全身性硬化症 / 硬皮病和弥漫性全身性硬化症 / 硬皮病。在局限性皮肤硬皮病中，皮肤的纤维化大体上限制于接近肘部的区域。局限性皮肤硬皮病患者通常经历血管损害。皮肤和器官纤维化在局限性硬皮病患者中通常进展缓慢。弥漫性硬皮病患者通常经历与在局限性硬皮病中相比进展更快速的皮肤和器官的纤维化和 / 或广泛的炎症和 / 或与在局限性硬皮病中可见相比更严重的内部器官涉及。

[0079] 通常认为间质肺疾病（其引起肺纤维化）是硬皮病相关死亡的主要原因（卢德维卡 - 布拉德莱 A.(Ludwicka-Bradley,A.）等人硬皮病间质肺疾病中的凝血和自身免疫性 (Coagulation and autoimmunity in scleroderma interstitial lung disease). 关节炎与风湿病论文集 (Semin Arthritis Rheum),41(2),212-22,2011)。引起硬皮病相关死亡的其它并发症包括（但不限于）癌症、心力衰竭、肺高血压、肾衰竭和吸收障碍或其任何组合。

[0080] 硬皮病最常通过检查皮肤症状来诊断。诊断的测试包括（但不限于）目视和 / 或手动检查皮肤、血压测试、胸 x 射线、肺 CT、超声心动图、尿分析、皮肤活检和血液测试（包括抗核抗体测试、抗拓扑异构酶抗体测试、抗着丝粒抗体测试、抗 U3 抗体测试、抗 RNA 抗体测试、其它类型的抗体测试、红细胞沉降速率以及类风湿因子）。

[0081] 抗 CCL2 抗体

[0082] 本发明提供基于投与抗 CCL2 抗体（具体来说是高亲和力抗 CCL2 抗体）来治疗硬皮病和相关纤维化和 / 或发炎性疾病、病症和病况的方法和组合物。

[0083] CCL2

[0084] CCL2 是由多种细胞类型产生的趋化因子。其也称为单核趋化蛋白 -1(MCP-1)。已知 CCL2 是免疫系统许多细胞类型的强力引诱剂，包括（但不限于）单核细胞、CD4 和 CD8 记忆 T 淋巴细胞和 NK 细胞（卡鲁利 M.(Carulli,M.）等人 CCL2 血清水平可在全身性硬化症中用于风险分层或监测治疗反应吗？ (Can CCL2serum levels be used in risk stratification or to monitor treatment response in systemic sclerosis？）风湿病年鉴 (Ann Rheum Dis),67,105-109,2008, 山本 T.(Yamamoto,T.）硬皮病 - 病理生理学 (Scleroderma-Pathophysiology). 欧洲皮肤病学杂志 (Eur J Dermatol),19(1),14-24)。CCL2 已展示出促进白细胞跨内皮单层迁移，表明在促进单核细胞的血管周围浸润中的作用（同上）。CCL2 也已展示出在体外大鼠成纤维细胞中促进成纤维细胞活化并上调 I 型胶原蛋白 mRNA 表达。在硬皮病患者以及硬皮病动物模型中已展示出 CCL2 水平升高（同上）。确切地说，在硬皮病皮肤中已展示 CCL2 表达水平增加，并且在硬皮病成纤维细胞中已展示

CCL2RNA 和蛋白质增加（同上）。

[0085] 人类 CCL2 是含有 76 个氨基酸残基的 8.6kDa 蛋白，其氨基酸序列展示在表 1 中。其由多种细胞类型（尤其包括单核细胞、血管内皮细胞、平滑肌细胞、某些上皮细胞）表达并且结合其受体 CCR2。CCL2 属于 CC 趋化因子家族，其含有邻近的两个半胱氨酸残基（邻近半胱氨酸残基在表 1 中带下划线）。

[0086] 表 1

[0087]

人类 **CCL2** 蛋白序列（**GeneBank：NP_002973**）	MKVSAALLCLLLIAATFIPQGLAQPDAINAPVTCCYNFTN RKISVQRLASYRRITSSKCPKEAVIFKTIVAKEICADPKQK WVQDSMDHLDKQTQTPKT（**SEQ ID NO: 1**）

[0088] CCL2 也已从非人类来源纯化、表征、克隆并测序并且可以重组方式产生或以化学方式合成。如本文中所用，术语 CCL2 涵盖其它物种（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、羊驼等等）中天然产生的任何 CCL2 蛋白以及与人类 CCL2 实质上同源或一致的任何重组或合成 CCL2。在一些实施例中，如本文中所用的 CCL2 蛋白的序列与 SEQ ID NO:1 至少 50％、55％、60％、65％、70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或更多同源。在一些实施例中，如本文中所用的 CCL2 蛋白的序列与 SEQ ID NO:1 至少 50％、55％、60％、65％、70％、75％、80％、85％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或更多一致。与人类 CCL2 实质上同源或一致的 CCL2 蛋白通常还保留人类 CCL2 的实质性活性。就本文中鉴别的 CCL2 序列来说的“氨基酸序列一致性百分比（％）”被定义为在比对序列且必要时引入空隙以实现最大序列一致性百分比，且不将任何保守取代视作序列一致性的一部分之后候选序列中与 CCL2 序列中氨基酸残基一致的氨基酸残基的百分比。为了测定氨基酸序列一致性百分比的比对可以在所属领域技能内的各种方式实现，例如使用公开可供使用的计算机软件，如 BLAST、ALIGN 或 Megalign(DNASTAR) 软件。所属领域的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内实现最大比对所需的任何算法。优选地，WU-BLAST-2 软件用于测定氨基酸序列一致性（奥特查尔 (Altschul) 等人，酶学方法 (Methods in Enzymology)266,460-480(1996)；http://blast.wustl/edu/blast/README.html)。WU-BLAST-2 使用数种搜索参数，其中大部分被设定成默认值。可调节的参数用以下值设定：重叠跨度＝ 1，重叠分率＝ 0.125，世界阈值 (T) ＝ 11。HSP 分数 (S) 和 HSP S2 参数是动态值且由程序自身确定，取决于具体序列的组成，然而，最小值可经调节且如上文所指出设定。

[0089] 上述 CCL2 蛋白中的任一者均可用于产生并鉴别特异性结合于 CCL2 的单特异性抗体。参见以下抗 CCL2 抗体章节。

[0090] 抗 CCL2 抗体

[0091] 本文中所述的 CCL2 蛋白或其片段可用于通过所属领域的技术人员熟知的方法产生抗体。如本文中所用，抗 CCL2 抗体包括特异性结合于 CCL2 的任何表位的任何抗体或抗体片段。如本文所用，术语“抗体”为打算包括对指定蛋白质或肽或其片段具有特异反应性的免疫球蛋白以及其片段。举例来说，术语“抗体”包括完整单克隆抗体、多克隆抗体、单域抗体（例如鲨鱼单域抗体（例如 IgNAR 或其片段））、以及抗体片段（只要其展现所需生物

活性即可）。适合的抗体还包括（但不限于）人类抗体、灵长类化抗体、嵌合抗体、双特异性抗体、人类化抗体、结合抗体（即，与其它蛋白质、放射性标记、细胞毒素结合或融合的抗体）、小型模块化免疫药物（“SMIPs™”）以及抗体片段。

[0092] 如本文所用，“抗体片段”包括完整抗体的一部分，如抗体的抗原结合区或可变区。抗体片段的实例包括 Fab、Fab’、F(ab’)2 以及 Fv 片段；三功能抗体；四功能抗体；线性抗体；单链抗体分子。术语“抗体片段”还包括像抗体一样通过与特定抗原结合以形成复合物来起作用的任何合成或基因工程改造的蛋白。举例来说，抗体片段包括经分离片段、由重链和轻链的可变区组成的“Fv”片段、轻链和重链可变区域通过肽连接子（“ScFv 蛋白”）连接的重组单链多肽分子以及由模拟高变区的氨基酸残基组成的最小识别单位。

[0093] 可以使用所属领域中众所周知的方法来产生抗 CCL2 抗体。举例来说，用于抗体产生的方案由哈洛 (Harlow) 和莱恩 (Lane)，抗体：实验指南 (Antibodies:A Laboratory Manual)，(1988) 描述。抗体通常可在小鼠、大鼠、天竺鼠、仓鼠、骆驼、羊驼、鲨鱼或其它适当宿主中产生。或者，抗体可在鸡中制得，产生 IgY 分子（谢德 (Schade) 等人，(1996)ALTEX 13(5):80-85)。在一些实施例中，适于本发明的抗体是低于人类的灵长类动物抗体。举例来说，在狒狒中产生治疗适用抗体的通用技术可见于例如戈登伯格 (Goldenberg) 等人，国际专利公开案第 WO 91/11465(1991) 号和洛斯曼 (Losman) 等人，国际癌症杂志 (Int. J.Cancer)46:310(1990) 中。在一些实施例中，单克隆抗体可使用杂交瘤方法制备（米尔斯坦 (Milstein) 和奎洛 (Cuello)，(1983) 自然 (Nature)305(5934):537-40)。在一些实施例中，单克隆抗体还可通过重组方法制得（美国专利第 4,166,452 号，1979)。

[0094] 与通过 B 细胞永生化产生单克隆抗体有关的许多困难可通过使用噬菌体展示在大肠杆菌 (E.coli) 中工程改造并表达抗体片段来解决。为了确保高亲和力单克隆抗体的回收，组合性免疫球蛋白文库必须通常含有较大组库大小。典型策略利用从经免疫小鼠的淋巴细胞或脾细胞获得的 mRNA 来使用逆转录酶合成 cDNA。重链和轻链基因分别通过 PCR 扩增并接合到噬菌体克隆载体中。产生两个不同文库，一个含有重链基因且一个含有轻链基因。从每一文库中分离噬菌体 DNA，且将重链和轻链序列接合在一起并包装以形成组合性文库。每一噬菌体含有随机的重链和轻链 cDNA 对，且在感染大肠杆菌后引导抗体链在经感染细胞中的表达。为了鉴别识别所关注抗原的抗体，接种噬菌体文库，且将存在于斑块中的抗体分子转移到过滤器。过滤器与放射性标记的抗原一起孵育，且接着洗涤以去除过量非结合配体。自动放射摄影上的放射性点鉴别含有会结合抗原的抗体的斑块。适用于产生人类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（加利福尼亚州拉荷亚 (La Jolla,Calif.) 获得。

[0095] 可以采用类似策略来获得高亲和力 scFv。参见例如沃恩 (Vaughn) 自然 •生物技术 (Nat.Biotechnol.),14:309314(1996)。具有较大组库的 scFv 文库可通过使用对应于所有已知 V_H、Vκ 和 Vλ 基因家族的 PCR 引物从非经免疫人类供体分离 V 基因来构建。在扩增之后，将 Vκ 和 Vλ 池组合以形成一个池。将这些片段接合到噬菌粒载体中。接着将 scFv 连接子 $(Gly_4,Ser)_3$ 接合到 V_L 片段上游噬菌粒中。将 V_H 和连接子 -V_L 片段扩增且装配在 J_H 区上。将所得 V_H- 连接子 -V_L 片段接合到噬菌粒载体中。噬菌粒文库可如上文所述使用过滤器或使用免疫管（能肯公司 (Nunc)；Maxisorp) 淘选。类似结果可通过从经免疫兔的淋巴细胞或脾细胞构建组合性免疫球蛋白文库并通过在巴斯德毕赤酵母 (P.pastoris) 中表达 scFv 构筑

体来实现。参见例如里德尔 (Ridder) 等人, 生物技术 (Biotechnology),13:255260(1995)。另外,在分离适当 scFv 之后,可通过亲和力成熟方法 (如 CDR3 诱变和链改组) 来获得具有较高结合亲和力和较慢解离速率的抗体片段。参见例如杰克逊 (Jackson) 等人, 英国癌症杂志 (Br.J.Cancer),78:181188(1998) ;奥斯本 (Osbourn) 等人, 免疫技术 (Immunotechnology),2:181196(1996)。

[0096] 另一形式的抗体片段是编码单一 CDR 的肽。CDR 肽 (“最小识别单位”) 可通过构筑编码所关注抗体的 CDR 的基因来获得。所述基因例如通过使用聚合酶链式反应合成来自产生抗体的细胞的 RNA 的可变区来制备。参见例如拉里克 (Larrick) 等人, 方法 :酶学方法伴侣 (Methods:A Companion to Methods in Enzymology)2:106(1991) ;考特尼 - 拉克 (Courtenay-Luck), “单克隆抗体的基因操纵 (Genetic Manipulation of Monoclonal Antibodies)”, 单克隆抗体 :产生,工程改造和临床应用 (MONOCLONAL ANTIBODIES:PRODUCTION,ENGINEERING AND CLINICAL APPLICATION), 里特 (Ritter) 等人 (编), 第 166179 页 (剑桥大学出版社 (Cambridge University Press)1995) ;和沃德 (Ward) 等人, “抗体的基因操纵和表达 (Genetic Manipulation and Expression of Antibodies)”, 单克隆抗体 :原理和应用 (MONOCLONAL ANTIBODIES:PRINCIPLES AND APPLICATIONS), 伯池 (Birch) 等人, (编), 第 137185 页 (威立 - 利斯公司 (Wiley-Liss,Inc.)1995)。

[0097] 在一些实施例中,适于本发明的抗体可以包括人类化或人类抗体。非人类抗体的人类化形式为含有来源于非人类 Ig 的最小序列的嵌合 Ig、Ig 链或片段 (如 Fv、Fab、Fab’、F(ab’)2 或 Ab 的其它抗原结合子序列)。人类化抗体通常具有一或多个由非人类来源引入的氨基酸残基。这些非人类氨基酸残基往往称为“进口”残基,其通常是从“进口”可变域获取。人类化通过啮齿动物互补决定区 (CDR) 或 CDR 序列取代相应人类抗体序列来实现 (莱克曼 (Riechmann) 等人, 自然 332(6162):323-7,1988 ;韦荷恩 (Verhoeyen) 等人, 科学 (Science).239(4847):1534-6,1988)。所述“人类化”抗体是嵌合 Ab(例如参见美国专利第 4,816,567 号、第 5,693,762 号和第 5,225,539 号),其中实质上少于完整的人类可变域已经来自非人类物种的相应序列取代。在一些实施例中,人类化抗体通常为一些 CDR 残基以及可能一些 FR 残基由来自啮齿动物 Ab 中的类似位点的残基取代的人类抗体。人类化抗体包括具有所需特异性、亲和力以及容量的人类 Ig(接受体抗体),其中来自接受体的 CDR 的残基由来自非人类物种 (供体抗体)(如小鼠、大鼠或兔) 的 CDR 的残基替代。在一些情况下,相应非人类残基代替人类 Ig 的 Fv 框架残基。人类化抗体可以包含在接受体抗体与引入 CDR 或框架序列中均不存在的残基。一般来说,人类化抗体包含实质上所有的至少一个并且通常两个可变域,其中大部分 (如果不是所有)CDR 区与非人类 Ig 的 CDR 区对应,并且大部分 (如果不是所有)FR 区为人类 Ig 共同序列的 FR 区。人类化抗体最佳还包含 Ig 恒定区 (Fc) 的至少一部分,通常是人类 Ig 的至少一部分 (莱克曼等人, 自然 332(6162):323-7,1988 ;韦荷恩等人, 科学 .239(4847):1534-6,1988)。

[0098] 人类抗体还可使用各种技术产生,包括噬菌体展示文库 (霍根布姆 (Hoogenboom) 等人, 分子免疫学 (Mol Immunol.)(1991)28(9):1027-37 ;马克斯 (Marks) 等人, 分子生物学杂志 (1991)222(3):581-97) 和制备人类单克隆抗体 (雷斯菲尔德 (Reisfeld) 和赛尔 (Sell),1985, 癌症调查 (Cancer Surv.)4(1):271-90)。类似地,可以采用将人类 Ig 基因引入内源性 Ig 基因已部分或完全失活的转基因动物中来合成人类抗体。在攻击之后,观

察人类抗体产生，其在所有方面与在人类中见到的极其类似，包括基因重组、装配以及抗体组库（费雪威尔德 (Fishwild) 等人，来自新颖品系的微型基因座转基因小鼠的高亲合力人 IgGκ 单克隆抗体 (High-avidity human IgG kappa monoclonal antibodies from a novel strain of minilocus transgenic mice)，自然•生物技术 (Nat Biotechnol.)1996 年 7 月 ;14(7):845-51 ;隆伯格 (Lonberg) 等人，来自包含四个不同基因修饰的小鼠的抗原特异性人类抗体 (Antigen-specific human antibodies from mice comprising four distinct genetic modifications)，自然 1994 年 4 月 28 日 ;368(6474):856-9 ;隆伯格和胡萨尔 (Huszar)，来自转基因小鼠的人类抗体 (Human antibodies from transgenic mice)，国际免疫学评论 (Int.Rev.Immunol.)1995 ;13(1):65-93 ;马克斯 (Marks) 等人，绕过免疫接种 :通过链改组构建高亲和力人类抗体 (By-passing immunization:building high affinity human antibodies by chain shuffling). 生 物 技 术（纽 约）(Biotechnology(N Y)).1992 年 7 月 ;10(7):779-83)。在一些实施例中，人类抗 CCL2 抗体通过免疫接种经工程改造以使人类抗体响应于抗原攻击的非人类动物来制得 ;例如用人类 CCL2 免疫接种（例如参见美国专利第 5,569,825 号、第 6,150,584 号和第 6,596,541 号）。

[0099] 使用高亲和力抗 CCL2 抗体治疗硬皮病是很重要的。如上文所述，CCL2 与 CCR2 受体之间的结合亲和力较高（即，60pM），并在血浆中存在高水平循环 CCL2。因此，大部分抗 CCL2 抗体很可能在投与后在血浆中螯合且仅一小部分可能定位到患病目标组织。因此，抗 CCL2 抗体不大可能有效竞争 CCL2 离开受体且抑制目标组织中的信号传导，除非其对 CCL2 还具有较高结合亲和力。此外，随着硬皮病进展，纤维化增加且血管渗透性和对目标组织的接近减少。使用高亲和力抗 CCL2 抗体确保留存在目标组织的抗体仍然能够结合 CCL2 并防止与其受体相互作用。

[0100] 因此，在一些实施例中，适于本发明的抗 CCL2 抗体或其片段的结合亲和力为或大于 约 500nM、100nM、10nM、1nM、500pM、100pM、50pM、10pM、1pM、500fM、400fM、300fM、200fM、100fM、50fM、10fM、1fM。在一些实施例中，适于本发明的抗 CCL2 抗体或其片段的结合亲和力在约 500nM 与 1fM 之间、在 500nM 与 10fM 之间、在 500nM 与 100fM 之间、在 500nM 与 1pM 之间、在 10nM 与 1fM 之间、在 10nM 与 100fM 之间、在 10nM 与 1pM 之间、在 1nM 与 1fM 之间、在 1nM 与 100fM 之间、在 1nM 与 500fM 之间、在 1nM 与 1pM 之间、在 1nM 与 10pM 之间、在 1nM 与 50pM 之间、在 1nM 与 100pM 之间、在 1nM 与 500pM 之间。

[0101] 生物分布和生物可用性

[0102] 在各种实施例中，在体内投与后，根据本发明的抗 CCL2 抗体可传递到各种目标组织。示例性所需目标组织包括（但不限于）皮肤、血管、肺、心、肾、胃肠道（包括肝）、食道、肌肉骨胳系统以及其组合。

[0103] 在各种实施例中，在体内投与后，根据本发明的抗 CCL2 抗体可在治疗上或临床上实现本文中所述的各种目标组织中的有效水平或活性。如本文中所用，治疗上或临床上有效水平或活性是足以在目标组织中带来治疗作用的水平或活性。治疗作用可为客观的（即，可通过一些测试或指标测量）或主观的（即，受试者给出作用的指示或感觉到作用）。举例来说，治疗上或临床上有效水平或活性可为足以改善目标组织中与硬皮病或相关疾病、病症或病况有关的症状的蛋白质水平或活性（例如 CCL2 水平）。在一些实施例中，根据本发明传递的本文中所述的抗 CCL2 抗体可使目标组织中与未经治疗的对照或治疗前状态

相比 CCL2 水平减小至少 5%、10%、20%、30%、40%、50%、60%、70%、80%、90%、95%。

[0104] 在一些实施例中，根据本发明传递的本文中所述的抗 CCL2 抗体可使 CCL2 血清水平减小到低于约 1000pg/ml、900pg/ml、800pg/ml、700pg/ml、600pg/ml、500pg/ml、400pg/ml、300pg/ml、250pg/ml、200pg/ml、180pg/ml、160pg/ml、140pg/ml、120pg/ml、100pg/ml 或更低。

[0105] 一般来说，在体内投与后，根据本发明的抗 CCL2 抗体在血清和 / 或目标组织（例如皮肤、血管、肺、心、肾、胃肠道（包括肝）、食道或肌肉骨胳系统）中具有足够长的半衰期。在一些实施例中，根据本发明的抗 CCL2 抗体的半衰期可能为至少约 30 分钟、45 分钟、60 分钟、90 分钟、2 小时、3 小时、4 小时、5 小时、6 小时、7 小时、8 小时、9 小时、10 小时、12 小时、16 小时、18 小时、20 小时、25 小时、30 小时、35 小时、40 小时、高达 3 天、高达 7 天、高达 14 天、高达 21 天或高达一个月。在一些实施例中，根据本发明的抗 CCL2 抗体可在投与后的 12 小时、24 小时、30 小时、36 小时、42 小时、48 小时、54 小时、60 小时、66 小时、72 小时、78 小时、84 小时、90 小时、96 小时、102 小时或一周之后在血清和 / 或目标组织中保持可检测水平或活性。可检测水平或活性可使用所属领域中已知的各种方法测定。

[0106] 在某些实施例中，本文中所述的抗 CCL2 抗体在（例如静脉内）投与所述受试者之后（例如在（例如静脉内）投与受试者之后的一周、3 天、48 小时、36 小时、24 小时、18 小时、12 小时、8 小时、6 小时、4 小时、3 小时、2 小时、1 小时、30 分钟或更短）在血清或靶向组织中实现至少 20μg/ml、至少 15μg/ml、至少 10μg/ml、至少 7.5μg/ml、至少 5μg/ml、至少 2.5μg/ml、至少 1.0μg/ml 或至少 0.5μg/ml 的浓度。

[0107] 硬皮病和相关疾病、病症或病况的治疗

[0108] 本文中所述的抗 CCL2 抗体可用于有效地治疗罹患或易患硬皮病或相关纤维化、发炎性疾病、病症或病况的个体。如本文中所用的术语“治疗（treat）”或“治疗”是指改善一或多种症状，预防或延缓一或多种症状的发作，和 / 或减轻相关疾病、病症或病况的一或多种症状的严重度或频率。

[0109] 本发明的各种抗体可以单独或与其它抗体或治疗剂组合投与。在一些实施例中，本文中所述的抗体可单独或与其它治疗剂（如适用于治疗纤维化或发炎性疾病、病症或病况的治疗剂）结合投与。所述治疗剂包括（但不限于）皮质类固醇、NSAID、免疫抑制药物（例如甲氨蝶呤和环磷氮介）、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、松弛素和环孢灵）和抗 TGFβ 治疗以及内皮素受体拮抗剂。

[0110] 在一些实施例中，本文中所述的抗体可使用常规剂量和传递方法（如对于其它可比治疗剂描述的那些）投与。待投与的剂量可通过所属领域的技术人员已知的常规程序确定。参见例如治疗学的药理学基础 (The Pharmacological Basis of Therapeutics), 古德曼 (Goodman) 和吉尔曼 (Gilman) 编，纽约的麦克米伦出版公司 (Macmillan Publishing Co.,New York)。一般来说，有效剂量是大到足以产生所需作用（例如中和 CCL2 和 / 或阻断 CCL2 与其同源受体结合）的剂量。剂量应不大到引起不良副作用，如非所需交叉反应、过敏性反应等。待考虑的因素包括所涉及的特定抗体 / 药剂的活性、其代谢稳定性和作用持续时间、投与的模式和时间、药物组合、排泄速率、以及进行疗法的宿主的年龄、体重、一般健康状况、性别、膳食以及具体疾病状态的严重度。

[0111] 本文中所述的抗体可以治疗上有效的任何给药方案投与。在一些实施例中，抗

CCL2 抗体以两月一次、每月一次、三周一次、两周一次、每周一次、每天一次或以可变的时间间隔投与。

[0112] 本文中所述的抗体可使用任何投与方法投与，包括肠胃外和非肠胃外投与途径。肠胃外途径包括例如静脉内、动脉内、门静脉内、肌内、皮下、腹膜内、脊柱内、鞘内、脑室内、颅内、胸膜内或其它注射途径。非肠胃外途径包括例如口服、经鼻、经皮、经肺、经直肠、颊内、经阴道、经眼。投与还可通过连续输注、局部投与、从植入物（凝胶、膜等）持续释放和/或静脉内注射进行。

[0113] 硬皮病

[0114] 在一些实施例中，本文中所述的方法和组合物可用于治疗罹患或易患所有形式硬皮病的受试者，包括局限性全身性硬化症/硬皮病、弥漫性全身性硬化症/硬皮病以及其它形式硬皮病。局限性全身性硬化症/硬皮病通常涉及主要影响手、臂以及面部的皮肤表现。其关系到以下并发症也称为 CREST 综合症：钙质沉着、雷诺氏现象 (Raynaud's phenomenon)、食道功能障碍、肢端皮肤硬化以及毛细血管扩张。另外，肺动脉高血压可能存在于高达三分之一的患者中，并且是此形式硬皮病的最严重的并发症。弥漫性全身性硬化症/硬皮病是快速进行性的且影响大面积皮肤和一或多种内脏，时常为肾、食道、心和肺。其它形式的硬皮病包括全身性无皮肤硬化的硬皮病 (systemic sine scleroderma)，其缺乏皮肤变化，但具有全身性表现；以及影响皮肤，但不影响内脏的两种局部化形式：硬斑病和带状硬皮病。

[0115] 在一些实施例中，治疗是指与未经治疗的对照或治疗前状态相比，部分或完全地将与硬皮病有关的一或多种症状缓解、改善、减轻、抑制、延缓发作、降低严重度和/或发病率，所述症状包括（但不限于）内皮细胞损伤；基底膜层增生；血管周围单核细胞浸润；纤维化；内脏器官架构紊乱；血管稀疏；缺氧；手指、背部和前臂肿胀；肢体感觉发冷；指端溃疡；指甲褶皱延长；指甲凹陷出血；指甲上凹陷疤痕；肺高血压；皮肤纤维化；脱发；皮肤绷紧；皮肤发硬；色素过多；色素过少；皮肤发痒；腕管综合症；肌肉无力；关节疼痛；关节僵硬；肾纤维化；食道纤维化；口腔纤维化；心纤维化；和肺纤维化；肝纤维化；肌肉纤维化；干咳；呼吸短促；呼吸困难；肺泡炎；肺炎；喘息；进餐后腹胀；便秘；腹泻；吞咽困难；胃窦血管扩张；食道回流；胃灼热；大便失禁；口腔中扁平白色斑点；附着齿龈粘膜损失；齿龈退缩；牙周韧带弥漫性加宽；吞咽困难；口腔无弹性；下颌骨、冠突和髁突的后支的吸收；癌症；心力衰竭；肺高血压；肾衰竭；吸收障碍；或其任何组合。

[0116] 在一些实施例中，治疗是指将纤维化部分或完全地缓解、改善、减轻、抑制、延缓发作、降低严重度和/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结缔组织。在不希望受具体理论束缚的情况下，认为纤维化可由某些成纤维细胞的活化引起。已知成纤维细胞的不同亚型执行不同的功能，即使是在单一组织内。举例来说，皮肤上层的乳头状成纤维细胞产生薄胶原蛋白束并具有较高增生速率，而来自皮肤更深真皮层的网状成纤维细胞产生厚胶原蛋白束和大量的多功能蛋白聚糖，并促进快速网格收缩。成纤维细胞可处于静息状态或处于活化的不同阶段。在正常细胞功能期间，成纤维细胞例如响应于损伤而变得活化以促进创伤愈合。活化的成纤维细胞产生增加的胞外基质组分，包括胶原蛋白和胶原蛋白调节酶。在患有的硬皮病个体中，通常观察到的成纤维细胞活化增加，伴随着 ECM 的过度产生。通常认为 ECM 的这种过度产生会引起纤维化，在器官或组织

中形成过量纤维结缔组织，这是硬皮病的特征。

[0117] 在一些实施例中，治疗是指将皮肤、肾、胃肠道（包括肝）、血管、胃肠道、肌肉骨胳系统、肺和/或食道中的纤维化部分或完全地缓解、改善、减轻、抑制、延缓发作、降低严重度和/或发病率。

[0118] 在一些实施例中，治疗引起皮肤纤维化部分或完全地缓解、改善、减轻、抑制、延缓发作、降低严重度和/或发病率。皮肤纤维化通常与皮肤变厚、硬化或形成疤痕（例如瘢痕瘤或烧伤疤痕等）有关。在一些实施例中，皮肤纤维化通过经修改的罗德南皮肤分数(Modified Rodnan Skin Score)评估。举例来说，如图1中所示，未涉及的皮肤给予分数0；轻度变厚给予分数1；中度变厚给予分数2；以及重度变厚给予分数3。在一些实施例中，治疗引起与治疗前状态相比，经修改的罗德南皮肤分数降低超过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。在一些实施例中，治疗引起皮肤纤维化的实质性消除。

[0119] 在不希望受理论束缚的情况下，还认为硬皮病患者中成纤维细胞的活化可由通过产生细胞因子活化免疫反应所引起。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产生，例如活化的T细胞、单核细胞或巨噬细胞，或者，细胞因子可由上皮细胞产生。促进成纤维细胞活化的一个因素可为与毛细管渗透性增加有关的真皮中单核细胞的血管周围浸润。成纤维细胞活化的替代性或其它方式包括与胞外基质相互作用和/或机械张力。因此，在一些实施例中，根据本发明治疗硬皮病患者引起一或多种促炎性细胞因子（如本文中所述的那些）的产生减少。在一些实施例中，治疗引起与治疗前状态相比，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）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是所属领域中已知的并可用于实践本发明。

[0120] 在一些实施例中，治疗引起CCL2血清水平降低。在一些实施例中，治疗引起与治疗前状态相比，CCL2血清水平降低超过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。在一些实施例中，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。在一些实施例中，治疗引起CCL2血清水平与实质上相同年龄或发展阶段的健康对照的CCL2血清水平相当。

[0121] 纤维化疾病、病症或病况

[0122] 除了硬皮病之外，根据本发明的方法和组合物可用于治疗纤维化疾病、病症或病况，通常包括（但不限于）多灶性纤维硬化、硬皮病样移植物抗宿主病、肾源性全身性纤维化、器官特异性纤维化等。说明性器官特异性纤维化病症包括（但不限于）肺纤维化、肺高血压、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、病症或病况在受影响组织中具有无序和/或放大的胞外基质沉积。纤维化可与炎症有

关，随着潜在疾病症状出现，和 / 或由手术程序或创伤愈合过程引起。未经检查的纤维化可引起底层器官或组织架构的破坏，通常被称为结疤。

[0123] NASH 通常是症状很少或没有的静寂疾病。患者在早期中通常感觉良好，且在疾病更晚期或肝硬化发展时仅开始具有如疲劳、体重减轻和无力的症状。NASH 的进展可耗时数年，甚至数十年。所述过程可终止并且在一些情况下可能甚至在无特定疗法的情况下自身开始逆转。或者 NASH 可缓慢恶化，引起结疤或纤维化出现并在肝中积聚。随着纤维化恶化，肝硬化发展，其中肝变得严重结疤、硬化且不能正常起作用。并不是所有患有 NASH 的个人都发展肝硬化，但一旦存在严重结疤或肝硬化，几乎没有治疗可停止所述进展。患有肝硬化的个人经历液体潴留、肌肉萎缩、肠出血和肝衰竭。肝移植是伴随肝衰竭的晚期肝硬化的唯一疗法，且移植在患有 NASH 的人中的执行渐增。在美国，NASH 排名为肝硬化的主要病因之一，在丙型肝炎和酒精性肝病之后。

[0124] 肾脏（肾）纤维化由肾中纤维结缔组织的过度形成造成。肾纤维化引起显著的发病率和死亡率且产生对透析或肾移植的需求。纤维化可出现在肾单位（肾脏的功能单位）的滤过或再吸收组分中。多种因素可造成肾脏结疤，尤其是涉及肾小球滤过自调节的生理学紊乱。这又引起积聚的胞外基质替代正常结构。个别细胞生理学的变化谱引起多种肽和非肽纤维蛋白原的产生，其刺激胞外基质合成与降解之间平衡的变化从而促进结疤。

[0125] 发炎性疾病、病症或病况

[0126] 在一些实施例中，根据本发明的方法和组合物用于治疗发炎性疾病、病症或病况，包括（但不限于）：全身性炎症反应 (SIRS)；阿尔茨海默氏病 (Alzheimer's Disease；和相关病况和症状，包括：慢性神经炎症、神经胶质活化；微神经胶质增加；神经炎性斑块形成；和对疗法的反应）；肌萎缩性侧索硬化症 (ALS)、关节炎（和相关病况和症状，包括（但不限于）：急性关节炎症、抗原诱导的关节炎、与慢性淋巴细胞性甲状腺炎有关的关节炎、胶原蛋白诱导的关节炎、青少年关节炎、类风湿性关节炎、骨关节炎、预后和链球菌诱导的关节炎、脊柱关节病、痛风性关节炎）、哮喘（和相关病况和症状，包括：支气管哮喘；慢性阻塞性气道疾病；慢性阻塞性肺病、青少年哮喘和职业性哮喘）；心血管疾病（和相关病况和症状，包括动脉粥样硬化；自身免疫心肌炎、慢性心肌缺氧、充血性心力衰竭、冠状动脉疾病、心肌病和心肌细胞功能障碍，包括：主动脉平滑肌细胞活化；心肌细胞凋亡；和心肌细胞功能的免疫调节；糖尿病和相关病况和症状，包括自身免疫糖尿病、胰岛素依赖性 (1 型）糖尿病、糖尿病性齿根骨膜炎、糖尿病性视网膜病变和糖尿病性肾病变）；胃肠炎症（和相关病况和症状，包括腹腔疾病、相关骨质减少、慢性结肠炎、克罗恩病 (Crohn's disease)、发炎性肠病和溃疡性结肠炎）；胃溃疡；肝炎症（如病毒和其它类型肝炎）、胆固醇胆石和肝纤维化、HIV 感染（和相关病况和症状，包括退化性反应，神经退化性反应和 HIV 相关的霍奇金氏病 (HIV associated Hodgkin's Disease))、川崎氏综合症 (Kawasaki's Syndrome；和相关疾病和病况，包括粘膜皮肤淋巴结综合症、颈椎淋巴结病、冠状动脉病变、水肿、发烧白细胞增加、轻度贫血、皮肤剥落、皮疹、结膜发红、血小板增多；多发性硬化、肾病变（和相关疾病和病况，包括糖尿病性肾病变、晚期肾病、急性和慢性丝球体肾炎、急性和慢性间质肾炎、狼疮肾炎、古德帕斯丘氏综合症 (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（和相关疾病和病况，包括急性神经退化、在老化和神经退化性疾病中的 IL-1 的诱生、IL-1 诱导的下丘脑神经元可塑性和慢性应力高反应性）、眼病变（和相

关疾病和病况，包括糖尿病性视网膜病变、格雷夫斯氏眼病变 (Graves' opthalmopathy) 和葡萄膜炎、骨质疏松症（和相关疾病和病况，包括牙槽、股骨、桡骨、椎骨或手腕骨质流失或破裂发生、绝经后骨质流失、质量、破裂发生或骨质流失速率）、中耳炎（成人或小儿）、胰脏炎或胰脏腺泡炎、牙周疾病（和相关疾病和病况，包括成人型、早发型和糖尿病型）；肺病，包括慢性肺病、慢性窦炎、透明膜病、缺氧和 SIDS 中的肺病；冠状动脉再狭窄或其它血管移植物；风湿，包括类风湿性关节炎、风湿性阿孝夫氏小体 (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；尿道感染，包括慢性前列腺炎、慢性骨盆疼痛综合症和尿石病；免疫病症，包括自身免疫性疾病，如斑秃、自身免疫心肌炎、格雷夫斯氏病、格雷夫斯眼病变、苔癣硬化症、多发性硬化、牛皮癣、全身性红斑狼疮、全身性硬化症、甲状腺疾病（例如淋巴瘤性甲状腺肿 (goiter and struma lymphomatosa)（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症和肥胖（非糖尿病性或与糖尿病有关）；对传染病的抵抗性，如利什曼体病 (Leishmaniasis)、麻风、莱姆病 (Lyme Disease)、莱姆心炎 (Lyme Carditis)、疟疾、脑型疟疾、脑膜炎、与疟疾有关的小管间质性肾炎），其由细菌、病毒（例如巨细胞病毒、脑炎病毒、埃－巴二氏病毒 (Epstein-Barr Virus)、人类免疫缺陷病毒、流感病毒）或原生动物（例如恶性疟原虫、锥虫）引起；对创伤的反应，包括脑创伤（包括中风和缺血、脑炎、脑病变、癫痫症、围产期脑损伤、长期发热性癫痫、SIDS 和蛛网膜下出血）、低出生体重（例如大脑性麻痹）、肺损伤（急性出血性肺损伤、古德帕斯丘氏综合症、急性缺血再灌注）、心肌功能障碍，由职业和环境污染物引起（例如易感毒油综合症硅粉沉着病）、辐射创伤、和创伤愈合反应效率（例如灼伤或热创伤、慢性创伤、手术创伤和脊髓损伤）；激素调节，包括生育力／繁殖力、怀孕的可能性、发生早产、产前和新生并发症，包括早产低出生体重、大脑性麻痹、败血症、甲状腺功能低下、氧气依赖性、颅脑异常、早发闭经；受试者对移植的反应（排斥或接受）、急性期反应（例如发热性反应）、一般炎症反应、急性呼吸窘迫反应、急性全身性炎症反应、创伤愈合、粘附、免疫发炎性反应、神经内分泌反应、发烧发展和抵抗、急性期反应、应激反应、疾病易感性、重复运动应激、网球肘以及疼痛管理和反应。

[0127] 患者分层、治疗监测和／或优化的生物指标或指示

[0128] 在一些实施例中，本文中所述的基于抗 CCL2 抗体的方法和组合物可与生物指标一起用于患者分层、治疗监测和／或优化。在一些实施例中，适合的生物指标是有差异地表达的生物指标。如本文中所用，术语“有差异地表达的生物指标”是指表达水平在罹患硬皮病的受试者（或受试者群体）中相对于其在健康或正常受试者（或健康或正常受试者群体）中的表达水平有所不同的生物指标。所述术语还涵盖表达水平对于不同疾病亚型（即局限性皮肤或弥漫性皮肤硬皮病）来说有所不同的生物指标。所述术语进一步涵盖表达水平在疾病的不同阶段（例如轻度或早期硬皮病、重度或晚期硬皮病）有所不同的生物指标。差异性表达包括生物指标的瞬时或细胞表达模式的定量以及定性差异。如下文更详细地描述，有差异地表达的生物指标单独或与其它有差异地表达的生物指标组合适用于诊断、分期、治疗、药物开发和相关领域中的多种不同应用。本文中所公开的有差异地表达的生物指标的表达模式可描述为硬皮病、硬皮病亚型、硬皮病阶段和硬皮病疾病严重度和／或进展的指纹或标签。其可用作参考点以比较和表征未知样品和寻求进一步信息的样品。如本文中所用的术语“降低的表达水平”是指如通过本文中所述的一或多种方法所测量，表达降低

至少 10％或更多，例如 20％、30％、40％、或 50％、60％、70％、80％、90％或更多，或者表达降低大于 1 倍、2 倍、3 倍、4 倍、5 倍、10 倍、50 倍、100 倍或更多。如本文中所用的术语“提高的表达水平”是指如通过一或多种方法（如本文中所述的方法）所测量，表达提高至少 10％或更多，例如 20％、30％、40％、或 50％、60％、70％、80％、90％或更多，或者表达提高大于 1 倍、2 倍、3 倍、4 倍、5 倍、10 倍、50 倍、100 倍或更多。

[0129] 皮肤基因表达分析

[0130] 鉴别硬皮病患者中有差异地表达的生物指标的各种方法是所属领域中已知的且可用于实践本发明。举例来说，皮肤基因表达分析对于对患者构造子集、鉴别响应患者子集的蛋白生物指标和指示来说可为强大的工具。在一些实施例中，在硬皮病患者中经有差异地调节的基因可通过比较健康个体与硬皮病个体的皮肤样品的转录概况来鉴别。此外，与疾病严重度相关的基因转录物可通过将各个进展程度阶段的硬皮病患者包括在内来鉴别。转录概况可通过微阵列分析进行分析，如已由例如米兰 (Milano) 等人“硬皮病皮肤的基因表达标签的分子子集 (Molecular Subsets in the Gene Expression Signatures of Scleroderma Skin)”（公共科学图书馆 •综合 (PLOS One)，3:7，1-18，2008) 描述，所述文献的全部内容以引用的方式并入本文中。举例来说，可在来自患有弥漫性硬皮病、局限性硬皮病、硬斑病（与硬皮病类似的无内部器官涉及的疾病）的患者和健康对照的皮肤样品（例如前臂和背部样品）上执行微阵列分析。为了鉴别与硬皮病最高度相关的基因，选择在复制品与样品位点之间最内部一致同时在个体之间最可变的基因以进行进一步分析。基于与硬皮病严重度相关的差异性基因表达的聚类分析可用于选择受硬皮病影响的基因。

[0131] 据报导，硬皮病中有差异地表达的示例性基因可聚类成 6 组。第一组包括在弥漫性硬皮病患者子组中和在硬斑病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。第二群组包括增殖标签，包括仅在细胞分裂时表达的基因。在这一聚类中展示出表达增加的基因包括细胞周期调节的基因，如 CKS1B、CDKS2、CDC2、MCM8 和 E2F7。增殖标签的存在与来自皮肤活检的报导一致，所述报导展示出弥漫性硬皮病组织的细胞经历增殖增加。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有关的基因，其与第三组类似地表达，且包括 PTPRC（其为 T 细胞活化所需）以及 CD2 和 CDW52（其在 T 淋巴细胞表面上表达）。第五组包括在弥漫性硬皮病中展示低表达的基因。这些基因在其它活检中展示较高表达水平且尤其包括 WIF1、四连接素、IGFBP6 和 IGFBP5。最后一组是异质的基因表达聚类，其在局限性硬皮病中较高且是弥漫性硬皮病的子组，包括（但不限于）UTS2R、GALR3、PARD6G、PSEN1、PHOX2A、CENTG3、HCN4、KLF16 和 GPR15G。其它有差异地表达的示例性基因描述在米兰 (Milano) 等人“硬皮病皮肤的基因表达标签的分子子集”（公共科学图书馆 • 综合，3:7，1-18，2008) 中，所述文献的全部内容以引用的方式并入本文中。

[0132] 多基因标签作为代理指标

[0133] 基因的组合可用作生物指标。生物指标鉴别的示例性方法提供在例如法瑞勒 (Farina) 等人，“四基因生物指标预测弥漫性皮肤全身性硬化症患者的皮肤疾病 (A Four-Gene Biomarker Predicts Skin Disease in Patients with Diffuse Cutaneous Systemic Sclerosis)”（关节炎与风湿病 (Arthritis Rheum.)62(2)，580-588，2010) 中，所述文献的全部内容以引用的方式并入本文中。开始于已知在硬皮病中经调节的目标（如

TGFβ 和干扰素），法瑞勒鉴别了四基因生物指标，包括基因 CTGF、THS1、COL4 和 PAI1。发现组合的这四种基因的转录与经修改的罗德南皮肤分数 (mRSS) 高度相关且高度预测弥漫性硬皮病。

[0134] mRSS 被用作硬皮病的一种临床指标。mRSS 通常如图 1 所示指定 :未涉及的皮肤指定分数 0 ;轻度变厚给予分数 1 ;中度变厚给予分数 2 ;以及重度变厚给予分数 3。通常，介于 0-51 范围内的总 mRSS 分数可基于在患者的 17 处皮肤区域 0-3 的定级来确定。mRSS 可用作单独或与其它生物指标组合诊断和监测治疗的指示。

[0135] 类似的策略可用于鉴别和验证硬皮病的潜在标签生物指标。确切地说，将被鉴别为在硬皮病中经正性或负性调节的基因转录物单独或组合测试，从而鉴别包含与硬皮病的临床指标最高度相关基因转录物或基因转录物组合的生物指标。除了 mRSS 之外，可使用其它临床指标，如健康评估调查表 (HAQ-DI)、一氧化碳的肺扩散能力 (DLCO) 或用力肺活量 (FVC)。

[0136] CCL2 水平

[0137] CCL2 水平（例如 CCL2 血清水平）可用作用于测定疾病严重度、器官涉及、选择适当治疗、监测疾病进展和患者反应的生物指标或指示。为了测定 CCL2 水平作为生物指标或指示，测定各种阶段硬皮病的患者和不受影响的个体的血清中的 CCL2 水平。这可通过利用例如 ELISA 分析血清中 CCL2 蛋白水平来进行，且与皮肤和其它器官（例如肺、肝、肾、食管）涉及相关。示例性方法描述在卡鲁利 (Carulli) 等人风湿病年鉴 (Ann Rheum Dis)67:105-109,2008 中。

[0138] 存在于如来自活检的皮肤和 / 或血清中的 CCL2 水平还可与 mRSS 或其它临床指标（如 HAQ-DI、DLCO 或 FVC) 相关。

[0139] 各种生物指标可单独或组合或可替代地与临床诊断指标（如 mRSS) 一起使用，从而基于硬皮病的严重度将患者分层、选择适当疗法或给药方案、评估疗法的有效性、监测对疗法的反应、预测疾病过程以及测量受试者中的疾病进展。通常，在所述方法中，将针对从一或多个时间点从受试者获得的生物样品测定的适合的生物指标水平（例如选自本文中所述的各种有差异地表达的基因的那些以及其它已知指标如 CCL2 水平）与从一或多个其它时间点来自受试者的水平相比。举例来说，生物指标水平可在治疗过程之前或在治疗过程开始时测量。生物指标水平可在整个治疗过程的一或多个时间点下测量且与在治疗之前或来自治疗过程的较早时间点的水平相比。鉴别或选择适当治疗，确定患者是否对治疗和 / 或治疗的优化具有积极反应可基于生物指标的评估加以确定。

[0140] 医药组合物

[0141] 本发明还提供包含一或多种所提供的抗体的组合物。在一些实施例中，本发明提供至少一种抗体和至少一种医药学上可接受的赋形剂。所述医药组合物可任选地包含一或多种其它治疗活性物质和 / 或与其组合投与。在一些实施例中，所提供的医药组合物适用于药物。在一些实施例中，所提供的医药组合物在治疗或预防硬皮病或与硬皮病关联或相关的负面影响中适用作防治性药剂（即疫苗）。在一些实施例中，所提供的医药组合物适用于治疗性应用，例如在罹患或易患硬皮病的个体中。在一些实施例中，医药组合物被调配成用于投与人类。

[0142] 举例来说，这里所提供的医药组合物可以无菌可注射形式（例如适于皮下注射或

静脉内输注的形式）提供。举例来说，在一些实施例中，医药组合物以适于注射的液体剂型提供。在一些实施例中，医药组合物以粉末（例如冻干和/或灭菌粉末）形式任选地在真空下提供，其在注射之前用水性稀释剂（例如水、缓冲液、盐溶液等）复原。在一些实施例中，医药组合物在水、氯化钠溶液、乙酸钠溶液、苯甲醇溶液、磷酸盐缓冲盐水等中稀释和/或复原。在一些实施例中，粉末应轻轻地与水性稀释剂混合（例如不振荡）。

[0143] 在一些实施例中，所提供的医药组合物包含一或多种医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）。在一些实施例中，医药组合物包含一或多种防腐剂。在一些实施例中，医药组合物不包含防腐剂。

[0144] 在一些实施例中，医药组合物以可冷藏和/或冷冻的形式提供。在一些实施例中，医药组合物以不可冷藏和/或冷冻的形式提供。在一些实施例中，复原的溶液和/或液体剂型在复原之后可储存一定的时间段（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）。在一些实施例中，抗体组合物储存长于指定时间会引起抗体降解。

[0145] 液体剂型和/或复原溶液在投与之前可包含微粒物质和/或变色。在一些实施例中，如果变色或混浊和/或如果在过滤之后微粒物质保留，那么不应使用溶液。

[0146] 本文中所述的医药组合物的组合物可通过药理学领域中已知或此后发展的任何方法制备。在一些实施例中，所述制备方法包括以下步骤：使活性成分与一或多种赋形剂和/或一或多种其它附加成分结合，且接着必要时和/或需要时，将产品成形和/或包装到所需单剂量或多剂量单位中。

[0147] 根据本发明的医药组合物可以散装、以单一单位剂量形式和/或以多个单一单位剂量形式制备、包装和/或出售。如本文中所用，“单位剂量”是包含预定量的活性成分的医药组合物的个别量。活性成分的量通常等于将投与受试者的剂量和/或所述剂量的方便分数，如例如所述剂量的二分之一或三分之一。

[0148] 根据本发明的医药组合物的活性成分、医药学上可接受的赋形剂和/或任何其它成分的相对量可变化，取决于所治疗的受试者的身份、身材和/或情况和/或取决于投与组合物的途径。作为举例，组合物可包含在 0.1％与 100％ (w/w) 之间的活性成分。

[0149] 本发明的医药组合物可另外包含医药学上可接受的赋形剂，其如本文中所用可为或包含溶剂、分散介质、稀释剂、或其它液体媒剂、分散或悬浮助剂、表面活性剂、等张剂、增稠或乳化剂、防腐剂、固体粘合剂、润滑剂等，如适于所需具体剂型。雷明顿氏药学科学和实践 (Remington's The Science and Practice of Pharmacy)，第 21 版，A.R. 根纳罗 (A.R.Gennaro)，(马里兰州巴尔的摩的利平科特威廉姆斯和威尔金斯公司 (Lippincott,Williams&Wilkins,Baltimore,MD),2006) 公开了用于调配医药组合物的各种赋形剂和用于制备其的已知技术。除非任何常规赋形剂介质均不与物质或其衍生物相容，如因产生任何非所需生物作用，或另外与医药组合物的任何其它组分以有害方式相互作用，否则预期其使用在本发明的范围内。

[0150] 实例

[0151] 实例 1. 制备高亲和力抗 CCL2 抗体

[0152] 本实例说明高亲和力抗 CCL2 抗体的制备。如上文所述，各种方法可用于产生并选择具有所需特异性和结合亲和力的抗体。

[0153] 在这一特定实例中，抗 CCL2 抗体由包含两个全长抗原结合臂的完整人类抗体组成。表达人类抗体基因的转基因小鼠最初经含纯化的人类重组 CCL2 的完全弗氏佐剂 (complete Freund's adjuvant) 经由皮下注射免疫。在初始免疫接种之后，每一小鼠一周一次接受额外的皮下注射持续三周。从小鼠收集具有较高抗体效价 (如通过 ELISA 测定) 的脾细胞，并如下将其与小鼠骨髓瘤细胞株融合。来自经免疫小鼠的脾细胞的单细胞悬浮液与四分之一数目的非分泌小鼠骨髓瘤细胞与 50％ PEG 融合。细胞以约 1×10^5 个 / 孔接种在平坦底部微量滴定板中，之后孵育一周。个别孔接着通过 ELISA 筛选人类抗 CCL2 单克隆 IgG 抗体。一旦出现广泛的杂交瘤生长，就再接种分泌抗体的融合瘤，再次筛选，并且如果对于人 IgG 仍然阳性，那么抗 CCL2 单克隆抗体通过限制稀释法亚克隆至少两次。

[0154] 或者，抗体可采用流式细胞术从来自经免疫转基因小鼠 (如上文所述) 的单抗原阳性 B 细胞的编码 V_H 和 V_L 域的 DNA 直接分离。简单来说，将人类 CCL2 免疫的转基因小鼠处死并收集脾细胞。通过裂解之后粒化所收集的脾细胞来去除红细胞。再悬浮的脾细胞首先与人 IgG、FITC- 抗 -mFc 和生物素化人类 CCL2 的混合物一起孵育 1 小时。染色的细胞用 PBS 洗涤两次，接着用人类和大鼠 IgG、APC- 抗 -mIgM 和 SA-PE 的混合物染色一小时。染色的细胞用 PBS 洗涤一次，并接着通过流式细胞术在 MOFLO™ XDP (贝克曼库尔特公司 (Beckman Coulter, Inc.) 上分析。分选出每一 IgG 阳性、IgM 阴性和抗原阳性的 B 细胞，并将其接种到 96 孔板上的另一孔中。来自这些 B 细胞的抗体基因的 RT-PCR 根据由王 (Wang) 等人 (2000, 免疫学方法杂志 (J. Immunol. Methods) 244:217-225) 描述的方法执行。将重链和轻链 PCR 产物分别克隆到含有人类重链恒定区 (例如 IgG_1) 和人类轻链恒定区 (例如 Cκ) 的载体中。接着将来自同一 B 细胞的具有重链可变区序列的经纯化重组质粒和具有轻链可变区序列的质粒组合并转染到宿主细胞株 (例如 CHO 细胞株) 中。

[0155] 除了典型小鼠免疫接种之外，还可使用基于骆驼 (camelids) 或噬菌体展示的其它抗体筛选方法。使用标准受体结合分析选择高亲和力抗体。纯化亲和力大于 10^{-12}M 的抗体。

[0156] 实例 2. 剂量范围测试

[0157] 本实例说明经设计以评估用于治疗硬皮病的抗 CCL2 抗体的有效剂量范围的剂量反应研究。

[0158] 博莱霉素 (bleomycin) 诱导的硬皮病小鼠模型用于本实例。通常，通过将博莱霉素、多聚肌苷酸 - 聚胞苷酸和 / 或 LPS 重复皮下注射到背侧皮肤中来在小鼠中诱导纤维化。确切地说，将含有浓度为 10-110 μg 以及高达 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 PBS 单独的渗透泵 (7 天) 皮下植入到 10 只 B6 小鼠组中。在这一小鼠模型中，皮肤中的组织病理学变化密切类似于在硬皮病中可见的组织病理学变化。早期单核细胞积聚以及 TGF-β 和趋化因子表达上调之后是特征在于厚胶原蛋白束和活化成纤维细胞积聚的真皮纤维化。小鼠还显示肺和肾纤维化的迹象。

[0159] 将递增浓度的抗 CCL2 抗体或对照抗体的剂量经由腹膜内注射投与到小鼠中。

[0160] 实例 3. 抗 CCL2 抗体的体内功效

[0161] 本实例说明经设计以评估用抗 CCL2 抗体治疗对用于硬皮病的博莱霉素小鼠模型中的炎症和纤维化的作用的研究。

[0162] 含有 PBS 单独或含 10-110 μg 以及高达 200 μg 博莱霉素的 PBS 的 7 或 28 天渗透

泵将被皮下植入到B6小鼠中。每两天，小鼠将经由腹膜内注射用如在实例2中所测定的适合浓度的抗CCL2抗体或用对照抗体处理。

[0163] 在7天（在7天渗透泵的情况下）或28天（在28天渗透泵的情况下）之后，将收集皮肤和肺组织用于转录和组织学分析。通过ELISA测量组织样品中的CCL2蛋白水平。对于转录分析，从皮肤组织提取RNA，且使用所属领域中通常已知的技术对分离的RNA进行半定量或定量逆转录酶-PCR。使用可商购的引物（TaqMan®）（塔克曼(TaqMan)）测量TGFβ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TNFα）的基因表达水平。对于组织学分析，通过显微镜下检查用苏木精和伊红(hematoxylin and eosin，H&E)染色的组织切片来分析皮肤纤维化。使用H&E染色观察组织形态是所属领域中众所周知的。免疫组织化学用于通过显微镜下检查使用所属领域中熟知的技术用单核特异性抗F4/80抗体探测的组织切片来定量单核浸润。

[0164] 预期用抗CCL2抗体治疗将减少单核细胞和巨噬细胞浸润，将减少炎性基因表达（例如IL-6、TNFα），且将降低TGFβ 诱导的标记基因表达。预期这会引起纤维化的普遍降低。

[0165] 实例4. 治疗性建模

[0166] 本实例说明各种组织和血浆中CCL2产生和流失的模型以预测组织目标水平。

[0167] CCL2通常在疾病组织中产生并分泌到血浆中。在健康个体中，皮肤中的CCL2合成较低或不可检测。CCL2合成随着非受影响和受影响皮肤两者中的总皮肤涉及而增加，引起血清CCL2水平增加。血清CCL2水平随着器官涉及进一步增加。健康个体的平均血清CCL2水平通常低于约100pg/ml。具有所谓的雷诺氏现象(Raynaud's phenomenon)的个体的平均血清CCL2水平略微增加。罹患硬化的患者的平均血清CCL2水平通常是约250pg/ml。罹患局限性皮肤全身性硬化症的患者的平均血清CCL2水平通常是约250pg/ml。罹患弥漫性皮肤全身性硬化症的患者的平均血清CCL2水平通常是约380pg/ml。罹患局限性皮肤全身性硬化症的患者的平均血清CCL2水平通常是约250pg/ml。

[0168] CCL2的分子量是约8.6kDa，其远小于约50kDa的肾小球滤过阈值，引起快速肾脏清除。CCL2通过活性受体介导的内化而内化。CCL2结合其受体CCR2的典型kd是约60pM-2nM。CCR2主要存在于淋巴来源细胞和淋巴内皮上。预期硬皮病在疾病进展早期引起血管渗透性增加，这允许CCL2和任何治疗性抗体在间质与血清之间的实质性平衡。因此，CCL2的血清半衰期基于来自小鼠和兔的数据是约10分钟。预期CCL2血清半衰期在人类中是类似的。相对可渗透的组织允许CCL2从组织到血清快速（例如在约2小时内）达到平衡（半最大值）。在一些情况下，血清CCL2水平在整个皮肤涉及但无器官涉及的情况下可能达到1000pg/ml(约75pM)。展示血清和组织CCL2平衡的目标特征曲线展示在图2中，其预测中和3nM组织CCL2并与其竞争离开其受体所需的抗体所需量。所说明的模型代表高CCL2水平的极端呈现。

[0169] 目前可供使用的静脉内注射的单克隆抗体通常并不有效，因为其在其到达患病组织之前结合血浆中的CCL2并形成复合物。参见图3。通过提供高亲和力的抗CCL2，我们可提供足够的抗CCL2抗体来结合组织中的CCL2并以60pM亲和力竞争CCR2。

[0170] 实例5. 临床设计

[0171] 基于动物治疗的成功，在健康个体和具有不同阶段硬皮病的个体中设计在表2-6

中详述的抗 CCL2 抗体的 I-III 期剂量范围和单剂量研究，从而评估抗 CCL2 疗法的安全性、耐受性、功效和药物动力学。

[0172] 人类临床试验 1 的第一目标包括确定在健康个体中投与的抗 CCL2 抗体的 4 种剂量水平的安全性。第二目标包括评估在健康个体中投与的抗 CCL2 抗体的 4 种不同剂量水平的药物动力学。这一临床试验的详细方案大纲展示在表 2 中。

[0173] 表 2 :人类临床试验 1

[0174]

期	1 期
试验数目	1
患者群体	健康志愿者
试验设计和终点	单剂量，剂量递增 第一：安全性 第二：PK
受试者数目	4 剂量组 每组 n=4 总共 16 个受试者
试验持续时间（FPI 到 LPV）	0.5 年 约 6 周给药 约 15 周随访 PK
注释	单一 1 期单位

[0175] 人类临床试验 2 的第一目标包括确定在具有硬皮病早期症状的个体中投与的抗 CCL2 抗体的 4 种剂量水平的安全性。第二目标包括 (1) 测定在具有硬皮病早期症状的个体中投与的抗 CCL2 抗体的 4 种不同剂量水平的药物动力学 ;(2) 通过分析连续皮肤活检中的基因表达来测定具有硬皮病早期症状的个体对抗 CCL2 抗体的 4 种不同剂量水平的药效学 (PD) 反应 ;以及 (3) 测定具有硬皮病早期症状的个体对抗 CCL2 抗体的 4 种不同剂量水平的临床反应，如通过经修改的罗德南皮肤分数 (mRSS) 测量。这一临床试验的详细方案大纲展示在表 3 中。

[0176] 表 3 :人类临床试验 2

[0177]

期	1/2 期
试验数目	1
患者群体	早期（从非雷诺氏现象（RP）症状发作起<2 年） 弥漫性 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 剂量水平

[0178]

	第一：安全性 第二：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）
受试者数目	4 剂量组 每组 n = 10（8 个活性剂/2 个安慰剂) 总共 40 个受试者
试验持续时间（FPI 到 LPV）	1.5 年
注释	在 1 年内高达 8 个地点募集

[0179] 人类临床试验 3 的第一目标包括测定在具有早期硬皮病症状的个体中投与的抗 CCL2 抗体的单一剂量水平的功效，如通过经修改的罗德南皮肤分数 (mRSS) 测量。第二目标包括 (1) 测定在具有早期硬皮病症状的个体中投与的抗 CCL2 抗体的单剂量水平的功效，如通过健康评估调查表 - 失能指数 (HAQ-DI) 测量 ；和 (2) 测定在具有早期硬皮病症状的个体中投与的抗 CCL2 抗体的单剂量水平的功效，如通过器官特异性评估测量。这一临床试验的详细方案大纲展示在表 4 中。

[0180] 表 4 ：人类临床试验 3

[0181]

期	2 期
试验数目	1
患者群体	早期（从非雷诺氏现象（RP）症状发作起<2 年） 弥漫性 SSc mRSS ≥ 15
试验设计和终点	1 剂量水平 双盲安慰剂对照平行组 治疗持续时间 6 个月 开放标签延长 第一：mRSS 第二：HAQ DI、器官特异性评估
受试者数目	2:1 随机化 总共 120 个受试者
试验持续时间（FPI 到 LPV）	1.5 年
注释	在 1 年内高达 20 个地点募集

[0182] 人类临床试验 4 的第一目标包括测定在患有局限性或弥漫性硬皮病伴有肺病的个体中投与的抗 CCL2 抗体的单剂量水平相对于口服环磷酰胺的功效，如通过用力肺活量 (FVC) 测量。第二目标包括 (1) 测定在患有局限性或弥漫性硬皮病伴有肺病的个体中投与的抗 CCL2 抗体的单剂量水平相对于口服环磷酰胺的功效，如通过 HAQ-DI 测量 ；(2) 测定在患有局限性或弥漫性硬皮病伴有肺病的个体中投与的抗 CCL2 抗体的单剂量水平相对于口服环磷酰胺的功效，如通过 mRSS 测量 ；以及 (3) 测定在患有局限性或弥漫性硬皮病伴有肺病的个体中投与的抗 CCL2 抗体的单剂量水平相对于口服环磷酰胺的功效，如通过一氧化

碳的肺扩散能力 (DLCO) 测量。这一临床试验的详细方案大纲展示在表 5 中。

[0183] 表 5 :人类临床试验 4

[0184]

期	2 期
试验数目	1
患者群体	局限性或弥漫性 SSc 伴有肺病： 由 HRCT 确定的活性肺泡炎 从非 RP 症状发作起<7 年 预测 FVC<85%>45%
试验设计和终点	1 剂量水平 双盲对照平行组 比较组：SoC（口服环磷酰胺） 治疗持续时间 12 个月 开放标签延长 第一：FVC 第二：DLCO、HAQ DI、mRSS
受试者数目	2:1 随机化 总共 120 个受试者
试验持续时间（FPI 到 LPV）	1.5 年
注释	在 6 个月内高达 10 个地点募集

[0185] 人类临床试验 5 的目标包括 (1) 测定在具有硬皮病早期症状和 / 或局限性或弥漫性硬皮病伴有肺病的个体中投与的抗 CCL2 抗体的单剂量水平相对于口服环磷酰胺的功效，如通过用力肺活量 (FVC) 测量 ;(2) 测定在具有硬皮病早期症状和 / 或局限性或弥漫性硬皮病伴有肺病的个体中投与的抗 CCL2 抗体的单剂量水平相对于口服环磷酰胺的功效，如通过 HAQ-DI 测量 ;(3) 测定在具有硬皮病早期症状和 / 或局限性或弥漫性硬皮病伴有肺病的个体中投与的抗 CCL2 抗体的单剂量水平相对于口服环磷酰胺的功效，如通过 mRSS 测量 ;以及 (4) 测定在具有硬皮病早期症状和 / 或局限性或弥漫性硬皮病伴有肺病的个体中投与的抗 CCL2 抗体的单剂量水平相对于口服环磷酰胺的功效，如通过 DLCO 测量。这一临床试验的详细方案大纲展示在表 6 中。

[0186] 表 6 :人类临床试验 5

[0187]

期	3 期
试验数目	各 1
试验设计和终点	单剂量水平，在早期 dSSc 或 SSc 肺病的任一者或两者中双盲头对头与 SoC 比较，取决于 2 期结果 如 2 相中的终点
受试者数目	各 120 个患者
试验持续时间（FPI 到 LPV）	2.0 年 0.5 到 1 年注册期
注释	治疗持续时间 12 个月

[0188] 用抗 CCL2 抗体治疗展现硬皮病早期症状的患者预期会展示症状的显著改善，如通过 mRSS 和 HAQ-DI 测量。用抗 CCL2 抗体治疗患有局限性或弥漫性硬皮病伴有肺病的患者预期会展示症状的显著改善，如通过 mRSS、HAQ-DI 和 FVC 测量。抗 CCL2 抗体预期在治疗具有硬皮病早期症状或患有局限性或弥漫性硬皮病伴有肺病的患者中比环磷酰胺更有效，如通过 mRSS、HAQ-DI 和 / 或 FVC 测量。

[0189] 等效物和范围

[0190] 所属领域的技术人员顶多使用常规实验即可识别或能够确定本文所述的本发明的具体实施例的许多等效物。本发明的范围并不打算限于以上说明书，而实际上是如所附权利要求书中所阐述。

[0191] 在权利要求书中，除非相反地指示或另外从上下文显而易见，否则如“一个 (a/an)”和“所述”的冠词可能意指一个或一个以上。因此，举例来说，提及“一个抗体”包括多个所述抗体，而提及“所述细胞”包括提及所属领域的技术人员已知的一或多个细胞等。除非相反地指示或另外从上下文显而易见，否则如果一个、一个以上或所有的群组成员存在、使用于给定产品或方法中或另外与其有关，那么在群组的一或多个成员之间包括“或”的权利要求书或说明书被视为满足。本发明包括恰好一个群组成员存在、使用于给定产品或方法中或另外与其有关的实施例。本发明包括一个以上或所有的群组成员存在、使用于给定产品或方法中或另外与其有关的实施例。此外，应理解本发明涵盖其中来自一或多个所列权利要求的一或多个限制、要素、条款、描述性术语等被引入到另一权利要求中的所有变化形式、组合以及排列。举例来说，附属于另一权利要求的任何权利要求可经修改以包括在附属于同一基本权利要求的任何其它权利要求中可见的一或多个限制。此外，在权利要求列举组合物时，应理解，除非另外指明，或者除非所属领域的技术人员将显而易见会产生矛盾或不一致，否则包括出于本文中所公开的任一目的使用组合物的方法，且包括根据本文中所公开的任一制造方法或所属领域中已知的其它方法制造组合物的方法。

[0192] 在要素作为清单（例如以马库西群组 (Markush group) 形式）呈现时，应理解所述要素的各子组也被公开，且任何元素都可从群组中去除。应理解，一般来说，在本发明或本发明的方面被称为包含具体要素、特征等时，本发明的或本发明的方面的某些实施例由所述要素、特征等组成或主要由所述要素、特征等组成。出于简单的目的，那些实施例尚未专门地以这些词语阐述在本文中。应指出，术语“包含”打算是开放的且允许包括额外要素或步骤。

[0193] 在给出范围时，包括终点。此外，应理解除非另外指明或另外从上下文显而易见且

为所属领域的技术人员所理解，否则表达为范围的值可在本发明的不同实施例中采用所述范围内的任何特定值或子范围，达到所述范围的下限单位的十分之一，除非上下文另外明确规定。

[0194] 另外，应理解，处于现有技术内的本发明的任何具体实施例可从任何一或多个权利要求中明确排除。由于所述实施例被认为是所属领域的技术人员已知的，故其可被排除，即使所述排除在本文中并未明确阐述。本发明的组合物的任何具体实施例（例如任何 HCV 基因型 / 亚型、任何 HCV 抗体、任何表位、任何医药组合物、任何投与方法、任何治疗性应用等）可出于任何原因从任何一或多个权利要求中排除，无论涉及存在的现有技术与否。

[0195] 提供上文所论述且贯穿本文的公开案仅仅出于其在本申请案的申请日之前的公开内容。不应将本文中的任何内容解释为承认本发明人无权先于借助于先前公开内容的此公开内容。

[0196] 其它实施例

[0197] 所属领域的技术人员将易于理解前文仅代表本发明的某些优选实施例。在不背离如随附权利要求书阐述的本发明范围的情况下可对上述程序和组合物作出各种变化和修改。

[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抗体

<130> 2006685-0330

<150> 61/650,149
<151> 2012-05-22

<160> 1

<170> PatentIn version 3.5

<210> 1
<211> 99
<212> PRT
<213> 智人

<400> 1

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

Pro Lys Thr

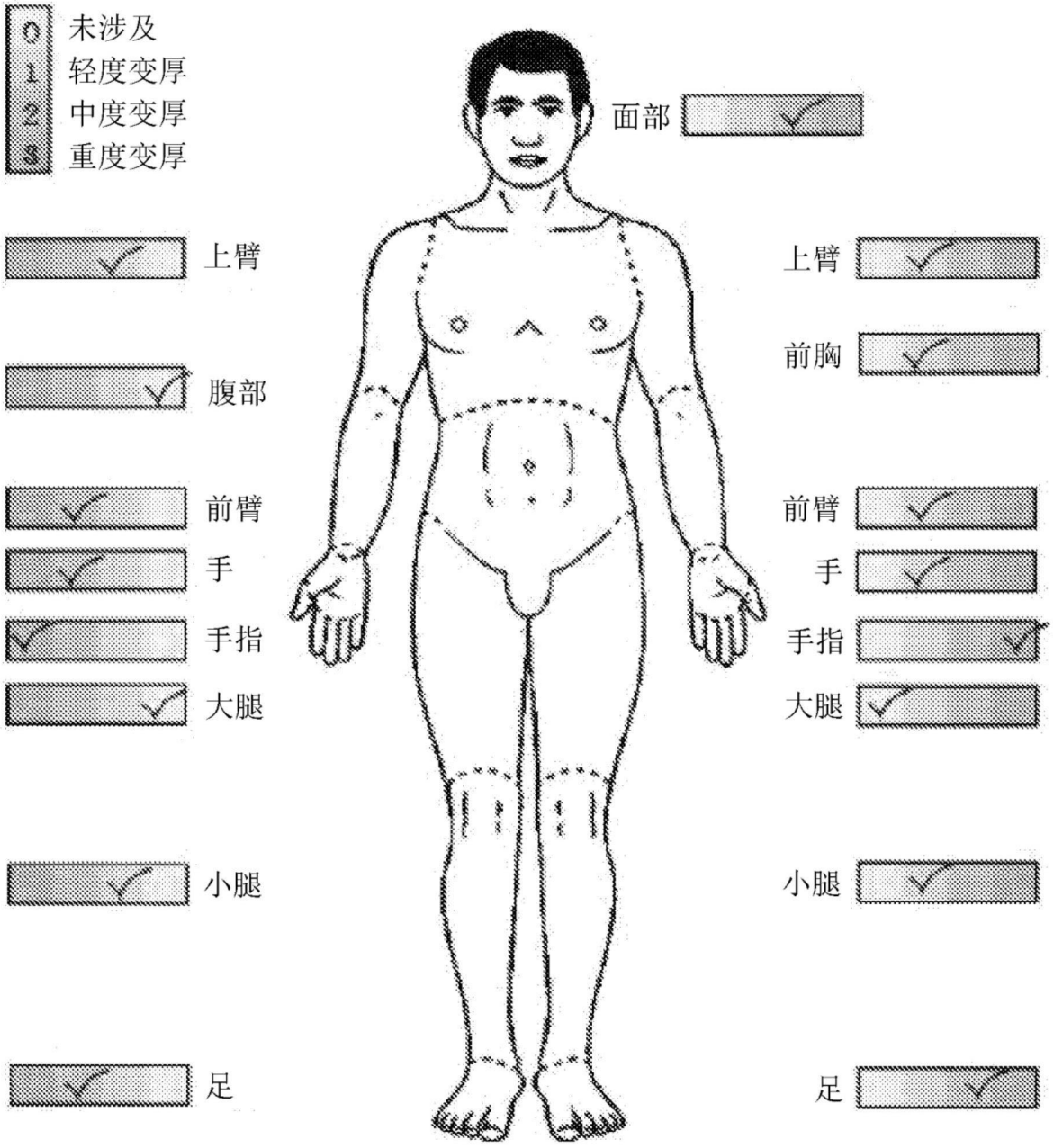
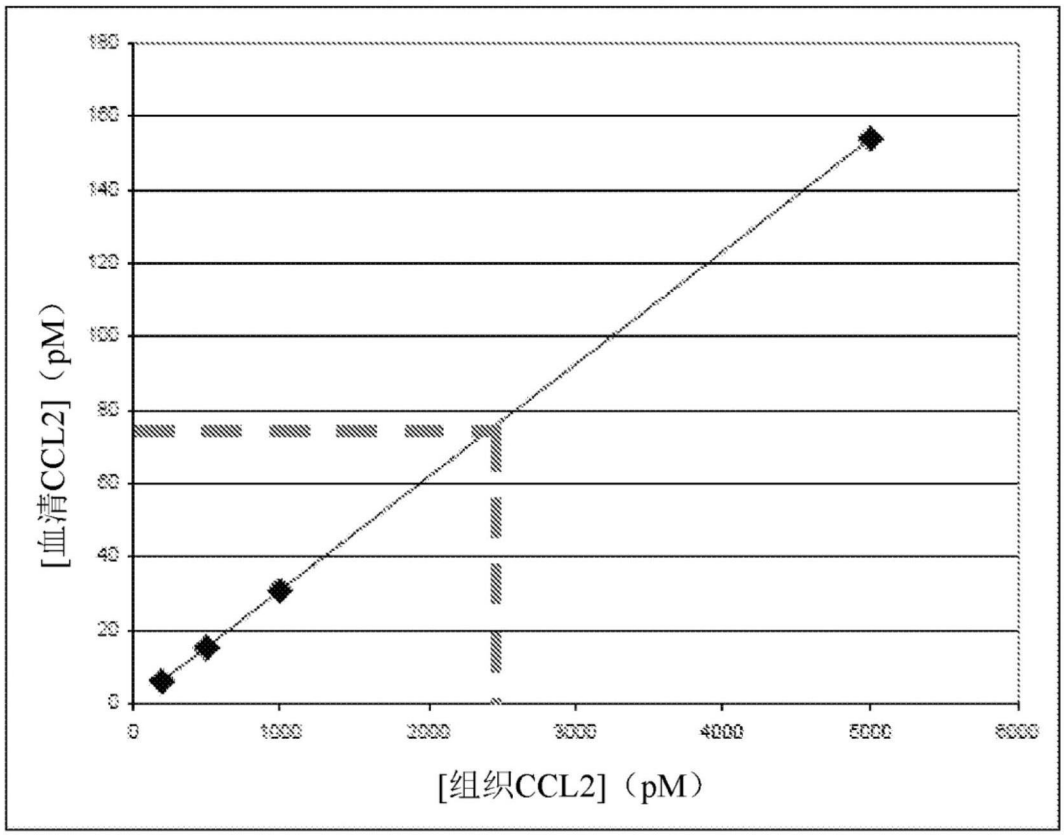


图 1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[组织CCL2]（pM）
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60
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100
120
140
160
180
0
1000
2000
3000
4000
5000
6000

图 2

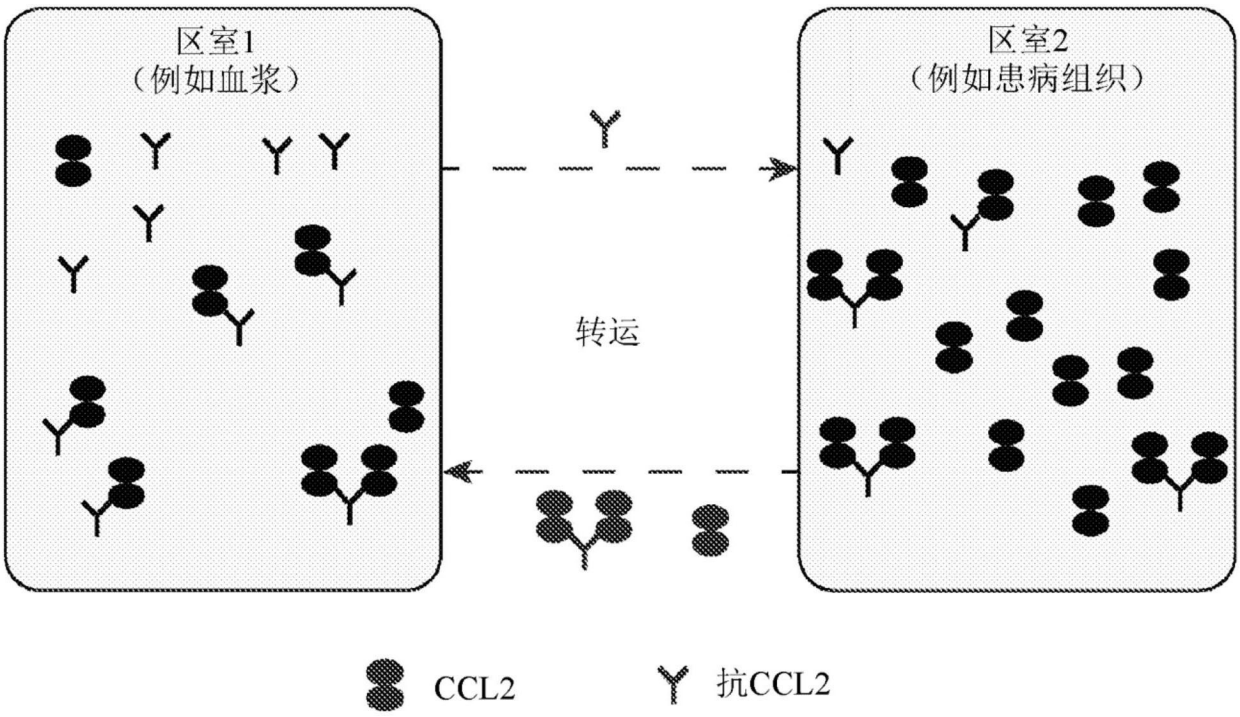
区室1
（例如血浆）
区室2
（例如患病组织）
转运
CCL2
抗CCL2

图 3

Abstract

The present invention provides, among other things, improved anti-CCL2 antibodies characterized with high affinity, potency, tissue selectivity and/or epitope specificity, 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 provides methods and compositions for treatment of scleroderma and related fibrotic and/or inflammatory diseases, disorders and conditions based on an anti-CCL2 antibody having an affinity of 10^{-12} M or greater.