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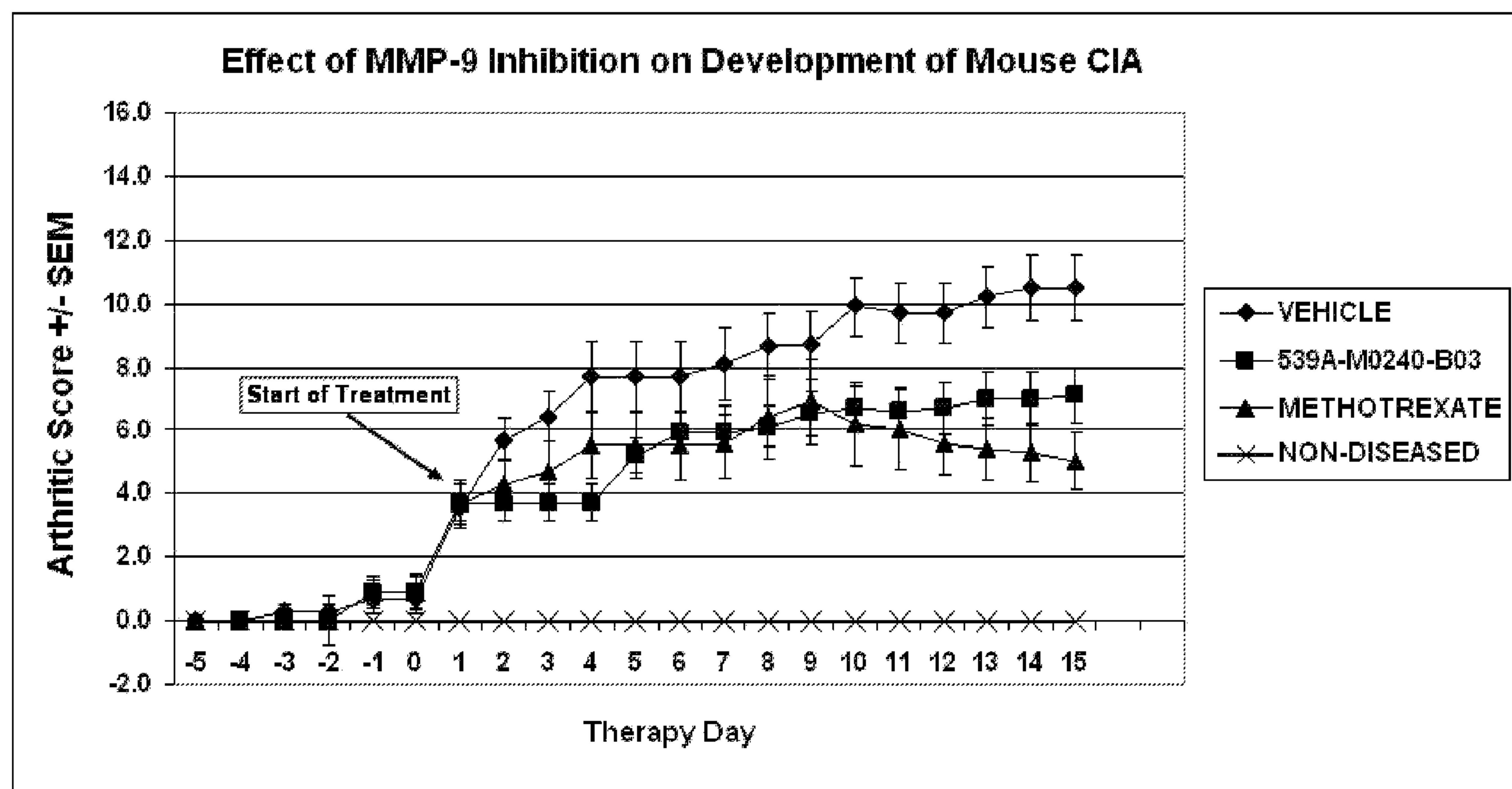


FIG. 9

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Proteins that bind to matrix metalloproteinase 9 and methods of using such proteins are described.

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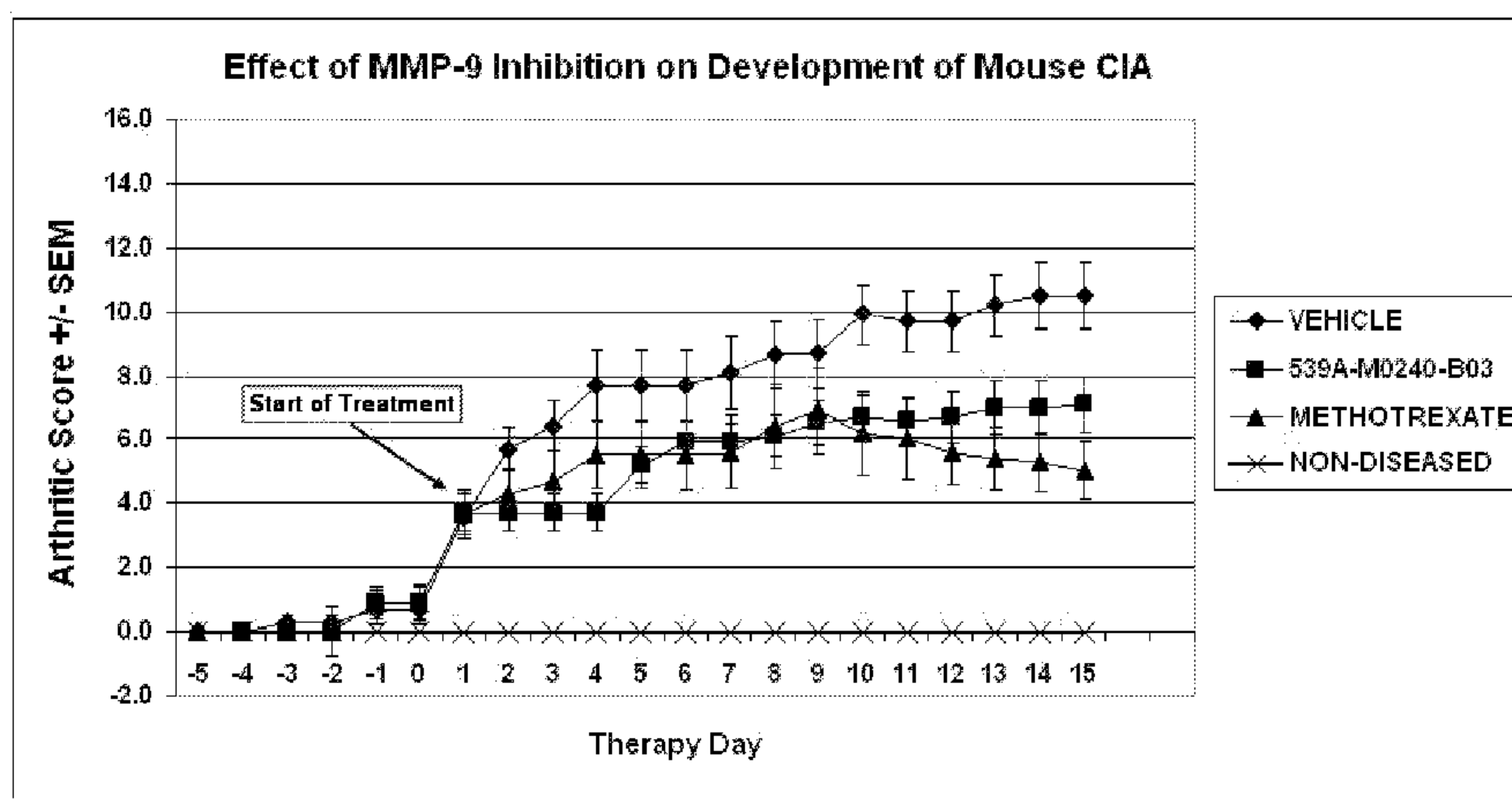


FIG. 9

(57) Abstract: Proteins that bind to matrix metalloproteinase 9 and methods of using such proteins are described.

METALLOPROTEINASE 9 BINDING PROTEINS

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Application Serial No. 61/033,075, filed on March 3, 2008, U.S. Application Serial No. 61/054,938, filed on May 21, 2008, and U.S.

5 Application Serial No. 61/138,297, filed on December 17, 2008. The disclosures of the prior applications are considered part of (and are incorporated by reference in) the disclosure of this application.

BACKGROUND

10 Matrix Metalloproteinases (MMPs) are a family of zinc metalloendopeptidases secreted by cells, and are responsible for much of the turnover of matrix components. The MMP family consists of at least 26 members, all of which share a common catalytic core with a zinc molecule in the active site.

SUMMARY

15 This disclosure relates, *inter alia*, to proteins that bind MMP-9, herein referred to as “MMP-9 binding proteins,” and methods of identifying and using such proteins. These proteins include antibodies and antibody fragments (e.g., primate antibodies and Fabs, especially human antibodies and Fabs) that bind to MMP-9 (e.g., human MMP-9). In some embodiments, these proteins include antibodies and antibody fragments (e.g., primate antibodies and Fabs, especially human antibodies and Fabs) that inhibit MMP-9 (e.g., human MMP-9) (e.g., inhibit the catalytic
20 activity of MMP-9). The MMP-9 binding proteins can be used in the treatment of diseases, particularly human disease, such as cancer, inflammation, heart failure, septic shock, neuropathic pain, inflammatory pain, or macular degeneration, in which excess or inappropriate activity of MMP-9 features. In many cases, the proteins have tolerable low or no toxicity.

25 In some aspects, the disclosure relates to proteins (e.g., antibodies, peptides and Kunitz domain proteins) that bind MMP-9, in particular, proteins (e.g., antibodies (e.g., human antibodies), peptides and Kunitz domain proteins) that bind and inhibit MMP-9.

In one embodiment, the disclosure provides an antibody (e.g., a human antibody) that binds to human MMP-9. In one embodiment, the human antibody is an inhibitor of the catalytic activity of MMP-9. The antibody can be, e.g., an IgG1, IgG2, IgG3, IgG4, Fab, Fab2', scFv, minibody, scFv::Fc fusion, Fab::HSA fusion, HSA::Fab fusion, Fab::HSA::Fab fusion, or other molecule that comprises the antigen combining site of one of the antibodies herein listed. In one embodiment, the antibody is used to guide a nano-particle or toxin to a cell expressing MMP-9 on the cell surface. In one embodiment, the antibody causes effector functions (CDC or ADCC) to kill the cell which expresses MMP-9.

In some embodiments, the VH and VL regions of the binding proteins (e.g., Fabs) can be provided as IgG, Fab, Fab2, Fab2', scFv, PEGylated Fab, PEGylated scFv, PEGylated Fab2, VH::CH1::HSA+LC, HSA::VH::CH1+LC, LC::HSA + VH::CH1, HSA::LC + VH::CH1, or other appropriate construct.

In another embodiment, the binding protein comprises a Kunitz domain protein or modified version (e.g., HSA fusion) or peptide-based MMP-9 binding protein that can inhibit MMP-9 activity.

In one aspect, the disclosure features a protein (e.g., an isolated protein) that binds to MMP-9 (e.g., human MMP-9) and includes at least one immunoglobulin variable region. For example, the protein includes a heavy chain (HC) immunoglobulin variable domain sequence and a light chain (LC) immunoglobulin variable domain sequence. In one embodiment, the protein binds to and inhibits MMP-9 (e.g., inhibits MMP-9 catalytic activity), e.g., human MMP-9.

In some embodiments, the protein binds to human MMP-9 specifically, and not to MMP-9 from another species (e.g., the protein does not bind to MMP-9 from another species with greater than background levels of binding).

In some embodiments, the protein binds MMP-9 specifically, and not to another matrix metalloproteinase (e.g., the protein does not bind to any other matrix metalloproteinase with greater than background levels of binding).

Such binding proteins can be conjugated to a drug (e.g., to form a MMP-9 binding protein-drug conjugate) and used therapeutically. This disclosure relates, in part, to MMP-9 binding protein-drug conjugates, the preparation of these conjugates, and uses thereof. The

conjugates can be used, e.g., in the treatment of disorders, e.g., for the treatment of cancer, inflammation, heart failure, septic shock, neuropathic pain, inflammatory pain, or macular degeneration. Targeting (e.g., an killing) of the MMP-9 expressing cells and/or tumors, e.g., with high affinity binding protein-drug conjugates can be a potent therapy in the treatment of
5 diseases, e.g., cancer, inflammation, heart failure, septic shock, neuropathic pain, inflammatory pain, or macular degeneration.

The protein can include one or more of the following characteristics: (a) a human CDR or human framework region; (b) the HC immunoglobulin variable domain sequence comprises one or more (e.g., 1, 2, or 3) CDRs that are at least 85, 88, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or
10 100% identical to a CDR of a HC variable domain described herein; (c) the LC immunoglobulin variable domain sequence comprises one or more (e.g., 1, 2, or 3) CDRs that are at least 85, 88, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% identical to a CDR of a LC variable domain described herein; (d) the LC immunoglobulin variable domain sequence is at least 85, 88, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% identical to a LC variable domain described herein; (e)
15 the HC immunoglobulin variable domain sequence is at least 85, 88, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% identical to a HC variable domain described herein; (f) the protein binds an epitope bound by a protein described herein, or an epitope that overlaps with such epitope; and (g) a primate CDR or primate framework region.

The protein can bind to MMP-9, e.g., human MMP-9, with a binding affinity of at least
20 10^5 , 10^6 , 10^7 , 10^8 , 10^9 , 10^{10} and 10^{11} M^{-1} . In one embodiment, the protein binds to MMP-9 with a K_{off} slower than 1×10^{-3} , 5×10^{-4} s^{-1} , or 1×10^{-4} s^{-1} . In one embodiment, the protein binds to MMP-9 with a K_{on} faster than 1×10^2 , 1×10^3 , or 5×10^3 $M^{-1}s^{-1}$. In one embodiment, the protein inhibits human MMP-9 activity, e.g., with a K_i of less than 10^{-5} , 10^{-6} , 10^{-7} , 10^{-8} , 10^{-9} , and 10^{-10} M. The protein can have, for example, an IC_{50} of less than 100 nM, 10 nM or 1 nM. In some
25 embodiments, the protein has an IC_{50} of about 1.8 nM. The affinity of the protein for MMP-9 can be characterized by a K_D of less than 100 nm, less than 10 nM, or about 3 nM (e.g., 3.1 nM), about 5 nM (e.g., 5 nM), about 6 nm (e.g., 5.9 nM), about 7 nM (e.g., 7.1 nM), or about 10 nM (e.g., 9.6 nM).

In some embodiments, the protein has a $K_D < 200$ nM.

In some embodiments, the protein has a $t_{1/2}$ of at least about 10 minutes (e.g., 11 minutes), at least about 20 minutes (e.g., 18 minutes), at least about 25 minutes (e.g., 23 minutes), at least about 35 minutes (e.g., 33 minutes), or at least about 60 minutes (e.g., 57 minutes).

In one embodiment, the protein binds the catalytic domain of human MMP-9, e.g., the protein contacts residues in or near the active site of MMP-9.

In some embodiments, the protein does not contact residues in or near the active site of MMP-9 but instead binds elsewhere on MMP-9 and causes a steric change in MMP-9 that affects (e.g., inhibits) its activity.

In one embodiment, the protein also binds to MMP-16 and/or MMP-24, e.g., with a binding affinity of at least 10^5 , 10^6 , 10^7 , 10^8 , 10^9 , 10^{10} and 10^{11} M^{-1} . For example, the protein binds to both MMP-9 and to MMP-16 or MMP-24 with a binding affinity of at least 10^5 , 10^6 , 10^7 , 10^8 , 10^9 , 10^{10} and 10^{11} M^{-1} .

In a preferred embodiment, the protein is a human antibody having the light and heavy chains of antibodies picked from the list comprising 539A-M0240-B03, 539A-X0034-C02, M0078-G07, M0081-D05, M0076-D03, M0072-H07, M0075-D12, and M0166-F10. In a preferred embodiment, the protein is a human antibody having its heavy chain picked from the list comprising 539A-M0240-B03, 539A-X0034-C02, M0078-G07, M0081-D05, M0076-D03, M0072-H07, M0075-D12, and M0166-F10. In a preferred embodiment, the protein is a human antibody having its light chain picked from the list comprising 539A-M0240-B03, 539A-X0034-C02, M0078-G07, M0081-D05, M0076-D03, M0072-H07, M0075-D12, and M0166-F10. In a preferred embodiment, the protein is a human antibody having one or more (e.g., 1, 2, or 3) heavy chain CDRs picked from the corresponding CDRs of the list of heavy chains comprising 539A-M0240-B03, 539A-X0034-C02, M0078-G07, M0081-D05, M0076-D03, M0072-H07, M0075-D12, and M0166-F10. In a preferred embodiment, the protein is a human antibody having one or more (e.g., 1, 2, or 3) light chain CDRs picked from the corresponding CDRs of the list of light chains comprising 539A-M0240-B03, 539A-X0034-C02, M0078-G07, M0081-D05, M0076-D03, M0072-H07, M0075-D12, and M0166-F10.

In a more preferred embodiment, the protein is a human antibody having the light and heavy chains of antibodies from M0166-F10. In another preferred embodiment, the protein is a human antibody having its heavy chain from M0166-F10. In yet another preferred embodiment, the protein is a human antibody having its light chain from M0166-F10. In an even more

preferred embodiment, the protein is a human antibody having one or more (e.g., 1, 2, or 3) heavy chain CDRs from the corresponding CDRs of the heavy chain comprising M0166-F10. In another even more preferred embodiment, the protein is a human antibody having one or more (e.g., 1, 2, or 3) light chain CDRs from the corresponding CDRs of the light chains comprising
5 M0166-F10.

In a more preferred embodiment, the protein is a human antibody having one or more heavy chain CDRs from the corresponding CDRs of the heavy chain comprising 539A-M0240-B03. In another more preferred embodiment, the protein is a human antibody having one or more (e.g., 1, 2, or 3) light chain CDRs from the corresponding CDRs of the light chain
10 comprising 539A-M0240-B03. In another even more preferred embodiment, the protein is a human antibody having one or more (e.g., 1, 2, or 3) light chain CDRs from the corresponding CDRs of the light chains of 539A-M0240-B03, and/or one or more heavy chain CDRs from the corresponding CDRs of the heavy chain of 539A-M0240-B03.

In a more preferred embodiment, the protein is a human antibody having one or more
15 heavy chain CDRs from the corresponding CDRs of the heavy chain comprising 539A-X0034-C02. In another more preferred embodiment, the protein is a human antibody having one or more (e.g., 1, 2, or 3) light chain CDRs from the corresponding CDRs of the light chain comprising 539A-X0034-C02. In another even more preferred embodiment, the protein is a human antibody having one or more (e.g., 1, 2, or 3) light chain CDRs from the corresponding
20 CDRs of the light chains of 539A-X0034-C02, and/or one or more heavy chain CDRs from the corresponding CDRs of the heavy chain of 539A-X0034-C02.

In one embodiment, the HC and LC variable domain sequences are components of the same polypeptide chain. In another, the HC and LC variable domain sequences are components of different polypeptide chains. For example, the protein is an IgG, e.g., IgG1, IgG2, IgG3, or
25 IgG4. The protein can be a soluble Fab (sFab). In other implementations the protein includes a Fab2', scFv, minibody, scFv::Fc fusion, Fab::HSA fusion, HSA::Fab fusion, Fab::HSA::Fab fusion, or other molecule that comprises the antigen combining site of one of the binding proteins herein. The VH and VL regions of these Fabs can be provided as IgG, Fab, Fab2, Fab2', scFv, PEGylated Fab, PEGylated scFv, PEGylated Fab2, VH::CH1::HSA+LC,
30 HSA::VH::CH1+LC, LC::HSA + VH::CH1, HSA::LC + VH::CH1, or other appropriate construction.

In one embodiment, the protein is a human or humanized antibody or is non-immunogenic in a human. For example, the protein includes one or more human antibody framework regions, e.g., all human framework regions. In one embodiment, the protein includes a human Fc domain, or an Fc domain that is at least 95, 96, 97, 98, or 99% identical to a human Fc domain.

In one embodiment, the protein is a primate or primatized antibody or is non-immunogenic in a human. For example, the protein includes one or more primate antibody framework regions, e.g., all primate framework regions. In one embodiment, the protein includes a primate Fc domain, or an Fc domain that is at least 95, 96, 97, 98, or 99% identical to a primate Fc domain. "Primate" includes humans (*Homo sapiens*), chimpanzees (*Pan troglodytes* and *Pan paniscus* (bonobos)), gorillas (*Gorilla gorilla*), gibbons, monkeys, lemurs, aye-ayes (*Daubentonia madagascariensis*), and tarsiers.

In certain embodiments, the protein includes no sequences from mice or rabbits (e.g., is not a murine or rabbit antibody).

In one embodiment, the protein is capable of binding to tumor cells expressing MMP-9, e.g., to Colo205 (a human colorectal carcinoma cell line), or MCF-7 (a human breast adenocarcinoma cell line) cells.

In one embodiment, protein is physically associated with a nanoparticle, and can be used to guide a nanoparticle to a cell expressing MMP-9 on the cell surface. In one embodiment, the protein causes effector cells (CDC or ADCC) to kill a cell which expresses MMP-9.

In another aspect, the disclosure features a MMP-9 binding protein that is a competitive inhibitor of MMP-9. In some embodiments, the binding protein competes with an MMP-9 substrate (e.g., collagen), e.g., binds to the same epitope as the substrate, e.g., and prevents substrate binding.

In some aspects, the disclosure features a method of inhibiting an interaction between MMP-9 and an MMP-9 substrate (e.g., collagen). The method includes contacting an MMP-9 binding protein described herein with MMP-9 (e.g., *in vitro* or *in vivo*), wherein the binding protein binds to MMP-9 and thereby prevents the binding of an MMP-9 substrate to MMP-9. In some embodiments, the binding protein binds to the same epitope on MMP-9 as the substrate,

e.g., the binding protein is a competitive inhibitor. In some embodiments, the binding protein does not bind the same epitope as the substrate but causes a steric change in MMP-9 that decreases or inhibits the ability of the substrate to bind.

5 In one aspect, the disclosure features a MMP-9 binding protein-drug conjugate that includes a MMP-9 binding protein and a drug.

In one embodiment, the binding protein comprises at least one immunoglobulin variable region, and/or the protein binds to and/or inhibits MMP-9, e.g., inhibits MMP-9 catalytic activity.

10 In one embodiment, the drug is a cytotoxic or cytostatic agent. The cytotoxic agent can be, e.g., selected from the group consisting of an auristatin, a DNA minor groove binding agent, a DNA minor groove alkylating agent, an enediyne, a lexitropsin, a duocarmycin, a taxane, a puromycin, a dolastatin, a podophyllotoxin, a baccatin derivative, a cryptophysin, a combretastatin, a maytansinoid, and a vinca alkaloid. In one embodiment, the cytotoxic agent is an auristatin and, e.g., the auristatin is selected from AFP, MMAF, MMAE, AEB, AEVB and auristatin E. In one embodiment, the auristatin is AFP or MMAF. In another embodiment, the cytotoxic agent is a maytansinoid and, e.g., the maytansinoid is selected from a maytansinol, maytansine, DM1, DM2, DM3 and DM4. In one embodiment, the maytansinoid is DM1. In another embodiment, the cytotoxic agent is selected from paclitaxel, docetaxel, CC-1065, SN-38, 15 topotecan, morpholino-doxorubicin, rhizoxin, cyanomorpholino-doxorubicin, dolastatin-10, echinomycin, combretastatin, calicheamicin, and netropsin. In one embodiment, the cytotoxin is an auristatin, a maytansinoid, or calicheamicin.

In one embodiment, the cytotoxic agent is an antitubulin agent and, e.g., the antitubulin agent is selected from AFP, MMAP, MMAE, AEB, AEVB, auristatin E, vincristine, vinblastine, 25 vindesine, vinorelbine, VP-16, camptothecin, paclitaxel, docetaxel, epothilone A, epothilone B, nocodazole, colchicines, colcimid, estramustine, cemadotin, discodermolide, maytansinol, maytansine, DM1, DM2, DM3, DM4 and eleutherobin.

In one embodiment, the MMP-9 binding protein (e.g., antibody) is conjugated to the drug (e.g., cytotoxic agent) via a linker. In one embodiment, the linker is cleavable under intracellular 30 conditions, e.g., the cleavable linker is a peptide linker cleavable by an intracellular protease. In one embodiment, the linker is a peptide linker, e.g., a dipeptide linker, e.g., a val-cit linker or a

phe-lys linker. In one embodiment, the cleavable linker is hydrolyzable at a pH of less than 5.5, e.g., the hydrolyzable linker is a hydrazone linker. In another embodiment, the cleavable linker is a disulfide linker.

5 A binding protein described herein can be provided as a pharmaceutical composition, e.g., including a pharmaceutically acceptable carrier. The composition can be at least 10, 20, 30, 50, 75, 85, 90, 95, 98, 99, or 99.9% free of other protein species. In some embodiments, the binding protein can be produced under GMP (good manufacturing practices). In some
10 embodiments, the binding protein is provided in pharmaceutically acceptable carriers, e.g., suitable buffers or excipients.

The dose of a binding protein (e.g., a pharmaceutical composition containing a binding protein described herein) is sufficient to block about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 85%, about 90%, about 95%, about 99%, or about
15 100% of the activity of MMP-9 in the patient, e.g., at the site of disease. Depending on the disease, this may require a dose, e.g., of between about 0.01 mg/Kg to about 100 mg/Kg, e.g., between about 0.1 and about 10 mg/Kg. For example, the dose can be a dose of about 0.1, about 1, about 3, about 6, or about 10 mg/Kg. For example, for an IgG having a molecular mass of 150,000 g/mole (2 binding sites), these doses correspond to approximately 18 nM, 180 nM, 540
nM, 1.08 microM, and 1.8 microM, respectively, of binding sites for a 5 L blood volume.

20 Medicine being partly an art, the optimal dose will be established by clinical trials, but will most likely lie in this range.

In another aspect, the disclosure features a method of detecting an MMP-9 in a sample, e.g., a sample from a patient (e.g., tissue biopsy or blood sample). The method includes:
25 contacting the sample with an MMP-9 binding protein; and detecting an interaction between the protein and the MMP-9, if present. In some embodiments, the protein includes a detectable label. An MMP-9 binding protein can be used to detect MMP-9 in a subject. The method includes: administering an MMP-9 binding protein to a subject; and detecting the protein in the subject. In some embodiments, the protein further includes a detectable label. For example, the
30 detecting comprises imaging the subject. For example, MMP-9 activity can be a marker of joint pathogenesis and/or disease progression in subjects with, or suspected of having, arthritis.

In another aspect, the disclosure features a method of modulating MMP-9 activity. The method includes: contacting an MMP-9 with an MMP-9 binding protein (e.g., in a human subject), thereby modulating MMP-9 activity. In some embodiments, the binding protein
5 inhibits MMP-9 activity (e.g., inhibits MMP-9 catalytic activity).

In another aspect, the disclosure features a method of treating cancer (e.g., metastatic cancer) (e.g., in a subject that has cancer or is suspected of having cancer). The method includes: administering, to a subject, an MMP-9 binding protein in an amount sufficient to treat a cancer in
10 the subject. For example, the cancer is head and neck cancer, oral cavity cancer, laryngeal cancer, chondrosarcoma, breast cancer (which may be estrogen receptor positive (ER+), estrogen receptor negative (ER-), Her2 positive (Her2+), Her2 negative (Her2-), or a combination thereof, e.g., ER+/Her2+, ER+/Her2-, ER-/Her2+, or ER-/Her2-), laryngeal cancer, ovarian cancer, lung cancer, prostate cancer, colon cancer (e.g., primary or metastatic colon cancer), testicular
15 carcinoma, melanoma, leukemia, B cell lymphoma, multiple myeloma, or a brain tumor (e.g., astrocytomas, glioblastomas, gliomas).

MMP-9 binding proteins can be useful for modulating metastatic activity in a subject (e.g., in a subject that has a metastatic cancer or is suspected of having a metastatic cancer). The protein can be administered, to the subject, in an amount effective to modulate
20 metastatic activity. For example, the protein inhibits one or more of: tumor growth, tumor embolism, tumor mobility, tumor invasiveness, and cancer cell proliferation.

The methods disclosed herein relating to the treatment cancer (e.g., treating cancer and/or modulation of metastatic activity) can further include providing (e.g., administering) to the subject a second therapy that is an anti-cancer therapy, e.g., administration of a
25 chemotherapeutic, e.g., an agent that antagonizes signaling through a VEGF pathway, e.g., bevacizumab (AVASTIN®). In one embodiment, the second therapy includes administering 5-FU, leucovorin, and/or irinotecan. In one embodiment, the second therapy includes administering a Tie1 inhibitor (e.g., an anti-Tie1 antibody). As another example, the second agent can be an anti-MMP14 binding protein (e.g., IgG or Fab, e.g., DX-2400, or a protein
30 described in U.S. Pub. App. No. 2007-0217997). In one embodiment, the second therapy is an inhibitor of plasmin (e.g., a kunitz domain disclosed in U.S. Patent No. 6,010,880, such as a

protein or polypeptide comprising the amino acid sequence

MHSFCAFKAE TGPCRARFDRWFFNIFTRQCEEFIYGGCEGNQNR FESLEECKKMCTRD
(SEQ ID NO:1).

5 In another aspect, the disclosure features a method of treating heart failure (e.g., in a subject that has heart failure or is suspected of having heart failure). The method includes: administering, to a subject, an MMP-9 binding protein in an amount sufficient to treat heart failure in the subject. The method can further include providing to the subject a second therapy that is a heart failure therapy.

10 In another aspect, the disclosure features a method of treating septic shock (e.g., in a subject that has septic shock or is suspected of having septic shock). The method includes: administering, to a subject, an MMP-9 binding protein in an amount sufficient to treat septic shock in the subject. The method can further include providing to the subject a second therapy
15 that is a therapy for septic shock.

 In another aspect, the disclosure features a method of treating neuropathic pain (e.g., in a subject that has neuropathic pain or is suspected of having neuropathic pain). The method includes: administering, to a subject, an MMP-9 binding protein in an amount sufficient to treat
20 neuropathic pain in the subject. The method can further include providing to the subject a second therapy that is a therapy for neuropathic pain.

 In another aspect, the disclosure features a method of treating inflammatory pain (e.g., in a subject that has inflammatory pain or is suspected of having inflammatory pain). The method
25 includes: administering, to a subject, an MMP-9 binding protein in an amount sufficient to treat inflammatory pain in the subject. The method can further include providing to the subject a second therapy that is a therapy for inflammatory pain.

 In another aspect, the disclosure features a method of treating an ocular condition (e.g.,
30 macular degeneration) (e.g., in a subject that has an ocular condition or is suspected of having an ocular condition). The method includes: administering, to a subject, an MMP-9 binding protein

in an amount sufficient to treat the ocular condition in the subject. In one embodiment, the method further includes administering a second agent an agent that antagonizes signaling through a VEGF pathway, e.g., bevacizumab or ranibizumab. In one embodiment where the second agent is a VEGF pathway inhibitor (e.g., bevacizumab or ranibizumab), the ocular
5 condition is macular degeneration, e.g., age-related macular degeneration, such as wet age-related macular degeneration.

In another aspect, the disclosure features a method of treating an inflammatory disease (e.g., chronic obstructive pulmonary disease (COPD), asthma, rhinitis, inflammatory bowel
10 disease, synovitis, rheumatoid arthritis) (e.g., in a subject that has an inflammatory disease or is suspected of having an inflammatory disease). The method includes: administering, to a subject, an MMP-9 binding protein in an amount sufficient to treat the inflammatory disease in the subject. The method can further include providing to the subject a second therapy that is an anti-inflammatory therapy. For example, particularly for rheumatoid arthritis, the second therapy
15 comprises administering one or more of the following agents: aspirin, naproxen, ibuprofen, etodolac, cortisone (corticosteroids), antacids, sucralfate, proton-pump inhibitors, misoprostol, gold (e.g., gold salts, gold thioglucose, gold thiomalate, oral gold), methotrexate, sulfasalazine, D-penicillamine, azathioprine, cyclophosphamide, chlorambucil, cyclosporine, leflunomide, etanercept, infliximab, anakinra, adalimumab, and/or hydroxychloroquine.

20 Other exemplary therapeutic methods that include administering an MMP-9 binding protein are described below. An MMP-9 binding protein described herein can be administered in combination with one or more other MMP inhibitors, e.g., small molecule inhibitors, e.g., broad specificity inhibitors. In one embodiment, the small molecule inhibitors are one or more of neovastat, marimastat, BAY 12-9566, or prinomastat. In another embodiment, the one or more
25 MMP inhibitors include another MMP-9 binding protein.

MMP-9 binding proteins are useful for targeted delivery of an agent to a subject (e.g., a subject who has or is suspected of having a tumor), e.g., to direct the agent to a tumor in the subject. For example, an MMP-9 binding protein that is coupled to an anti-tumor agent (such as a chemotherapeutic, toxin, drug, or a radionuclide (e.g., ^{131}I , ^{90}Y , ^{177}Lu)) can be administered to a
30 subject who has or is suspected of having a tumor.

In another aspect, the disclosure features a method of imaging a subject. The method includes administering an MMP-9 binding protein to the subject. In some embodiments, the protein is one that does not substantially inhibit MMP-9 catalytic activity. The MMP-9 binding protein may include a detectable label (e.g., a radionuclide or an MRI-detectable label). In one
5 embodiment, the subject has or is suspected of having a tumor. The method is useful for cancer diagnosis, intraoperative tumor detection, post-operative tumor detection, or monitoring tumor invasive activity.

In one aspect, the disclosure features the use of an MMP-9 binding protein described
10 herein for the manufacture of a medicament for the treatment of a disorder described herein, e.g., cancer, inflammation, heart failure, septic shock, neuropathic pain, inflammatory pain, or macular degeneration.

The details of one or more embodiments of the invention are set forth in the
15 accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

The contents of all cited references including literature references, issued patents, published or non-published patent applications cited throughout this application as well as those
20 listed below are hereby expressly incorporated by reference in their entireties. In case of conflict, the present application, including any definitions herein, will control.

DESCRIPTION OF DRAWINGS

FIGS. 1A and 1B: FIG. 1A is a line graph showing human MMP-9 activity (Fluo/sec) in the presence of increasing concentrations (nM) of an MMP-9 binding protein (539A-M0166-F10). FIG. 1B is a table showing that an MMP-9 binding protein (539A-M0166-F10) is specific
25 for human MMP-9.

FIG. 2 is a line graph showing measured IC_{50} (nM) versus substrate concentration (μ M) of an MMP-9 binding protein (539A-M0166-F10).

FIG. 3 is a series of line graphs showing IC_{50} measurements at various concentrations of
30 substrate of an MMP-9 binding protein (539A-M0166-F10).

FIG. 4 is a set of two line graphs showing IC_{50} measurements at 10 μ M concentration of substrate (human MMP-9- top panel, or mouse MMP-9- bottom panel) of an MMP-9 binding protein (539A-M0240-B03).

FIG. 5 is a table showing that an MMP-9 binding protein (539A-M0240-B03) inhibits human and mouse MMP-9 but not human MMP-1, -2, -3, -7, -8, -10, -12, and -14.

FIGS. 6A and 6B are two line graphs showing IC_{50} (nM) versus substrate concentration (μ M) of an MMP-9/-2 binding protein (539A-M0237-D02). In FIG. 6A, the substrate is human MMP-9. In FIG. 6B, the substrate is mouse MMP-9.

FIG. 7 is a line graph showing activity of MMP-9 binding proteins in a Colo205 colon xenograft cancer model.

FIG. 8 is a line graph showing the efficacy of MMP-9 binding proteins in a BxPC-3 pancreatic cancer model.

FIG. 9 is a line graph showing the efficacy of an MMP-9 binding protein (539A-M0240-B03) in a mouse collagen-induced arthritis model.

FIG. 10 is a diagram showing effects of disease and treatment on joint parameters of MMP-9 binding protein (539A-M0240-B03) in a mouse collagen-induced arthritis model.

DETAILED DESCRIPTION

Matrix metalloproteinase-2 (MMP-2) and matrix metalloproteinase-9 (MMP-9) are 72- and 92-kD, respectively, type IV collagenases that are members of a group of secreted zinc metalloproteases which, in mammals, degrade the collagens of the extracellular matrix. Other members of this group include interstitial collagenase (MMP-1) and stromelysin (MMP-3). MMP-2, the 72-kD type IV collagenase (also known as CLG4A), is secreted from normal skin fibroblasts, whereas MMP-9, the 92-kD collagenase (also known as CLG4B), is produced by normal alveolar macrophages and granulocytes. The present disclosure provides proteins that bind to MMP-9 and, in some instances, inhibit MMP-9 activity.

The term “binding protein” refers to a protein that can interact with a target molecule. This term is used interchangeably with “ligand.” An “MMP-9 binding protein” refers to a protein that can interact with MMP-9, and includes, in particular, proteins that preferentially interact with and/or inhibit MMP-9. For example, the MMP-9 binding protein is an antibody.

The term “antibody” refers to a protein that includes at least one immunoglobulin variable domain or immunoglobulin variable domain sequence. For example, an antibody can include a heavy (H) chain variable region (abbreviated herein as VH), and a light (L) chain variable region (abbreviated herein as VL). In another example, an antibody includes two heavy (H) chain variable regions and two light (L) chain variable regions. The term “antibody” encompasses antigen-binding fragments of antibodies (e.g., single chain antibodies, Fab and sFab fragments, F(ab')₂, Fd fragments, Fv fragments, scFv, and domain antibodies (dAb) fragments (de Wildt et al., Eur J Immunol. 1996; **26**(3):629-39.)) as well as complete antibodies. An antibody can have the structural features of IgA, IgG, IgE, IgD, IgM (as well as subtypes thereof). Antibodies may be from any source, but primate (human and non-human primate) and primatized are preferred

The VH and VL regions can be further subdivided into regions of hypervariability, termed “complementarity determining regions” (“CDR”), interspersed with regions that are more conserved, termed “framework regions” (“FR”). The extent of the framework region and CDRs has been precisely defined (see, Kabat, E.A., *et al.* (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242, and Chothia, C. et al. (1987) J. Mol. Biol. 196:901-917, see also www.hgmp.mrc.ac.uk). Kabat definitions are used herein. Each VH and VL is typically composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4.

As used herein, an “immunoglobulin variable domain sequence” refers to an amino acid sequence which can form the structure of an immunoglobulin variable domain such that one or more CDR regions are positioned in a conformation suitable for an antigen binding site. For example, the sequence may include all or part of the amino acid sequence of a naturally-occurring variable domain. For example, the sequence may omit one, two or more N- or C-terminal amino acids, internal amino acids, may include one or more insertions or additional terminal amino acids, or may include other alterations. In one embodiment, a polypeptide that includes immunoglobulin variable domain sequence can associate with another immunoglobulin variable domain sequence to form an antigen binding site, e.g., a structure that preferentially interacts with an MMP-9 protein, e.g., the MMP-9 catalytic domain.

The VH or VL chain of the antibody can further include all or part of a heavy or light chain constant region, to thereby form a heavy or light immunoglobulin chain, respectively. In one embodiment, the antibody is a tetramer of two heavy immunoglobulin chains and two light immunoglobulin chains, wherein the heavy and light immunoglobulin chains are inter-connected by, e.g., disulfide bonds. In IgGs, the heavy chain constant region includes three immunoglobulin domains, CH1, CH2 and CH3. The light chain constant region includes a CL domain. The variable region of the heavy and light chains contains a binding domain that interacts with an antigen. The constant regions of the antibodies typically mediate the binding of the antibody to host tissues or factors, including various cells of the immune system (e.g., effector cells) and the first component (C1q) of the classical complement system. The light chains of the immunoglobulin may be of types kappa or lambda. In one embodiment, the antibody is glycosylated. An antibody can be functional for antibody-dependent cytotoxicity and/or complement-mediated cytotoxicity.

One or more regions of an antibody can be human or effectively human. For example, one or more of the variable regions can be human or effectively human. For example, one or more of the CDRs can be human, e.g., HC CDR1, HC CDR2, HC CDR3, LC CDR1, LC CDR2, and LC CDR3. Each of the light chain CDRs can be human. HC CDR3 can be human. One or more of the framework regions can be human, e.g., FR1, FR2, FR3, and FR4 of the HC or LC. For example, the Fc region can be human. In one embodiment, all the framework regions are human, e.g., derived from a human somatic cell, e.g., a hematopoietic cell that produces immunoglobulins or a non-hematopoietic cell. In one embodiment, the human sequences are germline sequences, e.g., encoded by a germline nucleic acid. In one embodiment, the framework (FR) residues of a selected Fab can be converted to the amino-acid type of the corresponding residue in the most similar primate germline gene, especially the human germline gene. One or more of the constant regions can be human or effectively human. For example, at least 70, 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% of an immunoglobulin variable domain, the constant region, the constant domains (CH1, CH2, CH3, CL1), or the entire antibody can be human or effectively human.

All or part of an antibody can be encoded by an immunoglobulin gene or a segment thereof. Exemplary human immunoglobulin genes include the kappa, lambda, alpha (IgA1 and IgA2), gamma (IgG1, IgG2, IgG3, IgG4), delta, epsilon and mu constant region genes, as well as

the many immunoglobulin variable region genes. Full-length immunoglobulin “light chains” (about 25 KDa or about 214 amino acids) are encoded by a variable region gene at the NH₂-terminus (about 110 amino acids) and a kappa or lambda constant region gene at the COOH--terminus. Full-length immunoglobulin “heavy chains” (about 50 KDa or about 446 amino acids), are similarly encoded by a variable region gene (about 116 amino acids) and one of the other aforementioned constant region genes, e.g., gamma (encoding about 330 amino acids). The length of human HC varies considerably because HC CDR3 varies from about 3 amino-acid residues to over 35 amino-acid residues.

The term “antigen-binding fragment” of a full length antibody refers to one or more fragments of a full-length antibody that retain the ability to specifically bind to a target of interest. Examples of binding fragments encompassed within the term “antigen-binding fragment” of a full length antibody include (i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; (ii) a F(ab')₂ fragment, a bivalent fragment including two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the VH and CH1 domains; (iv) a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, (v) a dAb fragment (Ward *et al.*, (1989) *Nature* 341:544-546), which consists of a VH domain; and (vi) an isolated complementarity determining region (CDR) that retains functionality. Furthermore, although the two domains of the Fv fragment, VL and VH, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent molecules known as single chain Fv (scFv). See *e.g.*, US patents 5,260,203, 4,946,778, and 4,881,175; Bird *et al.* (1988) *Science* 242:423-426; and Huston *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:5879-5883.

Antibody fragments can be obtained using any appropriate technique including conventional techniques known to those with skill in the art. The term “monospecific antibody” refers to an antibody that displays a single binding specificity and affinity for a particular target, e.g., epitope. This term includes a “monoclonal antibody” or “monoclonal antibody composition,” which as used herein refer to a preparation of antibodies or fragments thereof of single molecular composition, irrespective of how the antibody was generated.

An “effectively human” immunoglobulin variable region is an immunoglobulin variable region that includes a sufficient number of human framework amino acid positions such that the

immunoglobulin variable region does not elicit an immunogenic response in a normal human.

An “effectively human” antibody is an antibody that includes a sufficient number of human amino acid positions such that the antibody does not elicit an immunogenic response in a normal human.

5 A “humanized” immunoglobulin variable region is an immunoglobulin variable region that is modified to include a sufficient number of human framework amino acid positions such that the immunoglobulin variable region does not elicit an immunogenic response in a normal human. Descriptions of “humanized” immunoglobulins include, for example, US 6,407,213 and US 5,693,762.

10 As used herein, “binding affinity” refers to the apparent association constant or K_a . The K_a is the reciprocal of the dissociation constant (K_d). A binding protein may, for example, have a binding affinity of at least 10^5 , 10^6 , 10^7 , 10^8 , 10^9 , 10^{10} and 10^{11} M^{-1} for a particular target molecule, e.g., MMP-9, MMP-16, or MMP-24. Higher affinity binding of a binding protein to a first target relative to a second target can be indicated by a higher K_a (or a smaller numerical value K_d) for binding the first target than the K_a (or numerical value K_d) for binding the second target. In such cases, the binding protein has specificity for the first target (e.g., a protein in a first conformation or mimic thereof) relative to the second target (e.g., the same protein in a second conformation or mimic thereof; or a second protein). Differences in binding affinity (e.g., for specificity or other comparisons) can be at least 1.5, 2, 3, 4, 5, 10, 15, 20, 37.5, 50, 70, 20 80, 91, 100, 500, 1000, or 10^5 fold.

Binding affinity can be determined by a variety of methods including equilibrium dialysis, equilibrium binding, gel filtration, ELISA, surface plasmon resonance, or spectroscopy (e.g., using a fluorescence assay). Exemplary conditions for evaluating binding affinity are in TRIS-buffer (50mM TRIS, 150mM NaCl, 5mM $CaCl_2$ at pH7.5). These techniques can be used to measure the concentration of bound and free binding protein as a function of binding protein (or target) concentration. The concentration of bound binding protein ([Bound]) is related to the concentration of free binding protein ([Free]) and the concentration of binding sites for the binding protein on the target where (N) is the number of binding sites per target molecule by the following equation:

30
$$[Bound] = N \cdot [Free] / ((1/K_a) + [Free]).$$

It is not always necessary to make an exact determination of K_a , though, since sometimes it is sufficient to obtain a quantitative measurement of affinity, e.g., determined using a method such as ELISA or FACS analysis, is proportional to K_a , and thus can be used for comparisons, such as determining whether a higher affinity is, e.g., 2-fold higher, to obtain a qualitative measurement of affinity, or to obtain an inference of affinity, e.g., by activity in a functional assay, e.g., an *in vitro* or *in vivo* assay.

An “isolated composition” refers to a composition (e.g., protein) that is removed from at least 90% of at least one component of a natural sample from which the isolated composition can be obtained. Compositions produced artificially or naturally can be “compositions of at least” a certain degree of purity if the species or population of species of interests is at least 5, 10, 25, 50, 75, 80, 90, 92, 95, 98, or 99% pure on a weight-weight basis.

An “epitope” refers to the site on a target compound that is bound by a binding protein (e.g., an antibody such as a Fab or full length antibody). In the case where the target compound is a protein, the site can be entirely composed of amino acid components, entirely composed of chemical modifications of amino acids of the protein (e.g., glycosyl moieties), or composed of combinations thereof. Overlapping epitopes include at least one common amino acid residue, glycosyl group, phosphate group, sulfate group, or other molecular feature.

Calculations of “homology” or “sequence identity” between two sequences (the terms are used interchangeably herein) are performed as follows. The sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-homologous sequences can be disregarded for comparison purposes). The optimal alignment is determined as the best score using the GAP program in the GCG software package with a Blossum 62 scoring matrix with a gap penalty of 12, a gap extend penalty of 4, and a frameshift gap penalty of 5. The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position (as used herein amino acid or nucleic acid “identity” is equivalent to amino acid or nucleic acid “homology”). The percent identity between the two sequences is a function of the number of identical positions shared by the sequences.

In a preferred embodiment, the length of a reference sequence aligned for comparison purposes is at least 30%, preferably at least 40%, more preferably at least 50%, even more preferably at least 60%, and even more preferably at least 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% of the length of the reference sequence. For
5 example, the reference sequence may be the length of the immunoglobulin variable domain sequence.

As used herein, the term “substantially identical” (or “substantially homologous”) is used herein to refer to a first amino acid or nucleic acid sequence that contains a sufficient number of identical or equivalent (e.g., with a similar side chain, e.g., conserved amino acid substitutions)
10 amino acid residues or nucleotides to a second amino acid or nucleic acid sequence such that the first and second amino acid or nucleic acid sequences have (or encode proteins having) similar activities, e.g., a binding activity, a binding preference, or a biological activity. In the case of antibodies, the second antibody has the same specificity and has at least 50%, at least 25%, or at least 10% of the affinity relative to the same antigen.

Sequences similar or homologous (e.g., at least about 85% sequence identity) to the sequences disclosed herein are also part of this application. In some embodiments, the sequence identity can be about 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or higher. In addition, substantial identity exists when the nucleic acid segments hybridize under selective hybridization conditions (e.g., highly stringent hybridization conditions), to the complement of
20 the strand. The nucleic acids may be present in whole cells, in a cell lysate, or in a partially purified or substantially pure form.

As used herein, the term “hybridizes under low stringency, medium stringency, high stringency, or very high stringency conditions” describes conditions for hybridization and washing. Guidance for performing hybridization reactions can be found in *Current Protocols in*
25 *Molecular Biology*, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. Aqueous and nonaqueous methods are described in that reference and either can be used. Specific hybridization conditions referred to herein are as follows: (1) low stringency hybridization conditions in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by two washes in 0.2X SSC, 0.1% SDS at least at 50°C (the temperature of the washes can be increased to 55°C for low stringency
30 conditions); (2) medium stringency hybridization conditions in 6X SSC at about 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 60°C; (3) high stringency hybridization

conditions in 6X SSC at about 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 65°C; and (4) very high stringency hybridization conditions are 0.5M sodium phosphate, 7% SDS at 65°C, followed by one or more washes at 0.2X SSC, 1% SDS at 65°C. Very high stringency conditions (4) are the preferred conditions and the ones that should be used unless
5 otherwise specified. The disclosure includes nucleic acids that hybridize with low, medium, high, or very high stringency to a nucleic acid described herein or to a complement thereof, e.g., nucleic acids encoding a binding protein described herein. The nucleic acids can be the same length or within 30, 20, or 10% of the length of the reference nucleic acid. The nucleic acid can correspond to a region encoding an immunoglobulin variable domain sequence described herein.

10 An MMP-9 binding protein may have mutations (e.g., at least one, two, or four, and/or less than 15, 10, 5, or 3) relative to a binding protein described herein (e.g., conservative or non-essential amino acid substitutions), which do not have a substantial effect on protein function. Whether or not a particular substitution will be tolerated, i.e., will not adversely affect biological properties, such as binding activity can be predicted, e.g., by evaluating whether the mutation is
15 conservative or by the method of Bowie, et al. (1990) *Science* 247:1306-1310.

A “conservative amino acid substitution” is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic
20 acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). It is possible for many framework and CDR amino acid residues to include one or more conservative
25 substitutions.

Motif sequences for biopolymers can include positions which can be varied amino acids. For example, the symbol “X” in such a context generally refers to any amino acid (e.g., any of the twenty natural amino acids or any of the nineteen non-cysteine amino acids). Other allowed amino acids can also be indicated for example, using parentheses and slashes. For example,
30 “(A/W/F/N/Q)” means that alanine, tryptophan, phenylalanine, asparagine, and glutamine are allowed at that particular position.

A “non-essential” amino acid residue is a residue that can be altered from the wild-type sequence of the binding agent, e.g., the antibody, without abolishing or more preferably, without substantially altering a biological activity, whereas changing an “essential” amino acid residue results in a substantial loss of activity.

5 The term “cognate ligand” refers to a naturally occurring ligand of an MMP-9, including naturally occurring variants thereof (e.g., splice variants, naturally occurring mutants, and isoforms).

Statistical significance can be determined by any art known method. Exemplary statistical tests include: the Students T-test, Mann Whitney U non-parametric test, and Wilcoxon
10 non-parametric statistical test. Some statistically significant relationships have a P value of less than 0.05 or 0.02. Particular binding proteins may show a difference, e.g., in specificity or binding, that are statistically significant (e.g., P value < 0.05 or 0.02). The terms “induce”, “inhibit”, “potentiate”, “elevate”, “increase”, “decrease” or the like, e.g., which denote distinguishable qualitative or quantitative differences between two states, and may refer to a
15 difference, e.g., a statistically significant difference, between the two states.

MMP-9 Binding Proteins

The disclosure provides proteins that bind to MMP-9 (e.g., human MMP-9) and include at least one immunoglobulin variable region. For example, the MMP-9 binding protein includes a
20 heavy chain (HC) immunoglobulin variable domain sequence and a light chain (LC) immunoglobulin variable domain sequence. A number of exemplary MMP-9 binding proteins are described herein.

The MMP-9 binding protein may be an isolated protein (e.g., at least 70, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% free of other proteins).

25 The MMP-9 binding protein may additionally inhibit MMP-9, e.g., human MMP-9. The binding protein can inhibit the catalytic activity of MMP-9 (e.g., human MMP-9). In one embodiment, the protein binds the catalytic domain of human MMP-9, e.g., the protein contacts residues in or near the active site of MMP-9. In some embodiments, the protein does not contact residues in or near the active site of MMP-9 but instead binds elsewhere on MMP-9 and causes a
30 steric change in MMP-9 that affects (e.g., inhibits) its activity.

Exemplary MMP-9 binding proteins include 539A-M0240-B03, 539A-X0034-C02, M0078-G07, M0081-D05, M0076-D03, M0072-H07, M0075-D12, and M0166-F10, or proteins that comprise the HC and/or LC CDRs of 539A-M0240-B03, 539A-X0034-C02, M0078-G07, M0081-D05, M0076-D03, M0072-H07, M0075-D12, and M0166-F10.

5 MMP-9 binding proteins may be antibodies. MMP-9 binding antibodies may have their HC and LC variable domain sequences included in a single polypeptide (e.g., scFv), or on different polypeptides (e.g., IgG or Fab).

Matrix Metalloproteinase 9 (MMP-9)

MMP-9 Sequences. MMP-9 is encoded by a gene designated as *MMP9* with full name
10 Matrix metalloproteinase-9 precursor. Synonyms for MMP-9 include matrix metalloproteinase 9, gelatinase B (GELB), 92kDa gelatinase (CLG4B), 92kDa type IV collagenase (EC 3.4.24.35). The DNA sequence is known for *Homo sapiens* and *Mus musculus*. An exemplary cDNA sequence encoding human *MMP9* and the amino acid sequence are shown below. Exemplary cDNA sequences encoding murine *MMP9* and amino acid sequences are also shown below. An
15 exemplary MMP-9 protein can include the human or mouse MMP-9 amino acid sequence, a sequence that is 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to one of these sequences, or a fragment thereof, e.g., a fragment without the signal sequence or prodomain.

Table 1 shows the similar genes in other organisms and the percentage of similarity with human MMP-9. No similarity-to-human data found for MMP9 for: chimpanzee (*Pan*
20 *troglodytes*), pig (*Sus scrofa*), cow (*Bos taurus*), fruit fly (*Drosophila melanogaster*), worm (*Caenorhabditis elegans*), baker's yeast (*Saccharomyces cerevisiae*), tropical clawed frog (*Silurana tropicalis*), African malaria mosquito (*Anopheles gambiae*), green algae (*Chlamydomonas reinhardtii*), soybean (*Glycine max*), barley (*Hordeum vulgare*), tomato (*Lycopersicon esculentum*), rice blast fungus (*Magnaporthe grisea*), sugarcane (*Saccharum*
25 *officinarum*), loblolly pine (*Pinus taeda*), corn (*Zea mays*), wheat (*Triticum aestivum*), Alicante grape (*Vitis vinifera*), bread mold (*Neurospora crassa*), fission yeast (*Schizosaccharomyces pombe*), sea squirt (*Ciona intestinalis*), amoeba (*Dictyostelium discoideum*), A. gossypii yeast (*Ashbya gossypii*), K. lactis yeast (*Kluyveromyces lactis*), medicago trunc (*Medicago truncatula*), malaria parasite (*Plasmodium falciparum*), schistosome parasite (*Schistosoma mansoni*),
30 sorghum (*Sorghum bicolor*), toxoplasmosis (*Toxoplasma gondii*).

cDNA and amino acid sequences of human *MMP9*

ACCESSION AK123156

VERSION AK123156.1 GI:34528630

5 **translation="**MARKGARRPRQGPGSHKWLQPGSRREKERIPQPPPPARPPRDAA
 PRRVLVPAVRRVPESGHFAGRPWAPQCHPKGLRRPSAESHVAQAGVQCHDLGSLQPP
 PPSSGDSPASASRVAGITSTVPGTLSALDDCCLITELPYKPPAVLY"**"**

1 acactttgcg ttccgcggcc ccggcccctt ggtttcctag tectggctcc attcccctct
 10 61 caggcctagg gctgggaccc ctccccgccc ccggtcttgg ccctgcccc ttcaacagac
 121 ggtccgcccc ggcccctccc cctcgctccc ccgggccctg gcaggccccg cccctgcgg
 181 cctctacctt tgacgtcttc ccccgggagg tggcgggggt ctgcgaccga atgccggcgg
 241 gactctgggt cagggcttct ggcgggccct gcggggggca gcgaggtgac cgtgaacctg
 301 cggctcatgg cgcggaaagg agccaggcgg ccgcggcaag gtccgggatc gcacaagtgg
 15 361 ctgcaaccag gctctaggag ggagaaagag cggatcccc aaccccctcc gcccgccgc
 421 ccccgcgag acgcggcgcc gcgcagggtc ctagtgcccg ctgtgcgaag ggttcctgaa
 481 tctggccact tcgctgggag gccctgggct cccagtgcc acccgaaggg cctgaggagg
 541 ccatctgcag aatctcactc tgctgcccag gccggagtgc agtgctcatga tcttggctca
 601 ctgcaacctc cgctcccag ttcaggagat tctcctgcct cagcctcccg ggtggctggg
 20 661 attacaagca cagtgcctgg cacattatcg gcacttgatg actgttgtct aataactgag
 721 cttccataca aaccacctgc cgtcctgtac tgaaggagaa agagcttcca gccggggagg
 781 caggaaatct gggctcctgg cttgggttgca tcctgactt cctaaatgac ctggagaagg
 841 cctctgcctc tgctgggatc ttgtctgtgc tggggcattt gtttccattt ccaagggctt
 901 tttcttcctc gctcagaatt tgaccactca ctaagaggag cttagtgtgg tgtctcacga
 25 961 agggatcctc ctcagccctc acctcggtac tggaagacgt cgtgcgtgtc caaaggcacc
 1021 ccggggaaca tccggtccac ctcgctggcg ctccggggat ccaccatctg cgccttcacg
 1081 tcgaacctgc gggcaggcgc ggaggagaca ggtgctgagc cggctagcgg acggaccgac
 1141 ggcgcccggg ctcccctgc cggcgccgc ggcggcgctc acctccagag gcgcgcccg
 1201 ctgaacagca gcacttccc cctgccactc cggaggggccc cggtcacctg ggccacgtcg
 30 1261 gcgcccaggc ccagcttgct cagacgcctc gggcccagca ccgacgcgcc tgtgtacacc
 1321 cacacctggc gccctgcagg ggaggagggt cacgtcgggt tgggggcgca gagggagcac
 1381 gtactcctag aacgcgagga gggagattcc ggcgaggcct ttctagccc gcgtgcccgc
 1441 agtccctgca acccaggggc agaggcgtg ggtagagcga cgcgagggcg tggagaggag
 1501 ggggcagaaa ctcagccgcc cctacgtttg ctaaactgcg tccgccaggg ggcgtatttt
 35 1561 tctaaaacgc acaagacgtt tcgtgggtta tcgatggtct cttgagcctc cttgactgat
 1621 ggggattgac cgggcggggg agggaaagta ggtaactaac cagagaagaa gaaaagcttc
 1681 ttggagagcg gctcctcaaa gaccgagtcc agcttgccgg gcagcgcggg ccacttgtcg
 1741 gcgataagga aggggccctg cggccggctc ccctgcctc cagagaatcg ccagtacttc

1801 ctgagaaagc gaggagggaa aggacgggct ctaagccttg gacacagggc cagtgggcgg
 1861 gaagggacgg gcagcccctc cgcaaagccc cctcccgcac ccacacaacc ccgcctcctc
 1921 acccatcctt gaacaaatac agctggttcc caatc

5 cDNA and amno acid sequences of mouse *MMP9*

ACCESSION NM_013599

VERSION NM_013599.2 GI:31560795

translation="MSPWQPLLLALLAFGCSSAAPYQRQPTFVVFPKDLKTSNLTDQ

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 10 PDVGRFQTFKGLKWDHNNITYWIQNYSEDLPRDMIDDAFARAFVWGEVAPLTFTRVY
 GPEADIVIQFGVAEHGDGYFPDGDGLLAHAFFPGAGVQGDHFDDELWSLGKGVVI
 PTYYGNSNGAPCHFPFTFEGRSYSACTTDGRNDGTPWCSTTADYDKDGKFGFCPSERL
 YTEHGNGEGKPCVFPFIFEGRSYSACTTKGRSDGYRWCATTANYDQDKLYGFCPTRVD
 ATVVGNSAGELCVFPFVFLGKQYSSCTSDGRRDGRWCATTSNFDTDKKWGFCPDQG
 15 YSLFLVAAHEFGHALGLDHSSVPEALMYPLYSYLEGFPLNKDDIDGIQYLYGRGSKPD
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 PGPTAPPTAGSSEASTESLSPADNPCNVDFDAIAEIQGALHFFKDGWYWKFLNHRGS
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 GPEVTHVSGLLPRRLGKALLFSKGRVWRFDLKSQKVDPQSVIRVDKEFSGVPWNSHDI
 20 FQYQDKAYFCHGKFFWRVSFQNEVNKVDHEVNQVDDVG YVTYDLLQCP"

1 ctcaccatga gtccctggca gcccctgctc ctggctctcc tggctttcgg ctgcagctct
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 121 aacctcacgg acaccagct ggcagaggca tacttgtacc gctatgggta caccggggcc
 25 181 gcccagatga tgggagagaa gcagtctcta cggccgggctt tgctgatgct tcagaagcag
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 301 cgctgtggtg tcccagacgt gggctcgattc caaaccttca aaggcctcaa gtgggaccat
 361 cataacatca catactggat ccaaaactac tctgaagact tgccgcgaga catgatcgat
 421 gacgccttcg cgcgcgctt cgcggtgtgg ggcgaggtgg caccctcac cttcaccgcg
 30 481 gtgtacggac ccgaagcgga cattgtcatc cagtttggtg tcgcggagca cggagacggg
 541 tatcccttcg acggcaagga cggccttctg gcacacgcct tccccctgg cgccggcggtt
 601 caggagatg cccatttcga cgacgacgag ttgtggctgc tgggcaaagg cgtcgtgatc
 661 cccacttact atggaaactc aaatggtgcc ccatgtcact ttccttcac cttcgaggga
 721 cgctcctatt cggcctgcac cacagacggc cgcaacgacg gcacgccttg gtgtagcaca
 35 781 acagctgact acgataagga cggcaaattt ggtttctgcc ctagtgagag actctacacg
 841 gagcacggca acggagaagg caaacctgt gtgttcccg tcatctttga gggccgctcc
 901 tactctgcct gcaccactaa aggccgctcg gatggttacc gctggtgcgc caccacagcc
 961 aactatgacc aggataaact gtatggcttc tgcctaccc gagtggacgc gaccgtagtt

1021 ggggggcaact cggcaggaga gctgtgcgtc ttccccttcg tcttcctggg caagcagtac
 1081 tcttcctgta ccagcgacgg ccgcagggat gggcgctctt ggtgtgcgac cacatcgaac
 1141 ttcgacactg acaagaagtg gggtttctgt ccagaccaag ggtacagcct gttcctggtg
 1201 gcagcgcacg agttcggcca tgcactgggc ttagatcatt ccagcgtgcc ggaagcgctc
 5 1261 atgtacccgc tgtatagcta cctcgagggc ttccctctga ataaagacga catagacggc
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 1381 actgaaccac agccgacagc acctcccact atgtgtccca ctatacctcc cacggcctat
 1441 cccacagtgg gccccacggg tggccctaca ggcgccccct cacctggccc cacaagcagc
 1501 ccgtcacctg gccctacagg cgccccctca cctggcccta cagcgcccc tactgcgggc
 10 1561 tcttctgagg cctctacaga gtctttgagt ccggcagaca atccttgcaa tgtggatggt
 1621 tttgatgcta ttgctgagat ccagggcgct ctgcatttct tcaaggacgg ttggtactgg
 1681 aagttcctga atcatagagg aagcccatta cagggccctt tccttactgc ccgcacgtgg
 1741 ccagccctgc ctgcaacgct ggactccgcc tttgaggatc cgcagaccaa gagggtttct
 1801 ttcttctctg gacgtcaaat gtgggtgtac acaggcaaga ccgtgctggg ccccaggagt
 15 1861 ctggataagt tgggtctagg ccagaggta acccacgtca gcgggcttct ccgcgctcgt
 1921 ctcggaagg ctctgctggt cagcaagggg cgtgtctgga gattcgactt gaagtctcag
 1981 aaggtggatc ccagagcgt cattcgctg gataaggagt tctctggtgt gccctggaac
 2041 tcacacgaca tcttcagta ccaagacaaa gcctatttct gccatggcaa attcttctgg
 2101 cgtgtgagtt tccaaaatga ggtgaacaag gtggaccatg aggtgaacca ggtggacgac
 20 2161 gtgggctacg tgacctacga cctcctgcag tgccttgaa ctagggtcc ttctttgctt
 2221 caaccgtgca gtgcaagtct ctagagacca ccaccaccac caccacacac aaaccccatc
 2281 cgagggaaag gtgctagctg gccaggtaca gactggtgat ctcttctaga gactgggaag
 2341 gagtggaggc aggcagggt ctctctgccc accgtccttt cttgttggac tgtttcta
 2401 aaacacggat ccccaacct ttccagctac tttagtcaat cagcttatct gtagttgcag
 25 2461 atgcatccga gcaagaagac aactttgtag ggtggattct gaccttttat ttttgtgtgg
 2521 cgtctgagaa ttgaatcagc tggcttttgt gacaggcact tcaccggcta aaccacctct
 2581 cccgactcca gcccttttat ttattatgta tgaggttatg ttcacatgca tgtatttaac
 2641 ccacagaatg ctactgtgt gtccggcgcg gctccaaccg ctgcataaat attaaggtat
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 30 2761 ccgagctatc cactcatcaa acatttattg agagcatccc tagggagcca ggctctctac
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 2881 tctgtaaat ctgctgaaac cagaccccag actcctctct ctcccagagag tccaactcac
 2941 tcaactgtgt tgctggcagc tgcagcatgc gtatacagca tgtgtgctag agaggtagag
 3001 ggggtctgtg cgttatggtt caggtcagac tgtgtcctcc aggtgagatg acccctcagc
 35 3061 tggaactgat ccaggaagga taaccaagt tcttcctggc agtctttttt aaataaatga
 3121 ataaatgaat atttacttaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa
 3181 aaaaa

//

ACCESSION NP_038627

VERSION NP_038627.1 GI:7305277

1 mspwqpllla llafgcssaa pyqrqptfvv fpkdlktsnl tdtqlaeayl yrygytraaq
 5 61 mmgekqslrp allmlqkqls lpqtgeldsq tlkairtpre gvpdvgrfqt fkgkwdhnn
 121 itywiqnyse dlprdmidda farafavwge vapltftrvy gpeadiviqf gvaehgdgyp
 181 fdgkdglah afppgagvqg dahfdddelw slgkgvvipt yygnsngapc hfpftfegrs
 241 ysacttdgrn dgtpwcstta dydkdgkfgf cpserlyteh gngegkpcvf pfifegrsys
 301 acttkgrsdg yrwcattany dqdklygfcg trvdatvvvg nsagelcvfp fvflgkqyss
 10 361 ctsdgrrdgr lwcattsndf tdkkwgfcpg qgyslflvaa hefghalgld hssvpealmy
 421 plysylegfp lnkddidgiq ylygrgskpd prppatttte poptapptmc ptipptaypt
 481 vgptvgptga pspgptssps pgptgapspg ptapptagss easteslspa dnpcnvdvfd
 541 aiaeiqgalh ffdkgwywkf lnhrsgplqg pflrtartwpa lpatldsafv dpqtkrvfff
 601 sgrqmwyvtg ktvlgrpsld klglgpevth vsgllprrlg kallfskgrv wrfdlksqkv
 15 661 dpqsvirvdk efsgvpwnsh difqyqdkay fchgkffwrv sfqnevnkvd hevngvddvg
 721 yvtydllqcp
 //

Table 1. MMP-9 orthologs from nine species

Organism	Gene	Locus	Description	Human Similarity	s
dog (Canis familiaris)	MMP9 ¹	--	matrix metalloproteinase 9 (gelatinase B, 92kDa gelatinase)	85.46(n) 80.97(a)	<u>403885</u> <u>NM_001003219.1</u> <u>NP_001003219.1</u>
rat (Rattus norvegicus)	Mmp9 ¹	--	matrix metalloproteinase 9	79.15(n) 74.89(a)	<u>81687</u> <u>NM_031055.1</u> <u>NP_112317.1</u>
mouse (Mus musculus)	Mmp9 ^{1,4}	<u>2 (96.00 cM)</u> ⁴	matrix metalloproteinase 9 ^{1,4}	78.69(n) ¹ 75(a) ¹	<u>17395</u> ¹ <u>NM_013599.2</u> ¹ <u>NP_038627.1</u> ¹ <u>AK004651</u> ⁴ <u>AK142787</u> ⁴ (see all 16)
chicken (Gallus gallus)	LOC395387 ¹	--	matrix metalloproteinase 9 (gelatinase B, 92kDa gelatinase)	66.96(n) 62.54(a)	<u>395387</u> <u>NM_204667.1</u> <u>NP_989998.1</u>
zebrafish (Danio rerio)	wufb02g06 ^{1~}	--	Danio rerio cDNA clone MGC64165	70.96(n)	<u>BC053292.1</u>

			IMAGE6797338, complete		
African clawed frog (Xenopus laevis)	MGC69080 ¹ ~	--	hypothetical protein MGC69080	72.25(n)	<u>BC057745.1</u>
rainbow trout (Oncorhynch us mykiss)	Omy.10476 ¹ ~	--	Oncorhynchus mykiss mRNA for matrix metalloproteinase	74.67(n)	<u>AJ320533.1</u>
thale cress (Arabidopsis thaliana)	MMP ¹	--	MMP (MATRIX METALLOPROTEINASE); metalloendopeptidase/	53(n) 46.85(a)	<u>843353</u> <u>NM 1056</u> <u>85.3</u> <u>NP 177174.</u> <u>1</u>
rice (Oryza sativa)	P0516G10.1 8 ¹	--	putative zinc metalloproteinase	51.98(n) 41.81(a)	<u>3063368</u> <u>XM 467</u> <u>714.1</u> <u>XP 467714</u> <u>.1</u>

Domains of MMP-9. MMP-9 belongs to the peptidase M10A family. MMP-9 consists of five domains; the amino-terminal and zinc-binding domains shared by all members of the secreted metalloprotease gene family, the collagen-binding fibronectin-like domain also present in the 72-kDa type IV collagenase, a carboxyl-terminal hemopexin-like domain shared by all known enzymes of this family with the exception of PUMP-1, and a unique 54-amino-acid- long proline-rich domain homologous to the alpha 2 chain of type V collagen (Wilhelm et al. (1989) *J. Biol. Chem.* **264**, 17213-17221) (**Table 2**).

Table 2. MMP-9 domains

FT	SIGNAL	<u>1</u>	<u>19</u>	
FT	PROPEP	<u>20</u>	<u>93</u>	Activation peptide.
FT	CHAIN	<u>94</u>	<u>?</u>	67 kDa matrix metalloproteinase-9.
FT	CHAIN	<u>107</u>	<u>707</u>	82 kDa matrix metalloproteinase-9.
15	FT	<u>?</u>	<u>707</u>	Removed in 64 kDa matrix
	FT			metalloproteinase-9 and 67 kDa matrix
	FT			metalloproteinase-9.
	FT	<u>225</u>	<u>273</u>	Fibronectin type-II 1.
	FT	<u>283</u>	<u>331</u>	Fibronectin type-II 2.
20	FT	<u>342</u>	<u>390</u>	Fibronectin type-II 3.
	FT	<u>513</u>	<u>707</u>	Hemopexin-like.

	FT	ACT_SITE	402	402	
	FT	METAL	131	131	Calcium 1.
	FT	METAL	165	165	Calcium 2 (via carbonyl oxygen).
	FT	METAL	175	175	Zinc 1 (structural).
5	FT	METAL	177	177	Zinc 1 (structural).
	FT	METAL	182	182	Calcium 3.
	FT	METAL	183	183	Calcium 3 (via carbonyl oxygen).
	FT	METAL	185	185	Calcium 3 (via carbonyl oxygen).
	FT	METAL	187	187	Calcium 3 (via carbonyl oxygen).
10	FT	METAL	190	190	Zinc 1 (structural).
	FT	METAL	197	197	Calcium 2 (via carbonyl oxygen).
	FT	METAL	199	199	Calcium 2 (via carbonyl oxygen).
	FT	METAL	201	201	Calcium 2.
	FT	METAL	203	203	Zinc 1 (structural).
15	FT	METAL	205	205	Calcium 3.
	FT	METAL	206	206	Calcium 1.
	FT	METAL	208	208	Calcium 1.
	FT	METAL	208	208	Calcium 3.
	FT	METAL	401	401	Zinc 2 (catalytic).
20	FT	METAL	405	405	Zinc 2 (catalytic).
	FT	METAL	411	411	Zinc 2 (catalytic).
	FT	SITE	59	60	Cleavage (by MMP3).
	FT	SITE	99	99	Cysteine switch (By similarity).
	FT	SITE	106	107	Cleavage (by MMP3).
25	FT	CARBOHYD	38	38	N-linked (GlcNAc...) (Potential).
	FT	CARBOHYD	120	120	N-linked (GlcNAc...) (Potential).
	FT	CARBOHYD	127	127	N-linked (GlcNAc...) (Potential).
	FT	DISULFID	230	256	By similarity.
	FT	DISULFID	244	271	By similarity.
30	FT	DISULFID	288	314	By similarity.
	FT	DISULFID	302	329	By similarity.
	FT	DISULFID	347	373	By similarity.
	FT	DISULFID	361	388	By similarity.
	FT	DISULFID	516	704	
35	FT	VARIANT	20	20	A -> V (in dbSNP:rs1805088).
	FT	VARIANT	82	82	E -> K (in dbSNP:rs1805089).
	FT	VARIANT	127	127	N -> K (in dbSNP:rs3918252).
	FT	VARIANT	239	239	R -> H.
	FT	VARIANT	279	279	R -> Q (common polymorphism;
40	FT				dbSNP:rs17576).
	FT	VARIANT	571	571	F -> V.
	FT	VARIANT	574	574	P -> R (in dbSNP:rs2250889).
	FT	VARIANT	668	668	R -> Q (in dbSNP:rs17577).
	FT	TURN	32	33	
45	FT	HELIX	41	51	
	FT	TURN	52	53	
	FT	HELIX	68	78	
	FT	TURN	79	79	
	FT	HELIX	88	94	
50	FT	TURN	95	95	
	FT	STRAND	103	105	
	FT	STRAND	119	125	
	FT	STRAND	130	132	
	FT	HELIX	134	149	
55	FT	TURN	150	150	
	FT	STRAND	151	153	
	FT	STRAND	155	158	

WO 2009/111450

PCT/US2009/035840

	FT	TURN	162	163
	FT	STRAND	164	171
	FT	STRAND	176	178
	FT	STRAND	183	186
5	FT	STRAND	189	191
	FT	STRAND	194	196
	FT	TURN	197	200
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	FT	TURN	206	207
10	FT	STRAND	213	219
	FT	HELIX	220	231
	FT	TURN	232	233
	FT	TURN	240	241
	FT	TURN	243	244
15	FT	STRAND	245	247
	FT	STRAND	255	261
	FT	HELIX	262	265
	FT	STRAND	268	270
	FT	TURN	274	276
20	FT	STRAND	279	283
	FT	TURN	284	285
	FT	STRAND	290	294
	FT	TURN	295	296
	FT	STRAND	297	301
25	FT	TURN	305	306
	FT	STRAND	313	319
	FT	HELIX	320	323
	FT	STRAND	326	328
	FT	HELIX	333	335
30	FT	TURN	340	344
	FT	STRAND	349	353
	FT	TURN	354	355
	FT	STRAND	356	358
	FT	TURN	364	365
35	FT	STRAND	372	378
	FT	HELIX	379	382
	FT	STRAND	385	387
	FT	HELIX	395	406
	FT	TURN	407	408
40	FT	TURN	415	416
	FT	TURN	418	419
	FT	HELIX	433	442
	FT	STRAND	512	517
	FT	HELIX	515	517
45	FT	STRAND	522	527
	FT	TURN	528	529
	FT	STRAND	530	535
	FT	TURN	536	537
	FT	STRAND	538	542
50	FT	STRAND	545	547
	FT	STRAND	551	555
	FT	HELIX	556	559
	FT	TURN	561	562
	FT	STRAND	568	572
55	FT	TURN	574	576
	FT	STRAND	579	583
	FT	TURN	584	585

	FT	STRAND	586	591
	FT	TURN	592	593
	FT	STRAND	594	600
	FT	HELIX	601	604
5	FT	TURN	605	605
	FT	TURN	608	609
	FT	STRAND	615	618
	FT	TURN	621	622
	FT	STRAND	623	628
10	FT	TURN	629	630
	FT	STRAND	631	636
	FT	TURN	637	640
	FT	HELIX	644	646
	FT	HELIX	650	653
15	FT	TURN	655	656
	FT	STRAND	662	667
	FT	TURN	668	669
	FT	STRAND	670	675
	FT	TURN	676	677
20	FT	STRAND	678	683
	FT	TURN	686	687
	FT	STRAND	690	696
	FT	TURN	697	700
	FT	TURN	702	703
25				

Factors that regulate MMP-9. The catalytic activity of MMP-9 is inhibited by histatin-3 1/24 (histatin-5). MMP-9 is activated by urokinase-type plasminogen activator; plasminogen; IL-1beta, 4-aminophenylmercuric acetate and phorbol ester. MMP-9 exists as monomer, disulfide-linked homodimer, and as a heterodimer with a 25 kDa protein. Macrophages and transformed cell lines produce only the monomeric MMP-9, the heterodimeric form is produced by normal alveolar macrophages and granulocytes. The processing of the precursor yields different active forms of 64, 67 and 82 kDa. Sequentially processing by MMP-3 yields the 82 kDa matrix metalloproteinase-9. In arthritis patients, this enzyme can contribute to the pathogenesis of joint destruction and can be a useful marker of disease status.

Endogenous inhibitors of MMP-9. MMP-9 has a number of endogenous inhibitors. Like other MMPs, MMP-9 is inhibited by TIMPs (Murphy, G., and Willenbrock, F. (1995) *Methods Enzymol.* **248**, 496-510). A characteristic of MMP-9 (and MMP-2) is the ability of their zymogens to form tight non-covalent and stable complexes with TIMPs. It has been shown that pro-MMP-2 binds TIMP-2 (Goldberg *et al.* (1989) *Proc. Natl. Acad. Sci. U. S. A.* **86**, 8207-8211), whereas pro-MMP-9 binds TIMP-1 (Wilhelm *et al.* (1989) *J. Biol. Chem.* **264**, 17213-17221). TIMPs typically are slow, tight binding inhibitors. A MMP-9 binding protein (e.g.,

antibody) selected from a library of phage-displayed proteins can be selected have more rapid kinetics. For example, recombinant TIMP-1 can be administered to inhibit MMP-9, e.g., in combination with a MMP-9 binding protein described herein.

5 Small molecule inhibitors of MMP-9. Skiles *et al.* (2004, Curr Med Chem, **11**:2911-77) reported that first generation small-molecule MMP inhibitors had poor bioavailability and the second generation had caused musculoskeletal pain and inflammation. Most small-molecule MMP inhibitors interact with the catalytic zinc but have fairly low affinity. Thus, a higher concentration is needed to have effect. The interaction with the catalytic zinc leads to inhibition
10 of other MMPs and toxic side effects. A MMP-9 binding protein described herein can be used in combination with a small molecule inhibitor. For example, because the inhibitors are used in combination, the dose of the small molecule used can be decreased and therefore result in fewer side effects. Examples of small molecule MMP-9 inhibitors include small synthetic anthranilic acid-based inhibitors (see, e.g., Calbiochem Inhibitor-I, catalogue #444278 and Levin et al.,
15 2001, *Bioorg. Med. Chem. Lett.* **11**:2975-2978).

Small interfering RNA inhibitors of MMP-9. MMP-9 can be inhibited by small interfering RNA (siRNA). Examples of siRNA that can be used include:

20 MMP-9 siRNA
5'- GACUUGCCGCGAGACAUGAtt -3'
3'- ttCUGAACGGCGCUCUGUACU -5'

25 Control RNA (mismatch)
5'- GACUUCGCGGGACACAUGAtt -3'
3'- ttCUGAAGCGCCCUGUGUACU -5'

See also Kawasaki et al., Feb. 10, 2008, *Nat. Med.* advance on-line publication doi:10.1038/nm1723. The siRNA can be administered to inhibit MMP-9, e.g., in combination with a MMP-9 binding protein described herein.

30

Drug Conjugates

The MMP-9 binding proteins described herein can be conjugated to a drug (e.g., a cytotoxic, cytostatic, or immunomodulatory agent). The conjugates can be used therapeutically

or prophylactically, e.g., the binding protein can target the drug, e.g., *in vivo*, e.g., to a site of disease (e.g., a tumor or site of inflammation), e.g., such that the drug affects the site of disease (e.g., causes a cytostatic or cytotoxic effect on targeted cells).

In some embodiments, the binding protein itself has therapeutic or prophylactic efficacy (e.g., the protein can modulate (e.g., antagonize) MMP-9, or cause a cytostatic or cytotoxic effect on a cell that expresses MMP-9 (e.g., an endothelial cell or tumor cell)). The binding protein-drug conjugate can be used such that the binding protein and drug both contribute (e.g., additively or synergistically) to an effect on MMP-9 (e.g., a therapeutic effect, e.g., *in vivo*, e.g., to a site of disease (e.g., a tumor or site of undesired angiogenesis or vascularization)). The drug and/or binding protein can be, for example, cytotoxic, cytostatic or otherwise prevent or reduce the ability of a targeted cell to divide and/or survive (e.g., when the drug is taken up or internalized by the targeted cell and/or upon binding of the binding protein to MMP-9). For example, if the targeted cell is a cancer cell, the drug and/or binding protein can prevent or reduce the ability of the cell to divide and/or metastasize.

Useful classes of drugs that can be used in the binding protein-drug conjugates described herein include cytotoxic or immunomodulatory agents such as, for example, antitubulin agents, auristatins, DNA minor groove binders, DNA replication inhibitors, alkylating agents (e.g., platinum complexes such as cis-platin, mono(platinum), bis(platinum) and tri-nuclear platinum complexes and carboplatin), anthracyclines, antibiotics, antifolates, antimetabolites, chemotherapy sensitizers, duocarmycins, etoposides, fluorinated pyrimidines, ionophores, lexitropsins, nitrosoureas, platinols, pre-forming compounds, purine antimetabolites, puromycins, radiation sensitizers, steroids, taxanes, topoisomerase inhibitors, vinca alkaloids, or the like.

Individual cytotoxic or immunomodulatory agents include, for example, an androgen, anthramycin (AMC), asparaginase, 5-azacytidine, azathioprine, bleomycin, busulfan, buthionine sulfoximine, camptothecin, carboplatin, carmustine (BSNU), CC-1065, chlorambucil, cisplatin, colchicine, cyclophosphamide, cytarabine, cytidine arabinoside, cytochalasin B, dacarbazine, dactinomycin (actinomycin), daunorubicin, decarbazine, docetaxel, doxorubicin, an estrogen, 5-fluorodeoxyuridine, 5-fluorouracil, gramicidin D, hydroxyurea, idarubicin, ifosfamide, irinotecan, lomustine (CCNU), mechlorethamine, melphalan, 6-mercaptopurine, methotrexate, mithramycin, mitomycin C, mitoxantrone, nitroimidazole, paclitaxel, plicamycin, procarbazine,

rapamycin (Sirolimus), streptozotocin, tenoposide, 6-thioguanine, thioTEPA, topotecan, vinblastine, vincristine, vinorelbine, VP-16 and VM-26.

In some typical embodiments, the drug comprises a cytotoxic agent. Suitable cytotoxic agents include, for example, dolastatins (e.g., auristatin E, AFP, MMAF, MMAE), DNA minor groove binders (e.g., enediynes and lexitropsins), duocarmycins, taxanes (e.g., paclitaxel and docetaxel), puromycins, vinca alkaloids, CC-1065, SN-38, topotecan, morpholino-doxorubicin, rhizoxin, cyanomorpholino-doxorubicin, echinomycin, combretastatin, netropsin, epothilone A and B, estramustine, cryptophysins, cemadotin, maytansinoids, discodermolide, eleutherobin, and mitoxantrone.

In some embodiments, the drug is a cytotoxic agent such as AFP, MMAF, MMAE, AEB, AEVB, auristatin E, paclitaxel, docetaxel, CC-1065, SN-38, topotecan, morpholino-doxorubicin, rhizoxin, cyanomorpholino-doxorubicin, dolastatin-10, echinomycin, combretastatin, calicheamicin, maytansine, DM-1, or netropsin.

In some embodiments, the drug is a cytotoxic agent that comprises a conventional chemotherapeutic such as, for example, doxorubicin, paclitaxel, melphalan, vinca alkaloids, methotrexate, mitomycin C or etoposide. In some embodiments, the drug can be a combined therapy, such as CHOP (Cyclophosphamide, Doxorubicin, Prednisolone and Vincristine), CHOP-R (Cyclophosphamide, Doxorubicin Vincristine, Prednisolone, and rituximab) or ABVD (Doxorubicin, Bleomycin, Vinblastine and Dacarbazine). Agents such as CC-1065 analogues (e.g., DC1), calicheamicin, maytansine, analogues of dolastatin 10, rhizoxin, and palytoxin can also be used.

In specific embodiments, the drug can be a cytotoxic or cytostatic agent that comprises auristatin E (also known in the art as dolastatin-10) or a derivative thereof. Typically, the auristatin E derivative is, e.g., an ester formed between auristatin E and a keto acid. For example, auristatin E can be reacted with paraacetyl benzoic acid or benzoylvaleric acid to produce AEB and AEVB, respectively. Other auristatin derivatives include AFP, MMAF, and MMAE. The synthesis and structure of auristatin E and its derivatives are described in US 20030083263 and US 20050009751, and U.S. Pat. Nos. 6,323,315; 6,239,104; 6,034,065; 5,780,588; 5,665,860; 5,663,149; 5,635,483; 5,599,902; 5,554,725; 5,530,097; 5,521,284; 5,504,191; 5,410,024; 5,138,036; 5,076,973; 4,986,988; 4,978,744; 4,879,278; 4,816,444; and 4,486,414. In some preferred embodiments, MMAF or AFP is used.

In specific embodiments, the drug is a cytotoxic agent that comprises a DNA minor groove binding agent. See, e.g., U.S. Pat. No. 6,130,237. For example, in some embodiments, the minor groove binding agent is a CBI compound. In other embodiments, the minor groove binding agent is an enediyne (e.g., calicheamicin).

5 Examples of anti-tubulin agents that can be used in the MMP-9 binding protein-drug conjugates include, but are not limited to, taxanes (e.g., TAXOL® (paclitaxel), TAXOTERE® (docetaxel)), T67 (Tularik), vinca alkyls (e.g., vincristine, vinblastine, vindesine, and vinorelbine), and dolastatins (e.g., auristatin E, AFP, MMAF, MMAE, AEB, AEVB). Other antitubulin agents include, for example, baccatin derivatives, taxane analogs (e.g., epothilone A
10 and B), nocodazole, colchicine and colcimid, estramustine, cryptophysins, cemadotin, maytansinoids, combretastatins, discodermolide, eleutherobin, rhizoxin/maytansine, auristatin dolastatin 10 MMAE, and peloruside A.

In some embodiments, the drug is a cytotoxic agent such as an anti-tubulin agent. In some embodiments, the anti-tubulin agent is an auristatin, a vinca alkaloid, a podophyllotoxin, a
15 taxane, a baccatin derivative, a cryptophysin, a maytansinoid, a combretastatin, or a dolastatin. In some embodiments, the antitubulin agent is AFP, MMAP, MMAE, AEB, AEVB, auristatin E, vincristine, vinblastine, vindesine, vinorelbine, VP-16, camptothecin, paclitaxel, docetaxel, epothilone A, epothilone B, nocodazole, colchicines, colcimid, estramustine, cemadotin, discodermolide, maytansine, DM1, DM2, DM3, DM4, or eleutherobin.

20 In some embodiments, the cytotoxic agent comprises a maytansinoid, another group of anti-tubulin agents. For example, in specific embodiments, the maytansinoid is maytansine or DM-1 (ImmunoGen, Inc.; see also Chari et al. *Cancer Res.* 52:127-131 (1992)). In some embodiments, sterically hindered thiol and disulfide-containing maytansinoids in which the alpha-carbon atom bearing the sulfur atom bears one or two alkyl substituents are used in the
25 binding protein-drug conjugate, e.g., US 2007-0292422; US 2007-0264266.

In some embodiments, the drug comprises an agent that acts to disrupt DNA. The drug may be selected from enediynes (e.g., calicheamicin and esperamicin) and non-enediyne small molecule agents (e.g., bleomycin, methidiumpropyl-EDTA-Fe(II)). Other useful drugs include daunorubicin, doxorubicin, distamycin A, cisplatin, mitomycin C, ecteinascidins,
30 duocarmycin/CC-1065, and bleomycin/pepleomycin.

In other embodiments, the drug can comprise an alkylating agent such as Asaley NSC 167780, AZQ NSC 182986, BCNU NSC 409962, Busulfan NSC 750, carboxyphthalatoplatinum NSC 271674, CBDCA NSC 241240, CCNU NSC 79037, CHIP NSC 256927, chlorambucil NSC 3088, chlorozotocin NSC 178248, cis-platinum NSC 119875, clomesone NSC 338947, 5 cyanomorpholinodoxorubicin NSC 357704, cyclodisone NSC 348948, dianhydrogalactitol NSC 132313, fluorodopan NSC 73754, hepsulfam NSC 329680, hycanthone NSC 142982, melphalan NSC 8806, methyl CCNU NSC 95441, mitomycin C NSC 26980, mitozolamide NSC 353451, nitrogen mustard NSC 762, PCNU NSC 95466, piperazine NSC 344007, piperazinedione NSC 135758, pipobroman NSC 25154, porfiromycin NSC 56410, spirohydantoin mustard NSC 10 172112, teroxirone NSC 296934, tetraplatin NSC 363812, thio-tepa NSC 6396, triethylenemelamine NSC 9706, uracil nitrogen mustard NSC 34462, or Yoshi-864 NSC 102627.

In some embodiments, the drug can comprise an antimetabolic agent such as allocolchicine NSC 406042, Halichondrin B NSC 609395, colchicine NSC 757, colchicine derivative NSC 33410, dolastatin 10 NSC 376128 (NG--auristatin derived), maytansine NSC 153858, rhizoxin 15 NSC 332598, taxol NSC 125973, taxol derivative NSC 608832, thiocolchicine NSC 361792, trityl cysteine NSC 83265, vinblastine sulfate NSC 49842, or vincristine sulfate NSC 67574.

In other embodiments, the drug can comprise an topoisomerase I inhibitor such as camptothecin NSC 94600, camptothecin, Na salt NSC 100880, aminocamptothecin NSC 603071, camptothecin derivative NSC 95382, camptothecin derivative NSC 107124, 20 camptothecin derivative NSC 643833, camptothecin derivative NSC 629971, camptothecin derivative NSC 295500, camptothecin derivative NSC 249910, camptothecin derivative NSC 606985, camptothecin derivative NSC 374028, camptothecin derivative NSC 176323, camptothecin derivative NSC 295501, camptothecin derivative NSC 606172, camptothecin derivative NSC 606173, camptothecin derivative NSC 610458, camptothecin derivative NSC 25 618939, camptothecin derivative NSC 610457, camptothecin derivative NSC 610459, camptothecin derivative NSC 606499, camptothecin derivative NSC 610456, camptothecin derivative NSC 364830, camptothecin derivative NSC 606497, or morpholinodoxorubicin NSC 354646.

In other embodiments, the drug can comprise an topoisomerase II inhibitor such as 30 doxorubicin NSC 123127, amonafide NSC 308847, m-AMSA NSC 249992, anthrapyrazole derivative NSC 355644, pyrazoloacridine NSC 366140, bisantrene HCL NSC 337766,

daunorubicin NSC 82151, deoxydoxorubicin NSC 267469, mitoxantrone NSC 301739, menogaril NSC 269148, N,N-dibenzyl daunomycin NSC 268242, oxanthrazole NSC 349174, rubidazole NSC 164011, VM-26 NSC 122819, or VP-16 NSC 141540.

In other embodiments, the drug can comprise an RNA or DNA antimetabolite such as L-
 5 alanosine NSC 153353, 5-azacytidine NSC 102816, 5-fluorouracil NSC 19893, acivicin NSC 163501, aminopterin derivative NSC 132483, aminopterin derivative NSC 184692, aminopterin derivative NSC 134033, an antifol NSC 633713, an antifol NSC 623017, Baker's soluble antifol NSC 139105, dichlorallyl lawsone NSC 126771, brequinar NSC 368390, ftorafur (pro-drug) NSC 148958, 5,6-dihydro-5-azacytidine NSC 264880, methotrexate NSC 740, methotrexate
 10 derivative NSC 174121, N-(phosphonoacetyl)-L-aspartate (PALA) NSC 224131, pyrazofurin NSC 143095, trimetrexate NSC 352122, 3-HP NSC 95678, 2'-deoxy-5-fluorouridine NSC 27640, 5-HP NSC 107392, alpha-TGDR NSC 71851, aphidicolin glycinate NSC 303812, ara-C NSC 63878, 5-aza-2'-deoxycytidine NSC 127716, beta-TGDR NSC 71261, cyclocytidine NSC 145668, guanazole NSC 1895, hydroxyurea NSC 32065, inosine glycodialdehyde NSC 118994,
 15 macbecin 11 NSC 330500, pyrazoloimidazole NSC 51143, thioguanine NSC 752, or thiopurine NSC 755. See also US 2007-0292441.

The abbreviation "AFP" refers to dimethylvaline-valine-dolaisoleuine-dolaproine-phenylalanine-p-phenylenediamine (e.g., see Formula XVI in US 2006-0233794).

The abbreviation "MAE" refers to monomethyl auristatin E (see Formula XI in US 2006-
 20 0233794).

The abbreviation "AEB" refers to an ester produced by reacting auristatin E with paraacetyl benzoic acid (e.g., see Formula XX in US 2006-0233794)

The abbreviation "AEVB" refers to an ester produced by reacting auristatin E with benzoylvaleric acid (e.g., see Formula XXI in US 2006-0233794).

25 The abbreviation "MMAF" refers to dovaline-valine-dolaisoleunine-dolaproine-phenylalanine (e.g., see Formula IVIV in US 2006-0233794).

The abbreviations "fk" and "phe-lys" refer to the linker phenylalanine-lysine.

The abbreviations "vc" and "val-cit" refer to the linker valine-citrulline.

In some embodiments, the drug is a cytotoxic agent selected from the group consisting of
 30 an auristatin, a DNA minor groove binding agent, a DNA minor groove alkylating agent, an

enediynes, a lexitropsin, a duocarmycin, a taxane, a puromycin, a dolastatin, a maytansinoid, and a vinca alkaloid.

In some embodiments, the drug is a cytotoxic agent such as AFP or MMAF.

5 In some embodiments, the drug is an immunosuppressive agent such as gancyclovir, etanercept, cyclosporine, tacrolimus, rapamycin, cyclophosphamide, azathioprine, mycophenolate mofetil, methotrexate, cortisol, aldosterone, dexamethasone, a cyclooxygenase inhibitor, a 5-lipoxygenase inhibitor, or a leukotriene receptor antagonist.

See generally US 2007-0292441; US 2007-0292422; US 2007-0264266; and US 2006-0233794.

10

Linkers

The binding proteins described herein can be associated with a drug to form a binding protein-drug conjugate by being linked to the drug directly. In some embodiments, the binding protein is directly conjugated to the drug. Alternatively, the binding proteins described herein
15 can be associated with a drug to form a binding protein-drug conjugate by use of a linker region between the drug and the binding protein. In some embodiments, the binding protein is conjugated to the drug via a linker. The linker can be cleavable under intracellular conditions, e.g., such that cleavage of the linker releases the drug from the binding protein in the intracellular environment. In some embodiments, the cleavable linker is a peptide linker
20 cleavable by an intracellular protease. In some embodiments, the peptide linker is a dipeptide linker.

In some embodiments, the dipeptide linker is a val-cit (vc) linker or a phe-lys (fk) linker. In some embodiments, the cleavable linker is hydrolyzable at a pH of less than 5.5. In some
25 embodiments, the hydrolyzable linker is a hydrazone linker. In some embodiments, the cleavable linker is a disulfide linker.

For example, in some embodiments, the linker is cleavable by a cleaving agent that is present in the intracellular environment (e.g., within a lysosome or endosome or caveolea). The linker can be, e.g., a peptidyl linker that is cleaved by an intracellular peptidase or protease enzyme, including, but not limited to, a lysosomal or endosomal protease. In some
30 embodiments, the peptidyl linker is at least two amino acids long or at least three amino acids long. Cleaving agents can include cathepsins B and D and plasmin, which are known to

hydrolyze dipeptide drug derivatives resulting in the release of active drug inside target cells (see, e.g., Dubowchik and Walker *Pharm. Therapeutics* 83:67-123 (1999)). In some embodiments, peptidyl linkers are cleavable by enzymes that are present in targeted cells (e.g., cancer cells). For example, a peptidyl linker that is cleavable by the thiol-dependent protease cathepsin-B, which is highly expressed in cancerous tissue, can be used (e.g., a Phe-Leu or a Gly-Phe-Leu-Gly linker). Other such linkers are described, e.g., in U.S. Pat. No. 6,214,345. In some embodiments, the peptidyl linker cleavable by an intracellular protease is a Val-Cit (vc) linker or a Phe-Lys linker (fk) (see, e.g., U.S. Pat. No. 6,214,345, which describes the synthesis of doxorubicin with the val-cit linker). One advantage of using intracellular proteolytic release of the drug is that the drug can be attenuated when conjugated and the serum stabilities of the conjugates are typically high.

In some preferred embodiments, a vc linker is used in the binding protein-drug conjugates described herein. For example, a binding protein-vcAFP or a binding protein-vcMMAF conjugate (e.g., a MMP-9 binding protein-vcAFP or a MMP-9 binding protein-vcMMAF conjugate) is prepared.

In other embodiments, the cleavable linker is pH-sensitive, i.e., sensitive to hydrolysis at certain pH values. For example, the pH-sensitive linker is hydrolyzable under acidic conditions. For example, an acid-labile linker that is hydrolyzable in the lysosome (e.g., a hydrazone, semicarbazone, thiosemicarbazone, cis-aconitic amide, orthoester, acetal., ketal., or the like) can be used. See, e.g., U.S. Pat. Nos. 5,122,368; 5,824,805; 5,622,929; Dubowchik and Walker *Pharm. Therapeutics* 83:67-123 (1999); Neville et al. *Biol. Chem.* 264:14653-14661 (1989). Such linkers are relatively stable under neutral pH conditions, such as those in the blood, but are unstable at below pH 5.5 or 5.0, the approximate pH of the lysosome. In certain embodiments, the hydrolyzable linker is a thioether linker (such as, e.g., a thioether attached to the therapeutic agent via an acylhydrazone bond (see, e.g., U.S. Pat. No. 5,622,929)).

In yet other embodiments, the linker is cleavable under reducing conditions (e.g., a disulfide linker). A variety of disulfide linkers are known in the art, including, for example, those that can be formed using SATA (N-succinimidyl-S-acetylthioacetate), SPDP (N-succinimidyl-3-(2-pyridyldithio)propionate), SPDB (N-succinimidyl-3-(2-pyridyldithio)butyrate) and SMPT (N-succinimidyl-oxycarbonyl-alpha-methyl-alpha-(2-pyridyl-dithio)toluene)-, SPDB and SMPT (See, e.g., Thorpe et al. *Cancer Res.* 47:5924-5931 (1987); Wawrzynczak et al., In

Immunoconjugates: Antibody Conjugates in Radioimagers and Therapy of Cancer (C. W. Vogel ed., Oxford U. Press, 1987). See also U.S. Pat. No. 4,880,935.

In yet other embodiments, the linker is a malonate linker (Johnson et al. *Anticancer Res.* 15:1387-93 (1995)), a maleimidobenzoyl linker (Lau et al. *Bioorg-Med-Chem.* 3(10):1299-1304 (1995), or a 3'-N-amide analog (Lau et al. *Bioorg-Med-Chem.* 3(10):1305-12 (1995)).

In some embodiments, the linker is not substantially sensitive to the extracellular environment. As used herein, "not substantially sensitive to the extracellular environment," in the context of a linker, means that no more than about 20%, typically no more than about 15%, more typically no more than about 10%, and even more typically no more than about 5%, no more than about 3%, or no more than about 1% of the linkers, in a sample of a binding protein-drug conjugate, are cleaved when the binding protein-drug conjugate is present in an extracellular environment (e.g., in plasma). Whether a linker is not substantially sensitive to the extracellular environment can be determined, for example, by incubating independently with plasma both (a) the binding protein-drug conjugate (the "conjugate sample") and (b) an equal molar amount of unconjugated binding protein or drug (the "control sample") for a predetermined time period (e.g., 2, 4, 8, 16, or 24 hours) and then comparing the amount of unconjugated binding protein or drug present in the conjugate sample with that present in control sample, as measured, for example, by high performance liquid chromatography.

In other, non-mutually exclusive embodiments, the linker promotes cellular internalization. In certain embodiments, the linker promotes cellular internalization when conjugated to the drug (i.e., in the milieu of the linker-drug moiety of the binding protein-drug conjugate described herein). In yet other embodiments, the linker promotes cellular internalization when conjugated to both the drug and the binding protein.

A variety of linkers that can be used with the present compositions and methods are described in WO 2004010957.

In some embodiments, the binding protein-drug conjugates described herein are used therapeutically in the treatment of a disorder (e.g., cancer or inflammation). In certain embodiments, it is desirable to only target a binding protein-drug conjugate to a cell that expresses the target to which the binding protein binds (e.g., to only target a MMP-9 expressing cell to which a MMP-9 binding protein binds, and not target a nearby "bystander" cell), e.g., to minimize toxicity. In other embodiments, it is desirable to target a binding protein-drug

conjugate to a cell expressing the target to which the binding protein binds and also to bystander cells (e.g., to elicit a “bystander effect”). In some embodiments, a binding protein-drug conjugate (e.g., a MMP-9 binding protein-drug conjugate can be engineered to exert a precise killing of only antigen-presenting cells without damaging proximal antigen-negative tissues, e.g.,
5 by preparing thioether-linked conjugates. Alternatively, it can be engineered to produce a bystander effect, e.g., by preparing disulfide-linked conjugates.

For example, many solid tumors express targets (e.g., antigens) in a heterogeneous fashion and are populated with both target-positive and target-negative cells. The bystander cytotoxicity associated with disulfide linker-containing conjugates provides a rationale for
10 treatment of sites of a disorder (e.g., tumors) with binding protein-drug conjugates even if the sites exhibit heterogeneous target expression. The bystander effect adds a degree of nonselective killing activity. Potentially, this could be a drawback if normal cells in tissues surrounding the site of disorder (e.g., tumor) are affected. However, as a potential advantage, the bystander cytotoxicity may damage tissues intricately involved in supporting the disorder, such as
15 endothelial cells and pericytes of tumor neovasculature, or tumor stromal cells, resulting, for example, in enhanced antitumor activity of the binding protein-drug conjugate against tumors expressing the antigen either homogeneously or heterogeneously. See also Kovtun et al. *Cancer Res.* 66:3214 (2006).

Techniques for conjugating therapeutic agents to proteins (such as binding proteins, e.g.,
20 MMP-9 binding proteins) are known. See, e.g., Arnon et al., “Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy,” in *Monoclonal Antibodies And Cancer Therapy* (Reisfeld et al eds., Alan R. Liss, Inc., 1985); Hellstrom et al., “Antibodies For Drug Delivery,” in *Controlled Drug Delivery* (Robinson et al. eds., Marcel Dekker, Inc., 2nd ed. 1987); Thorpe, “Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review,” in *Monoclonal*
25 *Antibodies '84: Biological And Clinical Applications* (Pinchera et al. eds., 1985); “Analysis, Results, and Future Prospective of the Therapeutic Use of Radiolabeled Antibody In Cancer Therapy,” in *Monoclonal Antibodies For Cancer Detection And Therapy* (Baldwin et al. eds., Academic Press, 1985); and Thorpe et al. *Immunol. Rev.* 62:119-58 (1982). See also, e.g., US 2006-0233794 and PCT publication WO 89/12624.

Display Libraries

A display library is a collection of entities; each entity includes an accessible polypeptide component and a recoverable component that encodes or identifies the polypeptide component. The polypeptide component is varied so that different amino acid sequences are represented.

5 The polypeptide component can be of any length, e.g. from three amino acids to over 300 amino acids. A display library entity can include more than one polypeptide component, for example, the two polypeptide chains of an sFab. In one exemplary implementation, a display library can be used to identify proteins that bind to MMP-9. In a selection, the polypeptide component of each member of the library is probed with MMP-9 (e.g., the catalytic domain of MMP-9 or other
10 fragment) and if the polypeptide component binds to the MMP-9, the display library member is identified, typically by retention on a support.

Retained display library members are recovered from the support and analyzed. The analysis can include amplification and a subsequent selection under similar or dissimilar conditions. For example, positive and negative selections can be alternated. The analysis can
15 also include determining the amino acid sequence of the polypeptide component and purification of the polypeptide component for detailed characterization.

A variety of formats can be used for display libraries. Examples include the following.

Phage Display: The protein component is typically covalently linked to a bacteriophage coat protein. The linkage results from translation of a nucleic acid encoding the protein
20 component fused to the coat protein. The linkage can include a flexible peptide linker, a protease site, or an amino acid incorporated as a result of suppression of a stop codon. Phage display is described, for example, in U.S. 5,223,409; Smith (1985) *Science* 228:1315-1317; WO 92/18619; WO 91/17271; WO 92/20791; WO 92/15679; WO 93/01288; WO 92/01047; WO 92/09690; WO 90/02809; de Haard *et al.* (1999) *J. Biol. Chem* 274:18218-30; Hoogenboom *et al.* (1998)
25 *Immunotechnology* 4:1-20; Hoogenboom *et al.* (2000) *Immunol Today* 2:371-8 and Hoet *et al.* (2005) *Nat Biotechnol.* **23**(3)344-8. Bacteriophage displaying the protein component can be grown and harvested using standard phage preparatory methods, e.g. PEG precipitation from growth media. After selection of individual display phages, the nucleic acid encoding the selected protein components can be isolated from cells infected with the selected phages or from
30 the phage themselves, after amplification. Individual colonies or plaques can be picked, the nucleic acid isolated and sequenced.

Other Display Formats. Other display formats include cell based display (see, e.g., WO 03/029456), protein-nucleic acid fusions (see, e.g., US 6,207,446), ribosome display (See, e.g., Mattheakis *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91:9022 and Hanes *et al.* (2000) *Nat Biotechnol.* 18:1287-92; Hanes *et al.* (2000) *Methods Enzymol.* 328:404-30; and Schaffitzel *et al.* (1999) *J Immunol Methods.* 231(1-2):119-35), and *E. coli* periplasmic display (*J Immunol Methods.* 2005 Nov 22;PMID: 16337958).

Scaffolds. Scaffolds useful for display include: antibodies (e.g., Fab fragments, single chain Fv molecules (scFV), single domain antibodies, camelid antibodies, and camelized antibodies); T-cell receptors; MHC proteins; extracellular domains (e.g., fibronectin Type III repeats, EGF repeats); protease inhibitors (e.g., Kunitz domains, ecotin, BPTI, and so forth); TPR repeats; trifoil structures; zinc finger domains; DNA-binding proteins; particularly monomeric DNA binding proteins; RNA binding proteins; enzymes, e.g., proteases (particularly inactivated proteases), RNase; chaperones, e.g., thioredoxin and heat shock proteins; intracellular signaling domains (such as SH2 and SH3 domains); linear and constrained peptides; and linear peptide substrates. Display libraries can include synthetic and/or natural diversity. See, e.g., US 2004-0005709.

Display technology can also be used to obtain binding proteins (e.g., antibodies) that bind particular epitopes of a target. This can be done, for example, by using competing non-target molecules that lack the particular epitope or are mutated within the epitope, e.g., with alanine. Such non-target molecules can be used in a negative selection procedure as described below, as competing molecules when binding a display library to the target, or as a pre-elution agent, e.g., to capture in a wash solution dissociating display library members that are not specific to the target.

Iterative Selection. In one preferred embodiment, display library technology is used in an iterative mode. A first display library is used to identify one or more binding proteins for a target. These identified binding proteins are then varied using a mutagenesis method to form a second display library. Higher affinity binding proteins are then selected from the second library, e.g., by using higher stringency or more competitive binding and washing conditions.

In some implementations, the mutagenesis is targeted to regions at the binding interface. If, for example, the identified binding proteins are antibodies, then mutagenesis can be directed to the CDR regions of the heavy or light chains as described herein. Further, mutagenesis can be

directed to framework regions near or adjacent to the CDRs. In the case of antibodies, mutagenesis can also be limited to one or a few of the CDRs, e.g., to make precise step-wise improvements. Exemplary mutagenesis techniques include: error-prone PCR, recombination, DNA shuffling, site-directed mutagenesis and cassette mutagenesis.

5 In one example of iterative selection, the methods described herein are used to first identify a protein from a display library that binds MMP-9 with at least a minimal binding specificity for a target or a minimal activity, e.g., an equilibrium dissociation constant for binding of less than 1 nM, 10 nM, or 100 nM. The nucleic acid sequence encoding the initial identified proteins are used as a template nucleic acid for the introduction of variations, e.g., to
10 identify a second protein that has enhanced properties (e.g., binding affinity, kinetics, or stability) relative to the initial protein.

Off-Rate Selection. Since a slow dissociation rate can be predictive of high affinity, particularly with respect to interactions between polypeptides and their targets, the methods described herein can be used to isolate binding proteins with a desired (e.g., reduced) kinetic
15 dissociation rate for a binding interaction to a target.

To select for slow dissociating binding proteins from a display library, the library is contacted to an immobilized target. The immobilized target is then washed with a first solution that removes non-specifically or weakly bound biomolecules. Then the bound binding proteins are eluted with a second solution that includes a saturating amount of free target or a target
20 specific high-affinity competing monoclonal antibody, i.e., replicates of the target that are not attached to the particle. The free target binds to biomolecules that dissociate from the target. Rebinding is effectively prevented by the saturating amount of free target relative to the much lower concentration of immobilized target.

The second solution can have solution conditions that are substantially physiological or
25 that are stringent. Typically, the solution conditions of the second solution are identical to the solution conditions of the first solution. Fractions of the second solution are collected in temporal order to distinguish early from late fractions. Later fractions include biomolecules that dissociate at a slower rate from the target than biomolecules in the early fractions.

Further, it is also possible to recover display library members that remain bound to the
30 target even after extended incubation. These can either be dissociated using chaotropic

conditions or can be amplified while attached to the target. For example, phage bound to the target can be contacted to bacterial cells.

Selecting or Screening for Specificity. The display library screening methods described herein can include a selection or screening process that discards display library members that
5 bind to a non-target molecule. Examples of non-target molecules include streptavidin on magnetic beads, blocking agents such as bovine serum albumin, non-fat bovine milk, any capturing or target immobilizing monoclonal antibody, or non-transfected cells which do not express the human MMP-9 target.

In one implementation, a so-called “negative selection” step is used to discriminate
10 between the target and related non-target molecule and a related, but distinct non-target molecules. The display library or a pool thereof is contacted to the non-target molecule. Members of the sample that do not bind the non-target are collected and used in subsequent selections for binding to the target molecule or even for subsequent negative selections. The negative selection step can be prior to or after selecting library members that bind to the target
15 molecule.

In another implementation, a screening step is used. After display library members are isolated for binding to the target molecule, each isolated library member is tested for its ability to bind to a non-target molecule (e.g., a non-target listed above). For example, a high-throughput ELISA screen can be used to obtain this data. The ELISA screen can also be used to obtain
20 quantitative data for binding of each library member to the target as well as for cross species reactivity to related targets or subunits of the target (e.g., mouse MMP-9) and also under different condition such as pH6 or pH 7.5. The non-target and target binding data are compared (e.g., using a computer and software) to identify library members that specifically bind to the target.

Other Exemplary Expression Libraries

25

Other types of collections of proteins (e.g., expression libraries) can be used to identify proteins with a particular property (e.g., ability to bind MMP-9 and/or ability to modulate MMP-9), including, e.g., protein arrays of antibodies (see, e.g., De Wildt et al. (2000) Nat. Biotechnol. 18:989-994), lambda gt11 libraries, two-hybrid libraries and so forth.

Exemplary Libraries

It is possible to immunize a non-human primate and recover primate antibody genes that can be displayed on phage (see below). From such a library, one can select antibodies that bind the antigen used in immunization. See, for example, Vaccine. (2003) 22(2):257-67 or

5 Immunogenetics. (2005) 57(10):730-8.. Thus one could obtain primate antibodies that bind and inhibit MMP-9 by immunizing a chimpanzee or macaque and using a variety of means to select or screen for primate antibodies that bind and inhibit MMP-9. One can also make chimeras of primatized Fabs with human constant regions, see Curr Opin Mol Ther. (2004) 6(6):675-83.

“PRIMATIZED antibodies, genetically engineered from cynomolgus macaque monkey and
10 human components, are structurally indistinguishable from human antibodies. They may, therefore, be less likely to cause adverse reactions in humans, making them potentially suited for long-term, chronic treatment” Curr Opin Investig Drugs. (2001) 2(5):635-8.

One exemplary type of library presents a diverse pool of polypeptides, each of which includes an immunoglobulin domain, e.g., an immunoglobulin variable domain. Of interest are
15 display libraries where the members of the library include primate or “primatized” (e.g., such as human, non-human primate or “humanized”) immunoglobulin domains (e.g., immunoglobulin variable domains) or chimeric primatized Fabs with human constant regions. Human or humanized immunoglobulin domain libraries may be used to identify human or “humanized” antibodies that, for example, recognize human antigens. Because the constant and framework
20 regions of the antibody are human, these antibodies may avoid themselves being recognized and targeted as antigens when administered to humans. The constant regions may also be optimized to recruit effector functions of the human immune system. The in vitro display selection process surmounts the inability of a normal human immune system to generate antibodies against self-antigens.

25 A typical antibody display library displays a polypeptide that includes a VH domain and a VL domain. An “immunoglobulin domain” refers to a domain from the variable or constant domain of immunoglobulin molecules. Immunoglobulin domains typically contain two β -sheets formed of about seven β -strands, and a conserved disulphide bond (see, e.g., A. F. Williams and A. N. Barclay, 1988, *Ann. Rev. Immunol.* 6:381-405). The display library can display the
30 antibody as a Fab fragment (e.g., using two polypeptide chains) or a single chain Fv (e.g., using a single polypeptide chain). Other formats can also be used.

As in the case of the Fab and other formats, the displayed antibody can include one or more constant regions as part of a light and/or heavy chain. In one embodiment, each chain includes one constant region, e.g., as in the case of a Fab. In other embodiments, additional constant regions are displayed.

5 Antibody libraries can be constructed by a number of processes (see, e.g., de Haard et al., 1999, *J. Biol. Chem.* 274:18218-30; Hoogenboom et al., 1998, *Immunotechnology* 4:1-20; Hoogenboom et al., 2000, *Immunol. Today* 21:371-378, and Hoet et al. (2005) *Nat Biotechnol.* 23(3)344-8. Further, elements of each process can be combined with those of other processes. The processes can be used such that variation is introduced into a single immunoglobulin domain
10 (e.g., VH or VL) or into multiple immunoglobulin domains (e.g., VH and VL). The variation can be introduced into an immunoglobulin variable domain, e.g., in the region of one or more of CDR1, CDR2, CDR3, FR1, FR2, FR3, and FR4, referring to such regions of either and both of heavy and light chain variable domains. The variation(s) may be introduced into all three CDRs of a given variable domain, or into CDR1 and CDR2, e.g., of a heavy chain variable domain.
15 Any combination is feasible. In one process, antibody libraries are constructed by inserting diverse oligonucleotides that encode CDRs into the corresponding regions of the nucleic acid. The oligonucleotides can be synthesized using monomeric nucleotides or trinucleotides. For example, Knappik et al., 2000, *J. Mol. Biol.* 296:57-86 describe a method for constructing CDR encoding oligonucleotides using trinucleotide synthesis and a template with engineered
20 restriction sites for accepting the oligonucleotides.

In another process, an animal, e.g., a rodent, is immunized with MMP-9. The animal is optionally boosted with the antigen to further stimulate the response. Then spleen cells are isolated from the animal, and nucleic acid encoding VH and/or VL domains is amplified and cloned for expression in the display library.

25 In yet another process, antibody libraries are constructed from nucleic acid amplified from naïve germline immunoglobulin genes. The amplified nucleic acid includes nucleic acid encoding the VH and/or VL domain. Sources of immunoglobulin-encoding nucleic acids are described below. Amplification can include PCR, e.g., with primers that anneal to the conserved constant region, or another amplification method.

30 Nucleic acid encoding immunoglobulin domains can be obtained from the immune cells of, e.g., a primate (e.g., a human), mouse, rabbit, camel, or rodent. In one example, the cells are

selected for a particular property. B cells at various stages of maturity can be selected. In another example, the B cells are naïve.

In one embodiment, fluorescent-activated cell sorting (FACS) is used to sort B cells that express surface-bound IgM, IgD, or IgG molecules. Further, B cells expressing different
5 isotypes of IgG can be isolated. In another preferred embodiment, the B or T cell is cultured in vitro. The cells can be stimulated in vitro, e.g., by culturing with feeder cells or by adding mitogens or other modulatory reagents, such as antibodies to CD40, CD40 ligand or CD20, phorbol myristate acetate, bacterial lipopolysaccharide, concanavalin A, phytohemagglutinin, or pokeweed mitogen.

10 In another embodiment, the cells are isolated from a subject that has a disease of condition described herein, e.g., a cancer (e.g., metastatic cancer, e.g., metastatic breast cancer), an inflammatory disease (e.g., synovitis, atherosclerosis), rheumatoid arthritis, osteoarthritis, an ocular condition (e.g., macular degeneration), diabetes, Alzheimer's Disease, cerebral ischemia, endometriosis, fibrin-invasive activity, angiogenesis, or capillary tube formation. In another
15 embodiment, the cells are isolated from a transgenic non-human animal that includes a human immunoglobulin locus.

In one preferred embodiment, the cells have activated a program of somatic hypermutation. Cells can be stimulated to undergo somatic mutagenesis of immunoglobulin genes, for example, by treatment with anti-immunoglobulin, anti-CD40, and anti-CD38
20 antibodies (see, e.g., Bergthorsdottir et al., 2001, *J. Immunol.* 166:2228). In another embodiment, the cells are naïve.

The nucleic acid encoding an immunoglobulin variable domain can be isolated from a natural repertoire by the following exemplary method. First, RNA is isolated from the immune cell. Full length (i.e., capped) mRNAs are separated (e.g. by degrading uncapped RNAs with
25 calf intestinal phosphatase). The cap is then removed with tobacco acid pyrophosphatase and reverse transcription is used to produce the cDNAs.

The reverse transcription of the first (antisense) strand can be done in any manner with any suitable primer. See, e.g., de Haard et al., 1999, *J. Biol. Chem.* 274:18218-30. The primer binding region can be constant among different immunoglobulins, e.g., in order to reverse
30 transcribe different isotypes of immunoglobulin. The primer binding region can also be specific to a particular isotype of immunoglobulin. Typically, the primer is specific for a region that is 3'

to a sequence encoding at least one CDR. In another embodiment, poly-dT primers may be used (and may be preferred for the heavy-chain genes).

A synthetic sequence can be ligated to the 3' end of the reverse transcribed strand. The synthetic sequence can be used as a primer binding site for binding of the forward primer during
5 PCR amplification after reverse transcription. The use of the synthetic sequence can obviate the need to use a pool of different forward primers to fully capture the available diversity.

The variable domain-encoding gene is then amplified, e.g., using one or more rounds. If multiple rounds are used, nested primers can be used for increased fidelity. The amplified nucleic acid is then cloned into a display library vector.

10 **Secondary Screening Methods**

After selecting candidate library members that bind to a target, each candidate library member can be further analyzed, e.g., to further characterize its binding properties for the target, e.g., MMP-9, or for binding to other protein, e.g., another metalloproteinase. Each candidate library member can be subjected to one or more secondary screening assays. The assay can be
15 for a binding property, a catalytic property, an inhibitory property, a physiological property (e.g., cytotoxicity, renal clearance, immunogenicity), a structural property (e.g., stability, conformation, oligomerization state) or another functional property. The same assay can be used repeatedly, but with varying conditions, e.g., to determine pH, ionic, or thermal sensitivities.

As appropriate, the assays can use a display library member directly, a recombinant
20 polypeptide produced from the nucleic acid encoding the selected polypeptide, or a synthetic peptide synthesized based on the sequence of the selected polypeptide. In the case of selected Fabs, the Fabs can be evaluated or can be modified and produced as intact IgG proteins. Exemplary assays for binding properties include the following.

ELISA. Binding proteins can be evaluated using an ELISA assay. For example, each
25 protein is contacted to a microtitre plate whose bottom surface has been coated with the target, e.g., a limiting amount of the target. The plate is washed with buffer to remove non-specifically bound polypeptides. Then the amount of the binding protein bound to the target on the plate is determined by probing the plate with an antibody that can recognize the binding protein, e.g., a tag or constant portion of the binding protein. The antibody is linked to a detection system (e.g.,

an enzyme such as alkaline phosphatase or horse radish peroxidase (HRP) which produces a colorimetric product when appropriate substrates are provided).

Homogeneous Binding Assays. The ability of a binding protein described herein to bind a target can be analyzed using a homogenous assay, i.e., after all components of the assay are added, additional fluid manipulations are not required. For example, fluorescence resonance energy transfer (FRET) can be used as a homogenous assay (see, for example, Lakowicz et al., U.S. Patent No. 5,631,169; Stavrianopoulos, et al., U.S. Patent No. 4,868,103). A fluorophore label on the first molecule (e.g., the molecule identified in the fraction) is selected such that its emitted fluorescent energy can be absorbed by a fluorescent label on a second molecule (e.g., the target) if the second molecule is in proximity to the first molecule. The fluorescent label on the second molecule fluoresces when it absorbs to the transferred energy. Since the efficiency of energy transfer between the labels is related to the distance separating the molecules, the spatial relationship between the molecules can be assessed. In a situation in which binding occurs between the molecules, the fluorescent emission of the 'acceptor' molecule label in the assay should be maximal. A binding event that is configured for monitoring by FRET can be conveniently measured through standard fluorometric detection means, e.g., using a fluorimeter. By titrating the amount of the first or second binding molecule, a binding curve can be generated to estimate the equilibrium binding constant.

Another example of a homogenous assay is ALPHASCREEN™ (Packard Bioscience, Meriden CT). ALPHASCREEN™ uses two labeled beads. One bead generates singlet oxygen when excited by a laser. The other bead generates a light signal when singlet oxygen diffuses from the first bead and collides with it. The signal is only generated when the two beads are in proximity. One bead can be attached to the display library member, the other to the target. Signals are measured to determine the extent of binding.

Surface Plasmon Resonance (SPR). The interaction of binding protein and a target can be analyzed using SPR. SPR or Biomolecular Interaction Analysis (BIA) detects biospecific interactions in real time, without labeling any of the interactants. Changes in the mass at the binding surface (indicative of a binding event) of the BIA chip result in alterations of the refractive index of light near the surface (the optical phenomenon of surface plasmon resonance (SPR)). The changes in the refractivity generate a detectable signal, which are measured as an indication of real-time reactions between biological molecules. Methods for using SPR are

described, for example, in U.S. Patent No. 5,641,640; Raether, 1988, Surface Plasmons Springer Verlag; Sjolander and Urbaniczky, 1991, *Anal. Chem.* 63:2338-2345; Szabo et al., 1995, *Curr. Opin. Struct. Biol.* 5:699-705 and on-line resources provide by BIAcore International AB (Uppsala, Sweden). BIAcore Flexchip can be used to compare and rank interactions in real time,
5 in terms of kinetics, affinity or specificity without the use of labels.

Information from SPR can be used to provide an accurate and quantitative measure of the equilibrium dissociation constant (K_d), and kinetic parameters, including K_{on} and K_{off} , for the binding of a binding protein to a target. Such data can be used to compare different biomolecules. For example, selected proteins from an expression library can be compared to
10 identify proteins that have high affinity for the target or that have a slow K_{off} . This information can also be used to develop structure-activity relationships (SAR). For example, the kinetic and equilibrium binding parameters of matured versions of a parent protein can be compared to the parameters of the parent protein. Variant amino acids at given positions can be identified that correlate with particular binding parameters, e.g., high affinity and slow K_{off} . This information
15 can be combined with structural modeling (e.g., using homology modeling, energy minimization, or structure determination by x-ray crystallography or NMR). As a result, an understanding of the physical interaction between the protein and its target can be formulated and used to guide other design processes.

Cellular Assays. Binding proteins can be screened for ability to bind to cells which
20 transiently or stably express and display the target of interest on the cell surface. For example, MMP-9 binding proteins can be fluorescently labeled and binding to MMP-9 in the presence of absence of antagonistic antibody can be detected by a change in fluorescence intensity using flow cytometry e.g., a FACS machine.

Other Exemplary Methods for Obtaining MMP-9 binding antibodies

25 In addition to the use of display libraries, other methods can be used to obtain a MMP-9 binding antibody. For example, MMP-9 protein or a region thereof can be used as an antigen in a non-human animal, e.g., a rodent.

In one embodiment, the non-human animal includes at least a part of a human immunoglobulin gene. For example, it is possible to engineer mouse strains deficient in mouse
30 antibody production with large fragments of the human Ig loci. Using the hybridoma

technology, antigen-specific monoclonal antibodies (Mabs) derived from the genes with the desired specificity may be produced and selected. See, e.g., XENOMOUSE™, Green et al., 1994, *Nat. Gen.* 7:13-21; U.S. 2003-0070185, WO 96/34096, published Oct. 31, 1996, and PCT Application No. PCT/US96/05928, filed Apr. 29, 1996.

5 In another embodiment, a monoclonal antibody is obtained from the non-human animal, and then modified, e.g., humanized or deimmunized. Winter describes a CDR-grafting method that may be used to prepare the humanized antibodies (UK Patent Application GB 2188638A, filed on March 26, 1987; US Patent No. 5,225,539. All of the CDRs of a particular human antibody may be replaced with at least a portion of a non-human CDR or only some of the CDRs
10 may be replaced with non-human CDRs. It is only necessary to replace the number of CDRs required for binding of the humanized antibody to a predetermined antigen.

Humanized antibodies can be generated by replacing sequences of the Fv variable region that are not directly involved in antigen binding with equivalent sequences from human Fv variable regions. General methods for generating humanized antibodies are provided by
15 Morrison, S. L., 1985, *Science* 229:1202-1207, by Oi et al., 1986, *BioTechniques* 4:214, and by Queen et al. US Patent Nos. 5,585,089, US 5,693,761 and US 5,693,762. Those methods include isolating, manipulating, and expressing the nucleic acid sequences that encode all or part of immunoglobulin Fv variable regions from at least one of a heavy or light chain. Numerous sources of such nucleic acid are available. For example, nucleic acids may be obtained from a
20 hybridoma producing an antibody against a predetermined target, as described above. The recombinant DNA encoding the humanized antibody, or fragment thereof, can then be cloned into an appropriate expression vector.

Reducing Immunogenicity of MMP-9 Binding Proteins

Immunoglobulin MMP-9 binding proteins (e.g., IgG or Fab MMP-9 binding proteins) may
25 be modified to reduce immunogenicity. Reduced immunogenicity is desirable in MMP-9 binding proteins intended for use as therapeutics, as it reduces the chance that the subject will develop an immune response against the therapeutic molecule. Techniques useful for reducing immunogenicity of MMP-9 binding proteins include deletion/modification of potential human T cell epitopes and 'germlining' of sequences outside of the CDRs (e.g., framework and Fc).

An MMP-9-binding antibody may be modified by specific deletion of human T cell epitopes or "deimmunization" by the methods disclosed in WO 98/52976 and WO 00/34317. Briefly, the heavy and light chain variable regions of an antibody are analyzed for peptides that bind to MHC Class II; these peptides represent potential T-cell epitopes (as defined in WO 98/52976 and WO 00/34317). For detection of potential T-cell epitopes, a computer modeling approach termed "peptide threading" can be applied, and in addition a database of human MHC class II binding peptides can be searched for motifs present in the VH and VL sequences, as described in WO 98/52976 and WO 00/34317. These motifs bind to any of the 18 major MHC class II DR allotypes, and thus constitute potential T cell epitopes. Potential T-cell epitopes detected can be eliminated by substituting small numbers of amino acid residues in the variable regions, or preferably, by single amino acid substitutions. As far as possible conservative substitutions are made, often but not exclusively, an amino acid common at this position in human germline antibody sequences may be used. Human germline sequences are disclosed in Tomlinson, I.A. et al., 1992, *J. Mol. Biol.* 227:776-798; Cook, G. P. et al., 1995, *Immunol. Today* Vol. 16 (5): 237-242; Chothia, D. et al., 1992, *J. Mol. Bio.* 227:799-817. The V BASE directory provides a comprehensive directory of human immunoglobulin variable region sequences (compiled by Tomlinson, I.A. et al. MRC Centre for Protein Engineering, Cambridge, UK). After the deimmunizing changes are identified, nucleic acids encoding V_H and V_L can be constructed by mutagenesis or other synthetic methods (e.g., de novo synthesis, cassette replacement, and so forth). Mutagenized variable sequence can, optionally, be fused to a human constant region, e.g., human IgG1 or κ constant regions.

In some cases a potential T cell epitope will include residues which are known or predicted to be important for antibody function. For example, potential T cell epitopes are usually biased towards the CDRs. In addition, potential T cell epitopes can occur in framework residues important for antibody structure and binding. Changes to eliminate these potential epitopes will in some cases require more scrutiny, e.g., by making and testing chains with and without the change. Where possible, potential T cell epitopes that overlap the CDRs were eliminated by substitutions outside the CDRs. In some cases, an alteration within a CDR is the only option, and thus variants with and without this substitution should be tested. In other cases, the substitution required to remove a potential T cell epitope is at a residue position within the framework that might be critical for antibody binding. In these cases, variants with and without

this substitution should be tested. Thus, in some cases several variant deimmunized heavy and light chain variable regions were designed and various heavy/light chain combinations tested in order to identify the optimal deimmunized antibody. The choice of the final deimmunized antibody can then be made by considering the binding affinity of the different variants in
5 conjunction with the extent of deimmunization, i.e., the number of potential T cell epitopes remaining in the variable region. Deimmunization can be used to modify any antibody, e.g., an antibody that includes a non-human sequence, e.g., a synthetic antibody, a murine antibody other non-human monoclonal antibody, or an antibody isolated from a display library.

MMP-9 binding antibodies are “germlined” by reverting one or more non-germline
10 amino acids in framework regions to corresponding germline amino acids of the antibody, so long as binding properties are substantially retained. Similar methods can also be used in the constant region, e.g., in constant immunoglobulin domains.

Antibodies that bind to MMP-9, e.g., an antibody described herein, may be modified in order to make the variable regions of the antibody more similar to one or more germline
15 sequences. For example, an antibody can include one, two, three, or more amino acid substitutions, e.g., in a framework, CDR, or constant region, to make it more similar to a reference germline sequence. One exemplary germlining method can include identifying one or more germline sequences that are similar (e.g., most similar in a particular database) to the sequence of the isolated antibody. Mutations (at the amino acid level) are then made in the
20 isolated antibody, either incrementally or in combination with other mutations. For example, a nucleic acid library that includes sequences encoding some or all possible germline mutations is made. The mutated antibodies are then evaluated, e.g., to identify an antibody that has one or more additional germline residues relative to the isolated antibody and that is still useful (e.g., has a functional activity). In one embodiment, as many germline residues are introduced into an
25 isolated antibody as possible.

In one embodiment, mutagenesis is used to substitute or insert one or more germline residues into a framework and/or constant region. For example, a germline framework and/or constant region residue can be from a germline sequence that is similar (e.g., most similar) to the non-variable region being modified. After mutagenesis, activity (e.g., binding or other
30 functional activity) of the antibody can be evaluated to determine if the germline residue or

residues are tolerated (i.e., do not abrogate activity). Similar mutagenesis can be performed in the framework regions.

5 Selecting a germline sequence can be performed in different ways. For example, a germline sequence can be selected if it meets a predetermined criteria for selectivity or similarity, e.g., at least a certain percentage identity, e.g., at least 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 99.5% identity. The selection can be performed using at least 2, 3, 5, or 10 germline sequences. In the case of CDR1 and CDR2, identifying a similar germline sequence can include selecting one such sequence. In the case of CDR3, identifying a similar germline sequence can include selecting one such sequence, but may including using two germline sequences that separately contribute to the amino-terminal portion and the carboxy-terminal portion. In other implementations more than one or two germline sequences are used, e.g., to form a consensus sequence.

15 In one embodiment, with respect to a particular reference variable domain sequence, e.g., a sequence described herein, a related variable domain sequence has at least 30, 40, 50, 60, 70, 80, 90, 95 or 100% of the CDR amino acid positions that are not identical to residues in the reference CDR sequences, residues that are identical to residues at corresponding positions in a human germline sequence (i.e., an amino acid sequence encoded by a human germline nucleic acid).

20 In one embodiment, with respect to a particular reference variable domain sequence, e.g., a sequence described herein, a related variable domain sequence has at least 30, 50, 60, 70, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% of the FR regions identical to FR sequence from a human germline sequence, e.g., a germline sequence related to the reference variable domain sequence.

25 Accordingly, it is possible to isolate an antibody which has similar activity to a given antibody of interest, but is more similar to one or more germline sequences, particularly one or more human germline sequences. For example, an antibody can be at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 99.5% identical to a germline sequence in a region outside the CDRs (e.g., framework regions). Further, an antibody can include at least 1, 2, 3, 4, or 5 germline residues in a CDR region, the germline residue being from a germline sequence of similar (e.g., most similar) to the variable region being modified. Germline sequences of primary interest are

30

human germline sequences. The activity of the antibody (e.g., the binding activity as measured by K_A) can be within a factor of 100, 10, 5, 2, 0.5, 0.1, and 0.001 of the original antibody.

Germline sequences of human immunoglobulin genes have been determined and are available from a number of sources, including the international ImMunoGeneTics information system® (IMGT), available via the world wide web at imgt.cines.fr, and the V BASE directory (compiled by Tomlinson, I.A. et al. MRC Centre for Protein Engineering, Cambridge, UK, available via the world wide web at vbase.mrc-cpe.cam.ac.uk).

Exemplary germline reference sequences for V_{kappa} include: O12/O2, O18/O8, A20, A30, L14, L1, L15, L4/18a, L5/L19, L8, L23, L9, L24, L11, L12, O11/O1, A17, A1, A18, A2, A19/A3, A23, A27, A11, L2/L16, L6, L20, L25, B3, B2, A26/A10, and A14. See, e.g., Tomlinson et al., 1995, *EMBO J.* 14(18):4628-3.

A germline reference sequence for the HC variable domain can be based on a sequence that has particular canonical structures, e.g., 1-3 structures in the H1 and H2 hypervariable loops. The canonical structures of hypervariable loops of an immunoglobulin variable domain can be inferred from its sequence, as described in Chothia et al., 1992, *J. Mol. Biol.* 227:799-817; Tomlinson et al., 1992, *J. Mol. Biol.* 227:776-798); and Tomlinson et al., 1995, *EMBO J.* 14(18):4628-38. Exemplary sequences with a 1-3 structure include: DP-1, DP-8, DP-12, DP-2, DP-25, DP-15, DP-7, DP-4, DP-31, DP-32, DP-33, DP-35, DP-40, 7-2, hv3005, hv3005f3, DP-46, DP-47, DP-58, DP-49, DP-50, DP-51, DP-53, and DP-54.

Protein Production

Standard recombinant nucleic acid methods can be used to express a protein that binds to MMP-9. Generally, a nucleic acid sequence encoding the protein is cloned into a nucleic acid expression vector. Of course, if the protein includes multiple polypeptide chains, each chain can be cloned into an expression vector, e.g., the same or different vectors, that are expressed in the same or different cells.

Antibody Production. Some antibodies, e.g., Fabs, can be produced in bacterial cells, e.g., *E. coli* cells. For example, if the Fab is encoded by sequences in a phage display vector that includes a suppressible stop codon between the display entity and a bacteriophage protein (or fragment thereof), the vector nucleic acid can be transferred into a bacterial cell that cannot

suppress a stop codon. In this case, the Fab is not fused to the gene III protein and is secreted into the periplasm and/or media.

Antibodies can also be produced in eukaryotic cells. In one embodiment, the antibodies (e.g., scFv's) are expressed in a yeast cell such as *Pichia* (see, e.g., Powers et al., 2001, *J.*

5 *Immunol. Methods*. 251:123-35), *Hansenula*, or *Saccharomyces*.

In one preferred embodiment, antibodies are produced in mammalian cells. Preferred mammalian host cells for expressing the clone antibodies or antigen-binding fragments thereof include Chinese Hamster Ovary (CHO cells) (including dhfr- CHO cells, described in Urlaub and Chasin, 1980, *Proc. Natl. Acad. Sci. USA* 77:4216-4220, used with a DHFR selectable
10 marker, e.g., as described in Kaufman and Sharp, 1982, *Mol. Biol.* 159:601-621), lymphocytic cell lines, e.g., NS0 myeloma cells and SP2 cells, COS cells, HEK293T cells (*J. Immunol. Methods* (2004) **289**(1-2):65-80.), and a cell from a transgenic animal, e.g., a transgenic mammal. For example, the cell is a mammary epithelial cell.

In addition to the nucleic acid sequence encoding the diversified immunoglobulin
15 domain, the recombinant expression vectors may carry additional sequences, such as sequences that regulate replication of the vector in host cells (e.g., origins of replication) and selectable marker genes. The selectable marker gene facilitates selection of host cells into which the vector has been introduced (see e.g., U.S. Patent Nos. 4,399,216, 4,634,665 and 5,179,017). For example, typically the selectable marker gene confers resistance to drugs, such as G418,
20 hygromycin or methotrexate, on a host cell into which the vector has been introduced. Preferred selectable marker genes include the dihydrofolate reductase (DHFR) gene (for use in *dhfr*⁻ host cells with methotrexate selection/amplification) and the *neo* gene (for G418 selection).

In an exemplary system for recombinant expression of an antibody, or antigen-binding portion thereof, a recombinant expression vector encoding both the antibody heavy chain and the
25 antibody light chain is introduced into *dhfr*⁻ CHO cells by calcium phosphate-mediated transfection. Within the recombinant expression vector, the antibody heavy and light chain genes are each operatively linked to enhancer/promoter regulatory elements (e.g., derived from SV40, CMV, adenovirus and the like, such as a CMV enhancer/AdMLP promoter regulatory element or an SV40 enhancer/AdMLP promoter regulatory element) to drive high levels of
30 transcription of the genes. The recombinant expression vector also carries a DHFR gene, which allows for selection of CHO cells that have been transfected with the vector using methotrexate

selection/amplification. The selected transformant host cells are cultured to allow for expression of the antibody heavy and light chains and intact antibody is recovered from the culture medium. Standard molecular biology techniques are used to prepare the recombinant expression vector, transfect the host cells, select for transformants, culture the host cells and recover the antibody
5 from the culture medium. For example, some antibodies can be isolated by affinity chromatography with a Protein A or Protein G coupled matrix.

For antibodies that include an Fc domain, the antibody production system may produce antibodies in which the Fc region is glycosylated. For example, the Fc domain of IgG molecules is glycosylated at asparagine 297 in the CH2 domain. This asparagine is the site for modification
10 with biantennary-type oligosaccharides. It has been demonstrated that this glycosylation is required for effector functions mediated by Fcγ receptors and complement C1q (Burton and Woof, 1992, *Adv. Immunol.* 51:1-84; Jefferis et al., 1998, *Immunol. Rev.* 163:59-76). In one embodiment, the Fc domain is produced in a mammalian expression system that appropriately glycosylates the residue corresponding to asparagine 297. The Fc domain can also include other
15 eukaryotic post-translational modifications.

Antibodies can also be produced by a transgenic animal. For example, U.S. Patent No. 5,849,992 describes a method of expressing an antibody in the mammary gland of a transgenic mammal. A transgene is constructed that includes a milk-specific promoter and nucleic acids encoding the antibody of interest and a signal sequence for secretion. The milk produced by
20 females of such transgenic mammals includes, secreted-therein, the antibody of interest. The antibody can be purified from the milk, or for some applications, used directly.

Characterization of MMP-9 Binding Proteins

Binding of MMP-9 binding proteins to cells expressing MMP-9 can be characterized in a number assays known in the art, including FACS (Fluorescence Activated Cell Sorting),
25 immunofluorescence, and immunocytochemistry. MMP-9 binding protein is contacted with cells and/or tissues which express or contain MMP-9, and binding is detected in accordance with the method being used. For example, a fluorescent detection system (e.g., fluorescent-labeled secondary antibody) employed for FACS and immunofluorescence analysis, or a enzymatic system is used for immunocytochemistry are generally used in these assays can be performed on
30 non-perm. MMP-9 binding proteins can be characterized as to cellular binding by FACS

(Fluorescence Activated Cell Sorting) using cells expressing MMP-9. Individual cells held in a thin stream of fluid are passed through one or more laser beams cause light to scatter and fluorescent dyes to emit light at various frequencies. Photomultiplier tubes (PMT) convert light to electrical signals and cell data is collected. Forward and side scatter are used for preliminary
5 identification of cells. Forward and side scatter are used to exclude debris and dead cells.

Fluorescent labeling allows investigation of cell structure and function. Cell autofluorescence is generated by labeling cell structures with fluorescent dyes. FACS collects fluorescence signals in one to several channels corresponding to different laser excitation and fluorescence emission wavelength. Immunofluorescence, the most widely used application, involves the staining of
10 cells with antibodies conjugated to fluorescent dyes such as fluorescein and phycoerythrin (PE).

This method can be used to label MMP-9 on the cell surface of MDA-MB-231 cells using biotinylated MMP-9 binding proteins. Biotin is used in these two-step detection systems in concert with conjugated streptavidin. Biotin is typically conjugated to proteins via primary amines (i.e., lysines). Usually, between 1.5 and 3 biotin molecules are conjugated to each
15 antibody. A second fluorescently conjugated antibody (streptavidin/PE) is added which is specific for biotin.

MMP-9 binding proteins can be characterized in cultured cells expressing the MMP-9 antigen. The method generally used is immunocytochemistry. Immunocytochemistry involves the use of antibodies that recognize parts of the receptor that are exposed to the outside
20 environment when expressed at the cell surface (the 'primary antibody'). If the experiment is carried out in intact cells, such an antibody will only bind to surface expressed receptors. Biotinylated or non-biotinylated MMP-9 binding proteins can be used. The secondary antibody is then either a streptavidin/HRP antibody (for biotinylated MMP-9 binding protein) or an anti-human IgG/HRP (for non-biotinylated MMP-9 binding protein). The staining can then be
25 detected using an inverted microscope. The assay can be performed in the absence of MMP-9 binding protein and in presence of 10µg/mL of MMP-9 binding protein.

MMP-9 binding proteins can be characterized in assays that measure their modulatory activity toward MMP-9 or fragments thereof *in vitro* or *in vivo*. For example, MMP-9 can be combined with a substrate such as Mca-Pro-Leu-Ala-Cys(Mob)-Trp-Ala-Arg-Dap(Dnp)-NH₂
30 under assay conditions permitting cleavage by MMP-9. The assay is performed in the absence of the MMP-9 binding protein, and in the presence of increasing concentrations of the MMP-9

binding protein. The concentration of binding protein at which 50% of the MMP-9 activity (e.g., binding to the substrate) is inhibited is the IC_{50} value (Inhibitory Concentration 50%) or EC_{50} (Effective Concentration 50%) value for that binding protein. Within a series or group of binding proteins, those having lower IC_{50} or EC_{50} values are considered more potent inhibitors of MMP-9 than those binding proteins having higher IC_{50} or EC_{50} values. Exemplary binding proteins have an IC_{50} value of less than 800 nM, 400 nM, 100 nM, 25 nM, 5 nM, or 1 nM, e.g., as measured in an *in vitro* assay for inhibition of MMP-9 activity when the MMP-9 is at 2 pM.

MMP-9 binding proteins may also be characterized with reference to the activity of MMP-9 on substrates (e.g., collagen, gelatin). For example, cleavage of gelatin by MMP-9 can be detected in zymography. The method is based on a SDS gel impregnated with a substrate, which is degraded by the proteases resolved during the incubation period. Coomassie blue staining of the gels reveals proteolytic fragments as white bands on a dark blue background. Within a certain range, the band intensity can be related linearly to the amount of the protease loaded. Cells expressing both MMP-9 and MMP-2 are used in this assay. The assay is performed in the absence of the MMP-9 binding protein, and in the presence of increasing concentrations of the MMP-9 binding protein. The concentration of binding protein at which 50% of the MMP-2 activity (e.g., binding to the substrate) is inhibited is the IC_{50} value (Inhibitory Concentration 50%) or EC_{50} (Effective Concentration 50%) value for that binding protein. Within a series or group of binding proteins, those having lower IC_{50} or EC_{50} values are considered more potent inhibitors of MMP-9 than those binding proteins having higher IC_{50} or EC_{50} values. Exemplary binding proteins have an IC_{50} value of less than 800 nM, 400 nM, 100 nM, 25 nM, 5 nM, or 1 nM, e.g., as measured in an *in vitro* assay for inhibition of MMP-9 activity.

The binding proteins can also be evaluated for selectivity toward MMP-9. For example, a MMP-9 binding protein can be assayed for its potency toward MMP-9 and a panel of MMPs and other enzymes, e.g., human and/or mouse enzymes, e.g., MMP-1, -2, -3, -7, -8, -12, -13, -14, -16, -17, -24, and TACE, and an IC_{50} value or EC_{50} value can be determined for each MMP. In one embodiment, a compound that demonstrates a low IC_{50} value or EC_{50} value for the MMP-9, and a higher IC_{50} value or EC_{50} value, e.g., at least 2-, 5-, or 10- fold higher, for another MMP within the test panel (e.g., MMP-1, -10) is considered to be selective toward MMP-9.

MMP-9 binding proteins can be evaluated for their ability to inhibit MMP-9 in a cell based assay, e.g., in situ zymography, e.g., in Colo205 cells or MCF-7 cells.

A pharmacokinetics study in rat, mice, or monkey can be performed with MMP-9 binding proteins for determining MMP-9 half-life in the serum. Likewise, the effect of the binding protein can be assessed *in vivo*, e.g., in an animal model for a disease, for use as a therapeutic, for example, to treat a disease or condition described herein, e.g., a cancer (e.g., metastatic cancer, e.g., metastatic breast cancer), an inflammatory disease (e.g., chronic obstructive pulmonary disease (COPD), asthma, rhinitis (e.g., allergic rhinitis), inflammatory bowel disease, synovitis, rheumatoid arthritis), heart failure, septic shock, neuropathic pain, inflammatory pain, osteoarthritis, or an ocular condition (e.g., macular degeneration).

Pharmaceutical Compositions

In another aspect, the disclosure provides compositions, e.g., pharmaceutically acceptable compositions or pharmaceutical compositions, which include an MMP-9-binding protein, e.g., an antibody molecule, other polypeptide or peptide identified as binding to MMP-9 described herein. The MMP-9 binding protein can be formulated together with a pharmaceutically acceptable carrier. Pharmaceutical compositions include therapeutic compositions and diagnostic compositions, e.g., compositions that include labeled MMP-9 binding proteins for *in vivo* imaging.

A pharmaceutically acceptable carrier includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. Preferably, the carrier is suitable for intravenous, intramuscular, subcutaneous, parenteral, spinal, or epidermal administration (e.g., by injection or infusion), although carriers suitable for inhalation and intranasal administration are also contemplated. Depending on the route of administration, the MMP-9 binding protein may be coated in a material to protect the compound from the action of acids and other natural conditions that may inactivate the compound.

A pharmaceutically acceptable salt is a salt that retains the desired biological activity of the parent compound and does not impart any undesired toxicological effects (see e.g., Berge, S.M., et al., 1977, *J. Pharm. Sci.* 66:1-19). Examples of such salts include acid addition salts and base addition salts. Acid addition salts include those derived from nontoxic inorganic acids, such

as hydrochloric, nitric, phosphoric, sulfuric, hydrobromic, hydroiodic, phosphorous, and the like, as well as from nontoxic organic acids such as aliphatic mono- and dicarboxylic acids, phenyl-substituted alkanoic acids, hydroxy alkanoic acids, aromatic acids, aliphatic and aromatic sulfonic acids, and the like. Base addition salts include those derived from alkaline earth metals, such as sodium, potassium, magnesium, calcium, and the like, as well as from nontoxic organic amines, such as N,N'-dibenzylethylenediamine, N-methylglucamine, chlorprocaine, choline, diethanolamine, ethylenediamine, procaine, and the like.

The compositions may be in a variety of forms. These include, for example, liquid, semi-solid and solid dosage forms, such as liquid solutions (e.g., injectable and infusible solutions), dispersions or suspensions, tablets, pills, powders, liposomes and suppositories. The form can depend on the intended mode of administration and therapeutic application. Many compositions are in the form of injectable or infusible solutions, such as compositions similar to those used for administration of humans with antibodies. An exemplary mode of administration is parenteral (e.g., intravenous, subcutaneous, intraperitoneal, intramuscular). In one embodiment, the MMP-9 binding protein is administered by intravenous infusion or injection. In another preferred embodiment, the MMP-9 binding protein is administered by intramuscular or subcutaneous injection.

The phrases "parenteral administration" and "administered parenterally" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion.

The composition can be formulated as a solution, microemulsion, dispersion, liposome, or other ordered structure suitable to high drug concentration. Sterile injectable solutions can be prepared by incorporating the binding protein in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying that yields a

powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. The proper fluidity of a solution can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prolonged absorption of injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin.

An MMP-9 binding protein can be administered by a variety of methods, although for many applications, the preferred route/mode of administration is intravenous injection or infusion. For example, for therapeutic applications, the MMP-9 binding protein can be administered by intravenous infusion at a rate of less than 30, 20, 10, 5, or 1 mg/min to reach a dose of about 1 to 100 mg/m² or 7 to 25 mg/m². The route and/or mode of administration will vary depending upon the desired results. In certain embodiments, the active compound may be prepared with a carrier that will protect the compound against rapid release, such as a controlled release formulation, including implants, and microencapsulated delivery systems.

Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Many methods for the preparation of such formulations are available. See, e.g., Sustained and Controlled Release Drug Delivery Systems, J.R. Robinson, ed., 1978, Marcel Dekker, Inc., New York.

Pharmaceutical compositions can be administered with medical devices. For example, in one embodiment, a pharmaceutical composition disclosed herein can be administered with a device, e.g., a needleless hypodermic injection device, a pump, or implant.

In certain embodiments, an MMP-9 binding protein can be formulated to ensure proper distribution *in vivo*. For example, the blood-brain barrier (BBB) excludes many highly hydrophilic compounds. To ensure that the therapeutic compounds disclosed herein cross the BBB (if desired), they can be formulated, for example, in liposomes. For methods of manufacturing liposomes, see, e.g., U.S. Patent Nos. 4,522,811; 5,374,548; and 5,399,331. The liposomes may comprise one or more moieties that are selectively transported into specific cells or organs, thus enhance targeted drug delivery (see, e.g., V.V. Ranade, 1989, *J. Clin. Pharmacol.* 29:685).

Dosage regimens are adjusted to provide the optimum desired response (e.g., a therapeutic response). For example, a single bolus may be administered, several divided doses

may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit contains a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms can be dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

An exemplary, non-limiting range for a therapeutically or prophylactically effective amount of an antibody disclosed herein is 0.1-20 mg/kg, more preferably 1-10 mg/kg. An anti-MMP-9 antibody can be administered, e.g., by intravenous infusion, e.g., at a rate of less than 30, 20, 10, 5, or 1 mg/min to reach a dose of about 1 to 100 mg/m² or about 5 to 30 mg/m². For binding proteins smaller in molecular weight than an antibody, appropriate amounts can be proportionally less. Dosage values may vary with the type and severity of the condition to be alleviated. For a particular subject, specific dosage regimens can be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions.

The pharmaceutical compositions disclosed herein may include a “therapeutically effective amount” or a “prophylactically effective amount” of an MMP-9 binding protein disclosed herein. A “therapeutically effective amount” refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result. A therapeutically effective amount of the composition may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the protein to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the composition is outweighed by the therapeutically beneficial effects.

A “therapeutically effective dosage” preferably modulates a measurable parameter, e.g., levels of circulating IgG antibodies or enzymatic activity, by a statistically significant degree or at least about 20%, more preferably by at least about 40%, even more preferably by at least about 60%, and still more preferably by at least about 80% relative to untreated subjects. The ability of

a compound to modulate a measurable parameter, e.g., a disease-associated parameter, can be evaluated in an animal model system predictive of efficacy in human disorders and conditions, e.g., a cancer (e.g., metastatic cancer, e.g., metastatic breast cancer), an inflammatory disease (e.g., chronic obstructive pulmonary disease (COPD), asthma, rhinitis (e.g., allergic rhinitis),
5 inflammatory bowel disease, synovitis, rheumatoid arthritis), heart failure, septic shock, neuropathic pain, inflammatory pain, osteoarthritis, or an ocular condition (e.g., macular degeneration). Alternatively, this property of a composition can be evaluated by examining the ability of the compound to modulate a parameter in vitro.

A “prophylactically effective amount” refers to an amount effective, at dosages and for
10 periods of time necessary, to achieve the desired prophylactic result. Typically, because a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount will be less than the therapeutically effective amount.

Stabilization and Retention

In one embodiment, an MMP-9 binding protein is physically associated with a moiety
15 that improves its stabilization and/or retention in circulation, e.g., in blood, serum, lymph, or other tissues, e.g., by at least 1.5, 2, 5, 10, or 50 fold. For example, an MMP-9 binding protein can be associated with a polymer, e.g., a substantially non-antigenic polymer, such as polyalkylene oxides or polyethylene oxides. Suitable polymers will vary substantially by weight. Polymers having molecular number average weights ranging from about 200 to about 35,000 (or
20 about 1,000 to about 15,000, and 2,000 to about 12,500) can be used. For example, an MMP-9 binding protein can be conjugated to a water soluble polymer, e.g., hydrophilic polyvinyl polymers, e.g. polyvinylalcohol and polyvinylpyrrolidone. A non-limiting list of such polymers include polyalkylene oxide homopolymers such as polyethylene glycol (PEG) or polypropylene glycols, polyoxyethylenated polyols, copolymers thereof and block copolymers thereof, provided
25 that the water solubility of the block copolymers is maintained.

An MMP-9 binding protein can also be associated with a carrier protein, e.g., a serum albumin, such as a human serum albumin. For example, a translational fusion can be used to associate the carrier protein with the MMP-9 binding protein.

Kits

An MMP-9 binding protein described herein can be provided in a kit, e.g., as a component of a kit. For example, the kit includes (a) an MMP-9 binding protein, e.g., a composition that includes an MMP-9 binding protein, and, optionally (b) informational material.

5 The informational material can be descriptive, instructional, marketing or other material that relates to the methods described herein and/or the use of an MMP-9 binding protein for the methods described herein.

The informational material of the kits is not limited in its form. In one embodiment, the informational material can include information about production of the compound, molecular
10 weight of the compound, concentration, date of expiration, batch or production site information, and so forth. In one embodiment, the informational material relates to using the binding protein to treat, prevent, or diagnosis of disorders and conditions, e.g., a cancer (e.g., metastatic cancer, e.g., metastatic breast or colon cancer), an inflammatory disease (e.g., chronic obstructive pulmonary disease (COPD), asthma, rhinitis (e.g., allergic rhinitis), inflammatory bowel disease,
15 synovitis, rheumatoid arthritis), heart failure, septic shock, neuropathic pain, inflammatory pain, osteoarthritis, or an ocular condition (e.g., macular degeneration).

In one embodiment, the informational material can include instructions to administer an MMP-9 binding protein in a suitable manner to perform the methods described herein, e.g., in a suitable dose, dosage form, or mode of administration (e.g., a dose, dosage form, or mode of
20 administration described herein). In another embodiment, the informational material can include instructions to administer an MMP-9 binding protein to a suitable subject, e.g., a human, e.g., a human having, or at risk for, a disorder or condition described herein, e.g., a cancer (e.g., metastatic cancer, e.g., metastatic breast cancer), an inflammatory disease (e.g., chronic obstructive pulmonary disease (COPD), asthma, rhinitis (e.g., allergic rhinitis), inflammatory
25 bowel disease, synovitis, rheumatoid arthritis), heart failure, septic shock, neuropathic pain, inflammatory pain, osteoarthritis, or an ocular condition (e.g., macular degeneration). For example, the material can include instructions to administer an MMP-9 binding protein to a patient with a disorder or condition described herein, e.g., a cancer (e.g., metastatic cancer, e.g., metastatic breast cancer), an inflammatory disease (e.g., chronic obstructive pulmonary disease
30 (COPD), asthma, rhinitis (e.g., allergic rhinitis), inflammatory bowel disease, synovitis, rheumatoid arthritis), heart failure, septic shock, neuropathic pain, inflammatory pain,

osteoarthritis, or an ocular condition (e.g., macular degeneration). The informational material of the kits is not limited in its form. In many cases, the informational material, e.g., instructions, is provided in print but may also be in other formats, such as computer readable material.

An MMP-9 binding protein can be provided in any form, e.g., liquid, dried or lyophilized form. It is preferred that an MMP-9 binding protein be substantially pure and/or sterile. When an MMP-9 binding protein is provided in a liquid solution, the liquid solution preferably is an aqueous solution, with a sterile aqueous solution being preferred. When an MMP-9 binding protein is provided as a dried form, reconstitution generally is by the addition of a suitable solvent. The solvent, e.g., sterile water or buffer, can optionally be provided in the kit.

The kit can include one or more containers for the composition containing an MMP-9 binding protein. In some embodiments, the kit contains separate containers, dividers or compartments for the composition and informational material. For example, the composition can be contained in a bottle, vial, or syringe, and the informational material can be contained association with the container. In other embodiments, the separate elements of the kit are contained within a single, undivided container. For example, the composition is contained in a bottle, vial or syringe that has attached thereto the informational material in the form of a label. In some embodiments, the kit includes a plurality (e.g., a pack) of individual containers, each containing one or more unit dosage forms (e.g., a dosage form described herein) of an MMP-9 binding protein. For example, the kit includes a plurality of syringes, ampules, foil packets, or blister packs, each containing a single unit dose of an MMP-9 binding protein. The containers of the kits can be air tight, waterproof (e.g., impermeable to changes in moisture or evaporation), and/or light-tight.

The kit optionally includes a device suitable for administration of the composition, e.g., a syringe, inhalant, dropper (e.g., eye dropper), swab (e.g., a cotton swab or wooden swab), or any such delivery device. In one embodiment, the device is an implantable device that dispenses metered doses of the binding protein. The disclosure also features a method of providing a kit, e.g., by combining components described herein.

Treatments

Proteins that bind to MMP-9 and identified by the method described herein and/or detailed herein have therapeutic and prophylactic utilities, particularly in human subjects. These

binding proteins are administered to a subject to treat, prevent, and/or diagnose a variety of disorders, including e.g., a cancer (e.g., metastatic cancer, e.g., metastatic breast or colon cancer), an inflammatory disease (e.g., chronic obstructive pulmonary disease (COPD), asthma, rhinitis (e.g., allergic rhinitis), inflammatory bowel disease, synovitis, rheumatoid arthritis), heart failure, septic shock, neuropathic pain, inflammatory pain, osteoarthritis, or an ocular condition (e.g., macular degeneration), or even to cells in culture, e.g. *in vitro* or *ex vivo*. Treating includes administering an amount effective to alleviate, relieve, alter, remedy, ameliorate, improve or affect the disorder, the symptoms of the disorder or the predisposition toward the disorder. The treatment may also delay onset, e.g., prevent onset, or prevent deterioration of a disease or condition.

Exemplary disorders include a cancer (e.g., metastatic cancer, e.g., metastatic breast or colon cancer), an inflammatory disease (e.g., chronic obstructive pulmonary disease (COPD), asthma, rhinitis (e.g., allergic rhinitis), inflammatory bowel disease, synovitis, rheumatoid arthritis), heart failure, septic shock, neuropathic pain, inflammatory pain, osteoarthritis, or an ocular condition (e.g., macular degeneration). Some of these disorders are discussed above. Still other disorders that can be treated using an MMP-9 binding protein include: aortic aneurysms, periodontitis, autoimmune blistering disorders of the skin, dermal photoaging.

As used herein, an amount of an target-binding agent effective to prevent a disorder, or a prophylactically effective amount of the binding agent refers to an amount of a target binding agent, e.g., an MMP-9 binding protein, e.g., an anti-MMP-9 antibody described herein, which is effective, upon single- or multiple-dose administration to the subject, for preventing or delaying the occurrence of the onset or recurrence of a disorder, e.g., a disorder described herein.

A binding agent described herein can be used to reduce angiogenesis in a subject, e.g., to treat a cancer (e.g., a solid tumor) or an angiogenesis-associated disorder. The method includes administering the binding to the subject, e.g., in an amount effective to modulate angiogenesis, a symptom of the disorder, or progression of the disorder. The agent (e.g., an MMP-9 binding protein, e.g., an anti-MMP-9 antibody) may be administered multiple times (e.g., at least two, three, five, or ten times) before a therapeutically effective amount is attained.

Methods of administering MMP-9 binding proteins and other agents are also described in “Pharmaceutical Compositions.” Suitable dosages of the molecules used can depend on the age and weight of the subject and the particular drug used. The binding proteins can be used as

competitive agents to inhibit, reduce an undesirable interaction, e.g., between a natural or pathological agent and the MMP-9. The dose of the MMP-9 binding protein can be the amount sufficient to block 90%, 95%, 99%, or 99.9% of the activity of MMP-9 in the patient, especially at the site of disease. Depending on the disease, this may require 0.1, 1.0, 3.0, 6.0, or 10.0 mg/Kg. For an IgG having a molecular mass of 150,000 g/mole (two binding sites), these doses correspond to approximately 18 nM, 180 nM, 540 nM, 1.08 μ M, and 1.8 μ M of binding sites for a 5 L blood volume.

In one embodiment, the MMP-9 binding proteins are used to inhibit an activity (e.g., inhibit at least one activity of, reduce proliferation, migration, growth or viability) of a cell, e.g., a cancer cell *in vivo*. The binding proteins can be used by themselves or conjugated to an agent, e.g., a cytotoxic drug, cytotoxin enzyme, or radioisotope. This method includes: administering the binding protein alone or attached to an agent (e.g., a cytotoxic drug), to a subject requiring such treatment. For example, MMP-9 binding proteins that do not substantially inhibit MMP-9 may be used to deliver nanoparticles containing agents, such as toxins, to MMP-9 associated cells or tissues, e.g., tumors.

Because the MMP-9 binding proteins recognize MMP-9-expressing cells and can bind to cells that are associated with (e.g., in proximity of or intermingled with) cancer cells, e.g., cancerous lung, liver, colon, breast, ovarian, epidermal, laryngeal, and cartilage cells, and particularly metastatic cells thereof, leukemia, B cell lymphoma, and multiple myeloma, MMP-9 binding proteins can be used to inhibit (e.g., inhibit at least one activity, reduce growth and proliferation, or kill) any such cells and inhibit carcinogenesis. Reducing MMP-9 activity near a cancer can indirectly inhibit (e.g., inhibit at least one activity, reduce growth and proliferation, or kill) the cancer cells which may be dependent on the MMP-9 activity for metastasis, activation of growth factors, and so forth.

Alternatively, the binding proteins bind to cells in the vicinity of the cancerous cells, but are sufficiently close to the cancerous cells to directly or indirectly inhibit (e.g., inhibit at least one activity, reduce growth and proliferation, or kill) the cancerous cells. Thus, the MMP-9 binding proteins (e.g., modified with a toxin, e.g., a cytotoxin) can be used to selectively inhibit cells in cancerous tissue (including the cancerous cells themselves and cells associated with or invading the cancer).

The binding proteins may be used to deliver an agent (e.g., any of a variety of cytotoxic and therapeutic drugs) to cells and tissues where MMP-9 is present. Exemplary agents include a compound emitting radiation, molecules of plants, fungal, or bacterial origin, biological proteins, and mixtures thereof. The cytotoxic drugs can be intracellularly acting cytotoxic drugs, such as
5 toxins short range radiation emitters, e.g., short range, high energy α -emitters.

To target MMP-9 expressing cells, particularly cancerous cells, a prodrug system can be used. For example, a first binding protein is conjugated with a prodrug which is activated only when in close proximity with a prodrug activator. The prodrug activator is conjugated with a second binding protein, preferably one which binds to a non competing site on the target
10 molecule. Whether two binding proteins bind to competing or non competing binding sites can be determined by conventional competitive binding assays. Exemplary drug prodrug pairs are described in Blakely et al., (1996) Cancer Research, 56:3287 3292.

The MMP-9 binding proteins can be used directly *in vivo* to eliminate antigen-expressing cells via natural complement-dependent cytotoxicity (CDC) or antibody dependent cellular
15 cytotoxicity (ADCC). The binding proteins described herein can include complement binding effector domain, such as the Fc portions from IgG1, -2, or -3 or corresponding portions of IgM which bind complement. In one embodiment, a population of target cells is ex vivo treated with a binding agent described herein and appropriate effector cells. The treatment can be supplemented by the addition of complement or serum containing complement. Further,
20 phagocytosis of target cells coated with a binding protein described herein can be improved by binding of complement proteins. In another embodiment target, cells coated with the binding protein which includes a complement binding effector domain are lysed by complement.

Methods of administering MMP-9 binding proteins are described in "Pharmaceutical Compositions." Suitable dosages of the molecules used will depend on the age and weight of the
25 subject and the particular drug used. The binding proteins can be used as competitive agents to inhibit or reduce an undesirable interaction, e.g., between a natural or pathological agent and the MMP-9.

The MMP-9 binding protein can be used to deliver macro and micromolecules, e.g., a gene into the cell for gene therapy purposes into the endothelium or epithelium and target only
30 those tissues expressing the MMP-9. The binding proteins may be used to deliver a variety of cytotoxic drugs including therapeutic drugs, a compound emitting radiation, molecules of plants,

fungal, or bacterial origin, biological proteins, and mixtures thereof. The cytotoxic drugs can be intracellularly acting cytotoxic drugs, such as short range radiation emitters, including, for example, short range, high energy α emitters, as described herein.

5 In the case of polypeptide toxins, recombinant nucleic acid techniques can be used to construct a nucleic acid that encodes the binding protein (e.g., antibody or antigen-binding fragment thereof) and the cytotoxin (or a polypeptide component thereof) as translational fusions. The recombinant nucleic acid is then expressed, e.g., in cells and the encoded fusion polypeptide isolated.

10 Alternatively, the MMP-9 binding protein can be coupled to high energy radiation emitters, for example, a radioisotope, such as ^{131}I , a γ -emitter, which, when localized at a site, results in a killing of several cell diameters. See, e.g., S.E. Order, "Analysis, Results, and Future Prospective of the Therapeutic Use of Radiolabeled Antibody in Cancer Therapy", *Monoclonal Antibodies for Cancer Detection and Therapy*, R.W. Baldwin et al. (eds.), pp 303 316 (Academic Press 1985). Other suitable radioisotopes include α emitters, such as ^{212}Bi , ^{213}Bi , and ^{211}At , and β emitters, such as ^{186}Re and ^{90}Y . Moreover, ^{177}Lu may also be used as both an imaging and
15 cytotoxic agent.

Radioimmunotherapy (RIT) using antibodies labeled with ^{131}I , ^{90}Y , and ^{177}Lu is under intense clinical investigation. There are significant differences in the physical characteristics of these three nuclides and as a result, the choice of radionuclide is very critical in order to deliver
20 maximum radiation dose to a tissue of interest. The higher beta energy particles of ^{90}Y may be good for bulky tumors. The relatively low energy beta particles of ^{131}I are ideal, but in vivo dehalogenation of radioiodinated molecules is a major disadvantage for internalizing antibody. In contrast, ^{177}Lu has low energy beta particle with only 0.2-0.3 mm range and delivers much lower radiation dose to bone marrow compared to ^{90}Y . In addition, due to longer physical half-
25 life (compared to ^{90}Y), the residence times are higher. As a result, higher activities (more mCi amounts) of ^{177}Lu labeled agents can be administered with comparatively less radiation dose to marrow. There have been several clinical studies investigating the use of ^{177}Lu labeled antibodies in the treatment of various cancers. (Mulligan T et al., 1995, *Clin. Canc. Res.* 1: 1447-1454; Meredith RF, et al., 1996, *J. Nucl. Med.* 37:1491-1496; Alvarez RD, et al., 1997, *Gynecol.*
30 *Oncol.* 65: 94-101).

Exemplary Diseases and Conditions

The MMP-9 binding proteins described herein are useful to treat diseases or conditions in which MMP-9 is implicated, e.g., a disease or condition described herein, or to treat one or more symptoms associated therewith. In some embodiments, the MMP-9 binding protein (e.g., MMP-9 binding IgG or Fab) inhibits MMP-9 activity, e.g., catalytic activity.

Examples of such diseases and conditions include a cancer (e.g., leukemia, B cell lymphoma, multiple myeloma, metastatic cancer, e.g., metastatic breast or colon cancer), an inflammatory disease (e.g., chronic obstructive pulmonary disease (COPD), asthma, rhinitis (e.g., allergic rhinitis), inflammatory bowel disease, synovitis, rheumatoid arthritis), heart failure, septic shock, neuropathic pain, inflammatory pain, osteoarthritis, or an ocular condition (e.g., macular degeneration). A therapeutically effective amount of a MMP-9 binding protein is administered to a subject having or suspected of having a disorder in which MMP-9 is implicated, thereby treating (e.g., ameliorating or improving a symptom or feature of a disorder, slowing, stabilizing or halting disease progression) the disorder.

The MMP-9 binding protein is administered in a therapeutically effective amount. A therapeutically effective amount of an MMP-9 binding protein is the amount which is effective, upon single or multiple dose administration to a subject, in treating a subject, e.g., curing, alleviating, relieving or improving at least one symptom of a disorder in a subject to a degree beyond that expected in the absence of such treatment. A therapeutically effective amount of the composition may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the compound to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the composition is outweighed by the therapeutically beneficial effects.

A therapeutically effective amount can be administered, typically an amount of the compound which is effective, upon single or multiple dose administration to a subject, in treating a subject, e.g., curing, alleviating, relieving or improving at least one symptom of a disorder in a subject to a degree beyond that expected in the absence of such treatment. A therapeutically effective amount of the composition may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the compound to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the composition is outweighed by the therapeutically beneficial effects. A

therapeutically effective dosage preferably modulates a measurable parameter, favorably, relative to untreated subjects. The ability of a compound to inhibit a measurable parameter can be evaluated in an animal model system predictive of efficacy in a human disorder.

Dosage regimens can be adjusted to provide the optimum desired response (e.g., a therapeutic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit contains a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier.

Cancer

Matrix metalloproteases (MMPs), such as MMP-9, are believed to contribute to cancer by cleaving components of the ECM and basement membranes, thereby allowing cancer cells to penetrate and infiltrate the subjacent stromal matrix. Additionally, a number of growth-factor receptors, cell adhesion molecules, chemokines, cytokines, apoptotic ligands, and angiogenic factors are substrates of MMPs. Hence, MMP activity may cause activation of growth factors, suppression of tumor cell apoptosis, destruction of chemokine gradients developed by host immune response, or release of angiogenic factors. MMPs may facilitate tumor growth by promoting the release of cell proliferation factors such as insulin-like growth factors which are bound to specific binding proteins (IGFBPs) (Manes et al., 1997 J. Biol. Chem. 272: 25706-25712).

Collagenases, including MMP-9 and MMP-2, have been found at elevated levels in melanoma and in cancers of the colon, breast, lung, prostate, and bladder. Usually, these elevated levels correlate with higher tumor grade and invasiveness. MMP-2 levels are significantly elevated in the serum of patients with metastatic lung cancer, and in those patients with high levels, response to chemotherapy is diminished. MMP-9 may contribute to tumor invasiveness and recurrence.

Accordingly, the disclosure provides methods of treating (e.g., slowing, eliminating, or reversing tumor growth, preventing or reducing, either in number or size, metastases, reducing or eliminating tumor cell invasiveness, providing an increased interval to tumor progression, or increasing disease-free survival time) cancer (e.g., breast cancer, including Her2+, Her2-, ER+, ER-, Her2+/ER+, Her2+/ER-, Her2-/ER+, and Her2-/ER- breast cancer), head and neck cancer, oral cavity cancer, laryngeal cancer, chondrosarcoma, ovarian cancer, lung cancer, prostate cancer, colon cancer (e.g., primary or metastatic colon cancer), testicular carcinoma, melanoma, leukemia, B cell lymphoma, multiple myeloma, brain tumors (e.g., astrocytomas, glioblastomas, gliomas)) by administering an effective amount of an MMP-9 binding protein (e.g., an anti-MMP-9 IgG or Fab). In some embodiments, the MMP-9 binding protein inhibits MMP-9 activity.

In certain embodiments, the MMP-9 binding protein is administered as a single agent treatment. In other embodiments, the MMP-9 binding protein is administered in combination with an additional anti-cancer agent.

Also provided are methods of preventing or reducing risk of developing cancer, by administering an effective amount of an MMP-9 binding protein to a subject at risk of developing cancer, thereby reducing the subject's risk of developing a cancer.

The disclosure further provides methods of modulating (e.g. reducing or preventing) angiogenesis at a tumor site by administering an effective amount of an MMP-9 binding protein, thereby reducing or preventing angiogenesis at the tumor site. The MMP-9 binding protein may be administered as a single agent therapy or in combination with additional agents.

Also provided are methods for reducing extracellular matrix (ECM) degradation by a tumor, comprising administering an effective amount of an MMP-9 binding protein to a subject, thereby reducing ECM degradation by a tumor in the subject.

The disclosed methods are useful in the prevention and treatment of solid tumors, soft tissue tumors, and metastases thereof. Solid tumors include malignancies (e.g., sarcomas, adenocarcinomas, and carcinomas) of the various organ systems, such as those of lung, breast, lymphoid, gastrointestinal (e.g., colon), and genitourinary (e.g., renal, urothelial, or testicular tumors) tracts, pharynx, prostate, and ovary. Exemplary adenocarcinomas include colorectal cancers, renal-cell carcinoma, liver cancer, non-small cell carcinoma of the lung, and cancer of the small intestine. Additional exemplary solid tumors include: fibrosarcoma, myxosarcoma,

liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, gastrointestinal system carcinomas, colon carcinoma, pancreatic cancer, breast cancer, genitourinary system carcinomas, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, endocrine system carcinomas, testicular tumor, lung carcinoma, small cell lung carcinoma, non-small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma, and retinoblastoma. Metastases of the aforementioned cancers can also be treated or prevented in accordance with the methods described herein. Blood cancers (e.g., leukemia, B cell lymphoma) and multiple myeloma can also be treated and/or prevented using the methods described herein.

Guidance for determination of a therapeutically effective amount for treatment of cancer may be obtained by reference to *in vivo* models of the cancer to be treated. For example, the amount of a MMP-9 binding protein that is a therapeutically effective amount in a rodent or Libechov minipig model of cancer may be used to guide the selection of a dose that is a therapeutically effective amount. A number of rodent models of human cancers are available, including nude mouse/tumor xenograft systems (e.g., melanoma xenografts; see, e.g., Trikha et al. Cancer Research 62:2824-2833 (2002)) and murine models of breast cancer or glioma (e.g., Kuperwasser et al., Cancer Research 65, 6130-6138, (2005); Bradford et al., Br J Neurosurg. 3(2):197-210 (1989)). A melanoblastoma-bearing Libechov minipig (MeLiM) is available as an animal model of melanoma (e.g., Boisgard et al., Eur J Nucl Med Mol Imaging 30(6):826-34 (2003)).

Synovitis

Synovitis is a condition characterized by inflammation of the synovium, a tissue normally only a few cell layers thick. In synovitis, the synovium can become thickened, more cellular,

and engorged with fluid. Synovitis can cause pain and inflammation within the affected joint, and is commonly seen in arthritic conditions (e.g., rheumatoid arthritis).

Active synovial MMP-2 is associated with radiographic erosions in patients with early synovitis (Goldbach-Mansky et al, 2000, Arthritis Res, 2:145-153). Synovial tissue expressions of MMP-2 and TIMP-2 are virtually undetectable in normal synovial tissue samples. The synovial tissue samples of patients with erosive disease have significantly higher levels of active MMP-2 than did those of patients without erosions. This may reflect augmented activation of MMP-2 by increased levels of MMP-9 and low levels of TIMP-2 seen in these tissues. Thus, active MMP-2 can contribute to the development and/or progression of rheumatoid arthritis and osteoarthritis.

Increased levels of MMP-9 have been found in the synovial fluid in subjects with arthritis (compared with normal individuals). The disclosure provides methods of treating (e.g., ameliorating, stabilizing, reducing, or eliminating a symptom of synovitis such as pain, joint swelling, synovial thickening, increased synovial fluid) synovitis by administering a therapeutically effective amount of a MMP-9 binding protein. Also provided are methods which combine MMP-9 binding protein therapy with additional therapies. Current therapies for synovitis include anti-inflammatory medications (e.g. NSAIDS and ibuprofen), cortisone injections into the joint, and surgical treatment (e.g., synovectomy). One or more of these treatments can be used in combination with an MMP-9 binding protein (e.g., an inhibitory MMP-9 binding protein, e.g., an anti-MMP-9 IgG or Fab) to treat this condition.

Guidance for determination of a therapeutically effective amount of an MMP-9 binding protein may be obtained from an animal model of synovitis. Rodent models of synovitis are available, including a rat model of synovitis-like inflammation (Cirino et al., J Rheumatol. 21(5):824-9 (1994)), and a model of carrageenan synovitis in male Wistar rats (Walsh et al. Lab Invest. 78(12):1513-21 (1998)).

Rheumatoid Arthritis and Associated Conditions

Rheumatoid arthritis (RA) is an autoimmune, chronic inflammatory disease that causes joint swelling and pain and normally results in joint destruction. RA generally follows a relapsing/remitting course, with “flares” of disease activity interspersed with remissions of disease symptoms. RA is associated with a number of additional inflammatory disorders,

including Sjogren's syndrome (dry eyes and mouth caused by inflammation of tear and saliva glands), pleuritis (inflammation of the pleura that causes pain upon deep breath and coughing), rheumatoid nodules (nodular sites of inflammation that develop within the lungs), pericarditis (inflammation of the pericardium that causes pain when lying down or leaning forward), Felty syndrome (splenomegaly and leucopenia observed in conjunction with RA, making the subject prone to infection), and vasculitis (an inflammation of the blood vessels which can block blood flow). MMP-9 and MMP-16 have been implicated in rheumatoid arthritis.

Symptoms of active RA include fatigue, lack of appetite, low grade fever, muscle and joint aches, and stiffness. Muscle and joint stiffness are usually most notable in the morning and after periods of inactivity. During flares, joints frequently become red, swollen, painful, and tender, generally as a consequence of synovitis.

Treatment for rheumatoid arthritis involves a combination of medications, rest, joint strengthening exercises, and joint protection. Two classes of medications are used in treating rheumatoid arthritis: anti-inflammatory "first-line drugs," and Disease-Modifying Antirheumatic Drugs (DMARDs)." The first-line drugs, include NSAIDS (e.g., aspirin, naproxen, ibuprofen, and etodolac) and cortisone (corticosteroids). DMARDS, such as gold (e.g., gold salts, gold thioglucose, gold thiomalate, oral gold), methotrexate, sulfasalazine, D-penicillamine, azathioprine, cyclophosphamide, chlorambucil, and cyclosporine, leflunomide, etanercept, infliximab, anakinra, and adalimumab, and hydroxychloroquine, promote disease remission and prevent progressive joint destruction, but they are not anti-inflammatory agents.

Increased levels of MMP-9 have been found in the synovial fluid in subjects with arthritis (compared with normal individuals). The disclosure provides methods of treating (e.g., ameliorating, stabilizing, or eliminating one or more symptoms or ameliorating or stabilizing the subject's score on a RA scale) rheumatoid arthritis by administering a therapeutically effective amount of a MMP-9 binding protein to a subject having or suspected of having RA. Additionally provided are methods of treating RA by administering a therapeutically effective amount of a MMP-9 binding protein and at least one NSAID and/or DMARDS.

Further provided are methods of treating (e.g., ameliorating, stabilizing, or eliminating one or more symptoms) rheumatoid arthritis associated disorders (Sjogren's syndrome, pleuritis, pulmonary rheumatoid nodules, pericarditis, Felty syndrome, and vasculitis) by administering a therapeutically effective amount of an MMP-9 binding protein.

Scales useful for assessing RA and symptoms of RA include the Rheumatoid Arthritis Severity Scale (RASS; Bardwell et al., (2002) *Rheumatology* **41**(1):38-45), SF-36 Arthritis Specific Health Index (ASHI; Ware et al., (1999) *Med. Care.* **37**(5 Suppl):MS40-50), Arthritis Impact Measurement Scales or Arthritis Impact Measurement Scales 2 (AIMS or AIMS2; Meenan et al. (1992) *Arthritis Rheum.* **35**(1):1-10); the Stanford Health Assessment Questionnaire (HAQ), HAQII, or modified HAQ (see, *e.g.*, Pincus et al. (1983) *Arthritis Rheum.* **26**(11):1346-53).

Guidance for the determination of the dosage that delivers a therapeutically effective amount of a MMP-9 binding protein may be obtained from animal models of rheumatoid arthritis, such as collagen-induced arthritis (CIA), which is induced, typically in rodents, by immunization with autologous or heterologous type II collagen in adjuvant (Williams et al. *Methods Mol Med.* 98:207-16 (2004)).

COPD

Chronic Obstructive Pulmonary Disease (COPD), also known as chronic obstructive airway disease (COAD), is a group of diseases characterized by the pathological limitation of airflow in the airway that is not fully reversible. COPD is the umbrella term for chronic bronchitis, emphysema and a range of other lung disorders. It is most often due to tobacco smoking, but can be due to other airborne irritants such as coal dust, asbestos or solvents, as well as congenital conditions such as alpha-1-antitrypsin deficiency.

The main symptoms of COPD include dyspnea (shortness of breath) lasting for months or perhaps years, possibly accompanied by wheezing, and a persistent cough with sputum production. It is possible the sputum may contain blood (hemoptysis) and become thicker, usually due to damage of the blood vessels of the airways. Severe COPD could lead to cyanosis caused by a lack of oxygen in the blood. In extreme cases it could lead to cor pulmonale due to the extra work required by the heart to get blood to flow through the lungs.

COPD is particularly characterised by the spirometric measurement of a ratio of forced expiratory volume over 1 second (FEV₁) to forced vital capacity (FVC) being < 0.7 and the FEV₁ < 80% of the predicted value as measured by a plethysmograph. Other signs include a rapid breathing rate (tachypnea) and a wheezing sound heard through a stethoscope. Pulmonary

emphysema is NOT the same as subcutaneous emphysema, which is a collection of air under the skin that may be detected by the crepitus sounds produced on palpation.

Treatment for COPD includes inhalers that dilate the airways (bronchodilators) and sometimes theophylline. The COPD patient must stop smoking. In some cases inhaled steroids
5 are used to suppress lung inflammation, and, in severe cases or flare-ups, intravenous or oral steroids are given. Antibiotics are used during flare-ups of symptoms as infections can worsen COPD. Chronic, low-flow oxygen, non-invasive ventilation, or intubation may be needed in some cases. Surgery to remove parts of the diseased lung has been shown to be helpful for some patients with COPD. Lung rehabilitation programs may help some patients. Lung transplant is
10 sometimes performed for severe cases. Bronchodilators that can be used include:

There are several types of bronchodilators used clinically with varying efficacy: for example, β_2 agonists, M_3 antimuscarinics, leukotriene antagonists, cromones, corticosteroids, and xanthines. These drugs relax the smooth muscles of the airway allowing for improved airflow. β_2 agonists include: Salbutamol (Ventolin), Bambuterol, Clenbuterol, Fenoterol, and Formoterol,
15 and long acting β_2 agonists (LABAs) such as Salmeterol. M_3 muscarinic antagonists (anticholinergics) include the quaternary M_3 muscarinic antagonist Ipratropium, which is widely prescribed with the β_2 agonist salbutamol, Ipratropium, and Tiotropium, which can be combined with a LABA and inhaled steroid. Cromones include Cromoglicate and Nedocromil. Leukotriene antagonists can be used and include Montelukast, Pranlukast, Zafirlukast. Xanthines include
20 theophylline, methylxanthines, theobromine. More aggressive EMR interventions include IV H_1 antihistamines and IV dexamethasone. Phosphodiesterase-4 antagonists include roflumilast and cilomilast. Corticosteroids can be used and include glucocorticoids, beclomethasone, mometasone, and fluticasone. Corticosteroids are often combined with bronchodilators in a single inhaler. Salmeterol and fluticasone can be combined (Advair). TNF antagonists include
25 cachexin, cachectin infliximab, adalimumab and etanercept.

The disclosure provides methods of treating COPD (e.g., ameliorating symptoms or the worsening of COPD) by administering a therapeutically effective amount of a MMP-9 binding protein (e.g., an inhibitory MMP-9 binding protein, e.g., an anti-MMP-9 IgG or Fab) to a subject having or suspected of having COPD. Also provided are methods of treating COPD by
30 administering a therapeutically effective amount of a MMP-9 binding protein with another

COPD treatment (e.g., β_2 agonists, M_3 antimuscarinics, leukotriene antagonists, cromones, corticosteroids, and xanthines).

Guidance regarding the efficacy and dosage an MMP-9 binding protein which will deliver a therapeutically effective amount of the protein can be obtained from an animal model of
5 COPD, see e.g., PCT publication WO 2007/084486 and references cited therein.

Asthma

Asthma is a chronic condition involving the respiratory system in which the airway occasionally constricts, becomes inflamed, and is lined with excessive amounts of mucus, often in response to one or more triggers. These episodes may be triggered by such things as exposure
10 to an environmental stimulant (or allergen) such as cold air, warm air, moist air, exercise or exertion, or emotional stress. In children, the most common triggers are viral illnesses such as those that cause the common cold. This airway narrowing causes symptoms such as wheezing, shortness of breath, chest tightness, and coughing. The airway constriction responds to bronchodilators.

15 In some individuals asthma is characterized by chronic respiratory impairment. In others it is an intermittent illness marked by episodic symptoms that may result from a number of triggering events, including upper respiratory infection, stress, airborne allergens, air pollutants (such as smoke or traffic fumes), or exercise. Some or all of the following symptoms may be present in those with asthma: dyspnea, wheezing, stridor, coughing, an inability for physical
20 exertion. Some asthmatics who have severe shortness of breath and tightening of the lungs never wheeze or have stridor and their symptoms may be confused with a COPD-type disease.

An acute exacerbation of asthma is commonly referred to as an asthma attack. The clinical hallmarks of an attack are shortness of breath (dyspnea) and either wheezing or stridor.

During an asthma episode, inflamed airways react to environmental triggers such as
25 smoke, dust, or pollen. The airways narrow and produce excess mucus, making it difficult to breathe. In essence, asthma is the result of an immune response in the bronchial airways.

The airways of asthmatics are "hypersensitive" to certain triggers/stimuli. In response to exposure to these triggers, the bronchi (large airways) contract into spasm (an "asthma attack"). Inflammation soon follows, leading to a further narrowing of the airways and excessive mucus
30 production, which leads to coughing and other breathing difficulties.

The most effective treatment for asthma is identifying triggers, such as pets or aspirin, and limiting or eliminating exposure to them. Desensitization is currently the only known "cure" to the disease.

Symptomatic control of episodes of wheezing and shortness of breath is generally
5 achieved with fast-acting bronchodilators.

Relief medication: Short-acting, selective beta₂-adrenoceptor agonists, such as salbutamol (*albuterol* USAN), levalbuterol, terbutaline and bitolterol, can be used. Older, less selective adrenergic agonists, such as inhaled epinephrine and ephedrine tablets, can be used. Anticholinergic medications, such as ipratropium bromide may be used.

10 Preventative medication: Current treatment protocols recommend prevention medications such as an inhaled corticosteroid, which helps to suppress inflammation and reduces the swelling of the lining of the airways, in anyone who has frequent (greater than twice a week) need of relievers or who has severe symptoms. If symptoms persist, additional preventive drugs are added until the asthma is controlled. With the proper use of prevention drugs, asthmatics can
15 avoid the complications that result from overuse of relief medications. Preventive agents include: inhaled glucocorticoids (e.g., ciclesonide, beclomethasone, budesonide, flunisolide, fluticasone, mometasone, and triamcinolone), leukotriene modifiers (e.g., montelukast, zafirlukast, pranlukast, and zileuton), mast cell stabilizers (e.g., cromoglicate (cromolyn), and nedocromil), antimuscarinics/anticholinergics (e.g., ipratropium, oxitropium, and tiotropium), methylxanthines
20 (e.g., theophylline and aminophylline), antihistamines, an IgE blocker such as omalizumab, methotrexate).

Long-acting beta₂-adrenoceptor agonists can be used and include salmeterol, formoterol, bambuterol, and sustained-release oral albuterol. Combinations of inhaled steroids and long-acting bronchodilators are becoming more widespread; the most common combination currently
25 in use is fluticasone/salmeterol (Advair in the United States, and Seretide in the United Kingdom). Another combination is budesonide/formoterol which is commercially known as Symbicort.

Concentrations of MMP-9 are increased in the bronchoalveolar lavage fluid (BAL), sputum, bronchi, and serum of asthmatic subjects compared with normal individuals. The
30 disclosure provides methods of treating asthma (e.g., ameliorating symptoms or the worsening of asthma) by administering a therapeutically effective amount of a MMP-9 binding protein (e.g.,

an inhibitory MMP-9 binding protein, e.g., an anti-MMP-9 IgG or Fab) to a subject having or suspected of having asthma. Also provided are methods of treating asthma by administering a therapeutically effective amount of a MMP-9 binding protein with another asthma treatment (e.g., glucocorticoids, leukotriene modifiers, mast cell stabilizers,

5 antimuscarinics/anticholinergics, antihistamines, an IgE blocker, methotrexate.

Guidance regarding the efficacy and dosage an MMP-9 binding protein which will deliver a therapeutically effective amount of the protein can be obtained from an animal model of asthma, see e.g., US Pat. No. 5,602,302, or European Pat. No. EP1192944 B1, and references cited therein.

10 Rhinitis

Rhinitis is the medical term describing irritation and inflammation of some internal areas of the nose. The primary symptom of rhinitis is a runny nose. It is caused by chronic or acute inflammation of the mucous membrane of the nose due to viruses, bacteria or irritants. The inflammation results in the generating of excessive amounts of mucus producing a runny nose, nasal congestion and post-nasal drip. Rhinitis has also been found to adversely affect more than just the nose, throat, and eyes. It has been associated with sleeping problems, problems with the ears, and even been linked to learning problems. Rhinitis is caused by an increase in histamine. This increase is likely caused by airborne allergens. These allergens may affect an individual's nose, throat, or eyes and cause an increase in fluid production within these areas. There are two types of Rhinitis that the general population may suffer from: allergic rhinitis and nonallergic rhinitis. Rhinitis is considered IgE-mediated when the sufferer is classified as having allergic rhinitis.

The typical method of diagnosis and monitoring of allergic rhinitis is skin testing, also known as "scratch testing" and "prick testing" due to the series of pricks and/or scratches made into the patient's skin. Small amounts of suspected allergens and/or their extracts (pollen, grass, mite proteins, peanut extract, etc.) are introduced to sites on the skin marked with pen or dye.

The management of rhinitis is mainly medical. Treatment for seasonal rhinitis is only needed during the appropriate time of the year. Current treatments include: antihistamine pills and sprays, leukotriene antagonists, nasal corticosteroid sprays, decongestant pills or sprays,

allergen immunotherapy, saline irrigation of sinus cavities through the use of a neti pot or by other means; nasal obstruction in perennial rhinitis may be treated by surgery.

The disclosure provides methods of treating rhinitis (e.g., allergic rhinitis) (e.g., ameliorating symptoms or the worsening of rhinitis) by administering a therapeutically effective amount of a MMP-9 binding protein (e.g., an inhibitory MMP-9 binding protein, e.g., an anti-MMP-9 IgG or Fab) to a subject having or suspected of having rhinitis. Also provided are methods of treating rhinitis by administering a therapeutically effective amount of a MMP-9 binding protein with another rhinitis treatment (e.g., β_2 agonists, M_3 antimuscarinics, leukotriene antagonists, cromones, corticosteroids, and xanthines).

Guidance regarding the efficacy and dosage of an MMP-9 binding protein which will deliver a therapeutically effective amount of the protein can be obtained from an animal model of rhinitis, see e.g., Zhao et al. (2005) *Rhinology* **43**:47-54, and references cited therein.

IBD

Inflammatory bowel disease (IBD) is a group of inflammatory conditions of the large intestine and, in some cases, the small intestine. The main forms of IBD are Crohn's disease and ulcerative colitis (UC). Accounting for far fewer cases are other forms of IBD: Collagenous colitis, Lymphocytic colitis, Ischaemic colitis, Diversion colitis, Behçet's syndrome, Infective colitis, and Indeterminate colitis.

The main difference between Crohn's disease and UC is the location and nature of the inflammatory changes. Crohn's can affect any part of the gastrointestinal tract, from mouth to anus (skip lesions), although a majority of the cases start in the terminal ileum. Ulcerative colitis, in contrast, is restricted to the colon and the rectum.

Microscopically, ulcerative colitis is restricted to the mucosa (epithelial lining of the gut), while Crohn's disease affects the whole bowel wall.

Finally, Crohn's disease and ulcerative colitis present with extra-intestinal manifestations (such as liver problems, arthritis, skin manifestations and eye problems) in different proportions.

Rarely, a definitive diagnosis of neither Crohn's disease nor ulcerative colitis can be made because of idiosyncrasies in the presentation. In this case, a diagnosis of indeterminate colitis may be made.

Diagnosis: Although very different diseases, both may present with any of the following symptoms: abdominal pain, vomiting, diarrhea, hematochezia, weight loss, weight gain and various associated complaints or diseases (arthritis, pyoderma gangrenosum, primary sclerosing cholangitis). Diagnosis is generally by colonoscopy with biopsy of pathological lesions.

5 Treatment: Depending on the level of severity, IBD may require immunosuppression to control the symptoms. Immunosuppressives such as azathioprine, methotrexate, or 6-mercaptopurine can be used. More commonly, treatment of IBD requires a form of mesalamine. Often, steroids are used to control disease flares and were once acceptable as a maintenance drug. In use for several years in Crohns disease patients and recently in patients with Ulcerative
10 Colitis, biologicals, such as Remicade, have been used. Severe cases may require surgery, such as bowel resection, strictureplasty or a temporary or permanent colostomy or ileostomy. Alternative medicine treatments for bowel disease exist in various forms, however such methods concentrate on controlling underlying pathology in order to avoid prolonged steroidal exposure or surgical excisement.

15 Usually the treatment is started by administering drugs, such as Prednisone, with high anti-inflammatory affects. Once the inflammation is successfully controlled, the patient is usually switched to a lighter drug, such as Asacol- a mesalamine, to keep the disease in remission. If unsuccessful, a combination of the aforementioned immunosurpression drugs with a mesalamine (which may also have an anti-inflammatory effect) may or may not be
20 administered, depending on the patient.

The disclosure provides methods of treating IBD (e.g., ameliorating symptoms or the worsening of IBD) by administering a therapeutically effective amount of a MMP-9 binding protein (e.g., an inhibitory MMP-9 binding protein, e.g., an anti-MMP-9 IgG or Fab) to a subject having or suspected of having IBD. Also provided are methods of treating IBD by administering
25 a therapeutically effective amount of a MMP-9 binding protein with another IBD treatment (e.g., azathioprine, methotrexate, 6-mercaptopurine, a mesalamine, Remicade).

Guidance regarding the efficacy and dosage an MMP-9 binding protein which will deliver a therapeutically effective amount of the protein can be obtained from animal models of IBD, see e.g., those described in US Pat. No. 6,114,382, PCT publication WO 2004/071186 and
30 references cited therein.

Ocular Conditions

Macular Degeneration. Macular degeneration progressively destroys the macula, the central portion of the retina, impairing central vision, leading to difficulty with reading, driving, and/or other daily activities that require fine central vision. While there are a number of different forms of macular degeneration, the most common is age-related macular degeneration (AMD). AMD presents as either “dry” or “wet”, with the wet type being far more common. In wet AMD, fluid leaking from newly formed subretinal blood vessels (subretinal neovascularization) distorts the macula and impairs vision. Symptoms of AMD include loss or impairment in central vision (generally slowing in dry AMD and rapidly in wet AMD) and abnormal visual perception of straight lines (*e.g.*, straight lines appear wavy). Supplements of zinc and the antioxidants vitamin C, vitamin E and beta-carotene reportedly slow the progression of wet AMD.

The disclosure provides methods of treating (*e.g.*, ameliorating vision, stabilizing vision degradation, or reducing the rate of vision degradation) AMD (wet AMD or dry AMD) by administering a therapeutically effective amount of a MMP-9 binding protein (*e.g.*, an inhibitory MMP-9 binding protein, *e.g.*, an anti-MMP-9 IgG or Fab) to a subject having or suspected of having AMD. Also provided are methods of treating AMD by administering a therapeutically effective amount of a MMP-9 binding protein with another AMD treatment (*e.g.*, zinc, vitamin C, vitamin E and/or beta-carotene).

Guidance regarding the efficacy and dosage an MMP-9 binding protein which will deliver a therapeutically effective amount of the protein can be obtained from an animal model of macular degeneration, *e.g.*, a *Coturnix coturnix japonica* (Japanese quail) model of macular degeneration (US Pat. No. 5,854,015), or wound creation on the Bruch's membrane of a C57BL/6J mouse, *e.g.*, with a krypton laser (US App. No. 20030181531).

Corneal Disease. Keratoconus is a progressive disease where the cornea thins and changes shape. The resulting distortion (astigmatism) frequently causes nearsightedness. Keratoconus may also cause swelling and scarring of the cornea and vision loss.

The disclosure provides methods of treating (*e.g.*, improving or stabilizing vision, or improving, stabilizing, reducing eliminating, or preventing corneal scarring) keratoconus in a subject having or suspected of having keratoconus by administering an effective amount of a MMP-9 binding protein (*e.g.*, an inhibitory MMP-9 binding protein, *e.g.*, an anti-MMP-9 IgG or Fab).

Guidance regarding the efficacy and dosage an MMP-9 binding protein which will deliver a therapeutically effective amount of the protein can be obtained from an animal model of keratoconus, e.g., the inbred SKC mouse line, which serves as a model for a subset of keratoconus (Tachibana et al. *Investig Ophthalmol Visual Sci*, 43:51-57 (2002)).

5 *Corneal Infection.* Also provided are methods of treating (e.g., preventing, reducing, stabilizing or eliminatnig corneal scarring as a result of the infection) corneal infection by administering an effective amount of a MMP-9 binding protein (e.g., an inhibitory MMP-9 binding protein, e.g., an anti-MMP-9 IgG or Fab) to a subject having or suspected of having a corneal infection. Additionally, methods are provided for treatment of corneal infection by
10 administering a MMP-9 binding protein and a therapeutic agent which treats the infectious agent (e.g., an antibiotic or anti-viral agent).

Guidance regarding the efficacy and dosage an MMP-9 binding protein which will deliver a therapeutically effective amount of the protein can be obtained from an animal model of corneal infection, e.g., a rabbit model of experimental keratomycosis, in which keratitis is
15 induced with a standardized inoculum of *Candida albicans* (SC 5314) placed on a debrided cornea (Goldblum et al. *Antimicrob Agents Chemother* 49:1359-1363 (2005)).

Osteoarthritis

Osteoarthritis, also known as degenerative arthritis, is characterized by the breakdown and eventual loss of the cartilage of one or more joints. Osteoarthritis commonly affects the
20 hands, feet, spine, and large weight-bearing joints, such as the hips and knees. The disclosure provides methods of treating (e.g., stabilizing, reducing, or eliminating joint pain, stabilizing or improving performance on general health or osteoarthritis scales) osteoarthritis by administering a therapeutically effective amount of a MMP-9 binding protein (e.g., an inhibitory MMP-9 binding protein, e.g., an anti-MMP-9 IgG or Fab) to a subject having or suspected of having
25 osteoarthritis.

Current medical treatment of osteoarthritis includes conservative measures (e.g., rest, weight reduction, physical and occupational therapy) and medications such as acetaminophen, pain-relieving creams applied to the skin over the joints such as capsaicin, salycin, methyl salicylate, and menthol, nonsteroidal anti-inflammatory drugs (NSAIDs) such as aspirin,
30 ibuprofen, nabumetone, and naproxen, and Cox-2 inhibitors. The disclosure further provides

methods of treating osteoarthritis by administering a therapeutically effective amount of a MMP-9 binding protein (e.g., an inhibitory MMP-9 binding protein, e.g., an anti-MMP-9 IgG or Fab) and another osteoarthritis therapy (e.g. acetaminophen, a topical pain-relieving cream, a nonsteroidal anti-inflammatory drug (NSAID) such as aspirin, ibuprofen, nabumetone, or
 5 naproxen, or a Cox-2 inhibitor).

Scales useful for the assessment of osteoarthritis include the Knee Injury and Osteoarthritis Outcome Score (KOOS; Roos et al. (1998) *J. Orthop. Sports Phys. Ther.* **28**(2):88-96), Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC; Roos et al. (2003) *Health Qual. Life Outcomes* **1**(1):17), and the 36-item Short Form General Health Scale
 10 (SF-36 GHS), as well as other assessment tools known in the art.

Guidance regarding the efficacy and dosage an MMP-9 binding protein which will deliver a therapeutically effective amount of the protein can be obtained from an animal model of osteoarthritis, e.g., injection of mono-iodoacetate (MIA) into the femorotibial joint of rodents which promotes loss of articular cartilage similar to that noted in human osteoarthritis (Guzman
 15 et al. *Toxicol Pathol.* **31**(6):619-24 (2003)), or transection of the anterior cruciate ligament (ACL) in canines to induce osteoarthritis (Fife and Brandt *J Clin Invest.* **84**(5): 1432–1439 (1989)).

Heart Failure

Heart failure is caused by any condition which reduces the efficiency of the myocardium,
 20 or heart muscle, through damage or overloading. As such, it can be caused by as diverse an array of conditions as myocardial infarction, hypertension and amyloidosis. Over time these increases in workload will produce changes to the heart itself. Congestive heart failure (CHF), congestive cardiac failure (CCF) or just heart failure, is a condition that can result from any structural or functional cardiac disorder that impairs the ability of the heart to fill with or pump a
 25 sufficient amount of blood through the body.

Other related terms include ischemic cardiomyopathy (implying that the cause of heart failure is coronary artery disease) and dilated cardiomyopathy (which is a description of echocardiographic findings characteristic of heart failure but which does not suggest any specific etiology).

Congestive heart failure exacerbation or decompensated heart failure (DHF) refer to episodes in which a patient with known chronic heart failure acutely develops symptoms.

Symptoms are dependent on two factors. The first is based on the side of the heart, right or left, that is involved. The second factor is based on the type of failure, either diastolic or
5 systolic. Symptoms and presentation may be indistinguishable making diagnosis impossible based on symptoms.

Given that the left side of the heart pumps blood from the lungs to the organs, failure to do so leads to congestion of the lung veins and symptoms that reflect this, as well as reduced supply of blood to the tissues. The predominant respiratory symptom is shortness of breath on
10 exertion (dyspnea, *dyspnée d'effort*) - or in severe cases at rest - and easy fatigueability. Orthopnea is increasing breathlessness on reclining. Paroxysmal nocturnal dyspnea is a nighttime attack of severe breathlessness, usually several hours after going to sleep. Poor circulation to the body leads to dizziness, confusion and diaphoresis and cool extremities at rest.

The right side of the heart pumps blood returned from the tissues to the lungs to exchange
15 CO₂ for O₂. Hence, failure of the right side leads to congestion of peripheral tissues. This may lead to peripheral edema or anasarca and nocturia. In more severe cases, ascites and hepatomegaly may develop.

Heart failure may decompensate easily; this may occur as the result of any intercurrent illness (such as pneumonia), but specifically myocardial infarction, anaemia, hyperthyroidism or
20 arrhythmias. These place additional strain on the heart muscle, which may cause symptoms to rapidly worsen. Excessive fluid or salt intake (including intravenous fluids for unrelated indications, but more commonly from dietary indiscretion), and medication that causes fluid retention (such as NSAIDs and thiazolidinediones), may also precipitate decompensation.

In examining a patient with possible heart failure, a health professional would look for
25 particular signs. General signs indicating heart failure are a laterally displaced apex beat (as the heart is enlarged) and a gallop rhythm (additional heart sounds) in case of decompensation. Heart murmurs may indicate the presence of valvular heart disease, either as a cause (e.g. aortic stenosis) or as a result (e.g. mitral regurgitation) of the heart failure.

Predominant left-sided clinical signs are tachypnea and increased work of breathing
30 (signs of respiratory distress not specific to heart failure), rales or crackles, which suggests the development of pulmonary edema, dullness of the lung fields to percussion and diminished

breath sounds at the bases of the lung, which suggests the development of a pleural effusion (fluid collection in the pleural cavity) that is transudative in nature, and cyanosis which suggests hypoxemia, caused by the decreased rate of diffusion of oxygen from fluid-filled alveoli to the pulmonary capillaries.

5 Right-sided signs are peripheral edema, ascites and hepatomegaly, an increased jugular venous pressure, which can be increased further by the hepatojugular reflux, and a parasternal heave.

Causes of left-side heart failure include: hypertension (high blood pressure), aortic and mitral valve disease, aortic coarctation. Causes of right-side heart failure include pulmonary
10 hypertension (e.g. due to chronic lung disease), pulmonary or tricuspid valve disease. Causes of both types include: Ischemic heart disease (due to insufficient vascular supply, usually as a result of coronary artery disease); this may be chronic or due to acute myocardial infarction (a heart attack), chronic arrhythmias (e.g. atrial fibrillation), cardiomyopathy of any cause, cardiac fibrosis, chronic severe anemia, thyroid disease (hyperthyroidism and hypothyroidism).

15 Treatments of heart failure include: moderate physical activity, bed rest, weight reduction, monitoring weight, sodium restriction, fluid restriction, diuretic agents, vasodilator agents, positive inotropes, ACE inhibitors, beta blockers, and aldosterone antagonists (e.g., spironolactone), angiotensin II receptor antagonist therapy (also referred to as AT₁-antagonists or angiotensin receptor blockers) (particularly using candesartan). Diuretics include loop diuretics
20 (e.g., furosemide, bumetanide), thiazide diuretics (e.g., hydrochlorothiazide, chlorthalidone, chlorthiazide), potassium-sparing diuretics (e.g., amiloride), spironolactone, eplerenone. Beta blockers include bisoprolol, carvedilol, and extended-release metoprolol. Positive inotropes include digoxin, dobutamine. Phosphodiesterase inhibitors such as milrinone are sometimes utilized in severe cardiomyopathy. Alternative vasodilators include the combination of
25 isosorbide dinitrate/hydralazine. Aldosterone receptor antagonists include spironolactone and the related drug eplerenone. Recombinant neuroendocrine hormones can also be used and include Nesiritide, a recombinant form of B-natriuretic peptide. Vasopressin receptor antagonists that can be used include tolvaptan and conivaptan. Devices and surgery options include cardiac resynchronization therapy (CRT; pacing both the left and right ventricles), through implantation
30 of an bi-ventricular pacemaker, or surgical remodelling of the heart, an implantable cardioverter-defibrillator (ICD), left ventricular assist devices (LVADs).

The disclosure provides methods of treating heart failure (e.g., ameliorating symptoms or the worsening of heart failure) by administering a therapeutically effective amount of a MMP-9 binding protein (e.g., an inhibitory MMP-9 binding protein, e.g., an anti-MMP-9 IgG or Fab) to a subject having or suspected of having heart failure. Also provided are methods of treating heart failure by administering a therapeutically effective amount of a MMP-9 binding protein with another heart failure treatment (e.g., a diuretic agent, a vasodilator agent, a positive inotrope, an ACE inhibitor, a beta blocker, and an aldosterone antagonist (e.g., spironolactone), angiotensin II receptor antagonist therapy (also referred to as AT₁-antagonists or angiotensin receptor blockers)).

Guidance regarding the efficacy and dosage an MMP-9 binding protein which will deliver a therapeutically effective amount of the protein can be obtained from an animal model of heart failure, see e.g., US Pat. No. 7,166,762 and references cited therein.

Septic Shock

Septic shock is a serious medical condition caused by decreased tissue perfusion and oxygen delivery as a result of infection and sepsis. It can cause multiple organ failure and death. Its most common victims are children, immunocompromised individuals, and the elderly, as their immune systems cannot cope with the infection as well as healthy adults are able. The mortality rate from septic shock is approximately 50%.

Symptoms include: Refractory hypotension - hypotension despite adequate fluid resuscitation. In adults it is defined as a systolic blood pressure < 90 mmHg, or a MAP < 60 mmHg, without the requirement for inotropic support, or a reduction of 40 mmHg in the systolic blood pressure from baseline. In children, it is BP < 2 SD of the normal blood pressure. In addition to the two criteria above, two or more of the following can be present: Hyperventilation (high respiratory rate) > 20 breaths per minute or, on blood gas, a P_aCO₂ less than 32 mmHg, and/or White blood cell count < 4000 cells/mm³ or > 12000 cells/mm³ (< 4 x 10⁹ or > 12 x 10⁹ cells/L).

A subclass of distributive shock, shock refers specifically to decreased tissue perfusion resulting in end-organ dysfunction. Cytokines TNF α , IL-1 β , IL-6 released in a large scale inflammatory response results in massive vasodilation, increased capillary permeability, decreased systemic vascular resistance, and hypotension. Hypotension reduces tissue perfusion

pressure and thus tissue hypoxia ensues. Finally, in an attempt to offset decreased blood pressure, ventricular dilatation and myocardial dysfunction will occur.

The process of infection by bacteria or fungi can result in systemic signs and symptoms that are variously described. In rough order of severity, these are bacteremia or fungemia; 5 septicemia; sepsis, severe sepsis or sepsis syndrome; septic shock; refractory septic shock; multiple organ dysfunction syndrome, and death.

The condition develops as a response to certain microbial molecules which trigger the production and release of cellular mediators, such as tumor necrosis factors (TNF); these act to stimulate immune response. Besides TNF α , other cytokines involved in the development of 10 septic shock include interleukin-1 β , and interferon γ .

Treatment primarily consists of 1) Volume resuscitation 2) Early antibiotic administration 3) Rapid source identification and control and 4) Support of major organ dysfunction. Among the choices for pressors, norepinephrine (optionally plus dobutamine as needed for cardiac output) or epinephrine can be used. Antimediator agents may be of some limited use in severe 15 clinical situations: Corticosteroids, especially if combined with a mineralocorticoid, can reduce mortality among patients who have relative adrenal insufficiency; or recombinant activated protein C (drotrecogin alpha). A sophorolipid mixture can be used.

The disclosure provides methods of treating septic shock by administering a therapeutically effective amount of a MMP-9 binding protein (e.g., an inhibitory MMP-9 binding 20 protein, e.g., an anti-MMP-9 IgG or Fab) to a subject having or suspected of having septic shock. Also provided are methods of treating septic shock by administering a therapeutically effective amount of a MMP-9 binding protein with another septic shock treatment (e.g., corticosteroid, sophorolipid mixture, or antibiotics).

Guidance regarding the efficacy and dosage an MMP-9 binding protein which will 25 deliver a therapeutically effective amount of the protein can be obtained from an animal model of septic shock, see e.g., US Pat. No. 7,262,178, and references cited therein.

Neuropathic Pain

Neuropathic pain is a complex, chronic pain state that usually is accompanied by tissue injury. With neuropathic pain, the nerve fibers themselves might be damaged, dysfunctional, or 30 injured. These damaged nerve fibers send incorrect signals to other pain centers. The impact of

a nerve fiber injury includes a change in nerve function both at the site of injury and areas around the injury.

Neuropathic pain often seems to have no obvious cause. It responds poorly to standard pain treatment and occasionally might get worse instead of better over time. For some people, it can lead to serious disability. One example of neuropathic pain is called phantom limb syndrome. This occurs when an arm or a leg has been removed because of illness or injury, but the brain still gets pain messages from the nerves that originally carried impulses from the missing limb. These nerves now seem to misfire and cause pain. Some common causes of neuropathic pain include: alcoholism, amputation, back, leg, and hip problems, cancer chemotherapy, diabetes, facial nerve problems, HIV infection or AIDS, multiple sclerosis, shingles, and spine surgery.

Some symptoms of neuropathic pain include shooting pain, burning pain, tingling, and numbness.

Improvement is often possible with proper treatment. Treatments include: administering an NSAID, an analgesic (e.g., with morphine), an anticonvulsant drug (e.g., an anticonvulsant described in US Pat. No. 5,760,007), an antidepressant drug, or other pain reliever. If another condition, such as diabetes, is involved, better management of that disorder might alleviate the neuropathic pain. In cases that are difficult to treat, a pain specialist might use invasive or implantable device therapies to effectively manage the pain. Electrical stimulation of the nerves involved in neuropathic pain generation might significantly control the pain symptoms.

MMP-9 and MMP-2 have been found to play roles in the development of neuropathic pain. MMP-9 is upregulated in the early phase of neuropathic pain development, while MMP-2 is upregulated in the late phase of neuropathic pain development. Targeting and inhibition of MMP-9 and/or MMP-2 is a therapeutic approach to treating neuropathic pain. The disclosure provides methods of treating neuropathic pain by administering a therapeutically effective amount of a MMP-9 binding protein (e.g., an inhibitory MMP-9 binding protein, e.g., an anti-MMP-9 IgG or Fab) to a subject having or suspected of having neuropathic pain. Also provided are methods of treating neuropathic pain by administering a therapeutically effective amount of a MMP-9 binding protein with another neuropathic pain treatment (e.g., an NSAID, an analgesic (e.g., with morphine), an anticonvulsant drug, an antidepressant drug, or other pain reliever; invasive or implantable device therapies).

Guidance regarding the efficacy and dosage an MMP-9 binding protein which will deliver a therapeutically effective amount of the protein can be obtained from an animal model of neuropathic pain, such as the L5 spinal nerve ligation (SNL) animal model, see e.g., Kawasaki et al., Feb. 10, 2008, *Nat. Med.* advance on-line publication doi:10.1038/nm1723. See also US Pat. No. 5,760,007 and references cited therein.

Inflammatory Pain

Inflammatory pain is precipitated by an insult to the integrity of tissues at a cellular level. This can happen, e.g., with penetration wounds, burns, extreme cold, fractures, arthritis, autoimmune conditions, excessive stretching, infections and vasoconstriction. Multiple chemicals can mediate the inflammatory process. For example, vascular components, fibroblastic components and tissue cell components can be involved. For example, mast cells release histamines and 5HT; macrophages activate fibroblasts, which in turn release interleukins and Tumor Necrosis Factor; cyclooxygenase activates prostaglandin and leukotrienes. The inflammatory mediators can directly affect nociceptors or may sensitize them to touch or movement, even some distance from the inflammatory field.

Treatments of inflammatory pain include nonsteroidal anti-inflammatory drugs (NSAIDs) and corticosteroids. Anti-inflammatory drugs include: ibuprofen, naproxen, naproxen sodium, aspirin, ketoprofen, valdecoxib, celecoxib, sulindac, oxaprozin, salsalate, piroxicam, indomethacin, etodolac, meloxicam, nabumetone, ketorolac tromethamine, rofecoxib.

Guidance regarding the efficacy and dosage an MMP-9 binding protein which will deliver a therapeutically effective amount of the protein can be obtained from an animal model of inflammatory pain, such as intraplantar injection of Carrageenan in an animal model, see e.g., Jabakhanji et al., *Molecular Pain* 2006, 2:1.

Endometriosis

Endometriosis is a common medical condition characterized by growth beyond or outside the uterus of endometrium, the tissue that normally lines the uterus. In endometriosis, the endometrium is found to be growing outside the uterus, on or in other areas of the body. Normally, the endometrium is shed each month during the menstrual cycle; however, in endometriosis, the misplaced endometrium is usually unable to exit the body. The endometriotic tissues still detach and bleed, but the result is far different: internal bleeding, degenerated blood

and tissue shedding, inflammation of the surrounding areas, pain, and formation of scar tissue may result. In addition, depending on the location of the growths, interference with the normal function of the bowel, bladder, small intestines and other organs within the pelvic cavity can occur. In very rare cases, endometriosis has also been found in the skin, the lungs, the eye, the
5 diaphragm, and the brain.

A major symptom of endometriosis is severe recurring pain. The amount of pain a woman feels is not necessarily related to the extent or stage (1 through 4) of endometriosis. Some women will have little or no pain despite having extensive endometriosis affecting large areas or having endometriosis with scarring. On the other hand, women may have severe pain
10 even though they have only a few small areas of endometriosis.

Symptoms of endometriosis can include (but are not limited to): Painful, sometimes disabling menstrual cramps (dysmenorrhea), pain may get worse over time (progressive pain), chronic pain (typically lower back pain and pelvic pain, also abdominal), painful sex (dyspareunia), painful bowel movements (dyschezia) or painful urination (dysuria), heavy
15 menstrual periods (menorrhagia), nausea and vomiting, premenstrual or intermenstrual spotting (bleeding between periods), and infertility and subfertility. Endometriosis may lead to fallopian tube obstruction. Bowel obstruction (possibly including vomiting, crampy pain, diarrhea, a rigid and tender abdomen, and distention of the abdomen, depending on where the blockage is and what is causing it) or complete urinary retention. In addition, women who are diagnosed with
20 endometriosis may have gastrointestinal symptoms that may mimic irritable bowel syndrome, as well as fatigue.

Patients who rupture an endometriotic cyst may present with an acute abdomen as a medical emergency. Endometriotic cysts in the thoracic cavity may cause some form of thoracic endometriosis syndrome, most often catamenial pneumothorax.

25 Diagnosis. Health history and a physical examination can in many patients lead the physician to suspect the diagnosis. Use of imaging tests (e.g., ultrasound and magnetic resonance imaging (MRI)) may identify larger endometriotic areas, such as nodules or endometriotic cysts. The only sure way to confirm an endometriosis diagnosis is by laparoscopy. The diagnosis is based on the characteristic appearance of the disease, if necessary corroborated by a biopsy.
30 Laparoscopy also allows for surgical treatment of endometriosis.

Treatment. Generally, endometriosis-directed drug therapy (other than the oral contraceptive pill) is utilized after a confirmed surgical diagnosis of endometriosis. Treatments include: NSAIDs and other pain medication, commonly used in conjunction with other therapy; Gonadotropin Releasing Hormone (GnRH) Agonist; Hormone suppression therapy; Progesterone or Progestins; avoiding products with xenoestrogens, which have a similar effect to naturally produced estrogen and can increase growth of the endometrium; continuous hormonal contraception; suppressive steroids such as Danazol (Danocrine) and gestrinone; aromatase inhibitors. Surgical treatment is usually a good choice if endometriosis is extensive, or very painful. Surgical treatments range from minor to major surgical procedures. Laparoscopy is very useful not only to diagnose endometriosis, but to treat it- endometriotic tissue can be ablated or removed in an attempt to restore normal anatomy. Laparotomy can be used for more extensive surgery either in attempt to restore normal anatomy. Other procedures include hysterectomy, bilateral salpingo-oophorectomy (removal of the fallopian tubes and ovaries), bowel resection. For patients with extreme pain, a presacral neurectomy may be indicated where the nerves to the uterus are cut.

MMP-9 is upregulated in endometriosis and may contribute to survival and invasion of endometriosis. The disclosure provides methods of treating endometriosis by administering a therapeutically effective amount of a MMP-9 binding protein (e.g., an inhibitory MMP-9 binding protein, e.g., an anti-MMP-9 IgG or Fab) to a subject having or suspected of having endometriosis. Also provided are methods of treating endometriosis by administering a therapeutically effective amount of a MMP-9 binding protein with another endometriosis treatment (e.g., corticosteroid, sophorolipid mixture, or antibiotics).

Guidance regarding the efficacy and dosage an MMP-9 binding protein which will deliver a therapeutically effective amount of the protein can be obtained from an animal model of endometriosis, see e.g., US Pat. Nos. 6,429,353 and 7,220,890, and references cited therein.

Combination Therapies

The MMP-9 binding proteins described herein, e.g., anti-MMP-9 Fabs or IgGs, can be administered in combination with one or more of the other therapies for treating a disease or condition associated with MMP-9 activity, e.g., a disease or condition described herein. For example, an MMP-9 binding protein can be used therapeutically or prophylactically with

surgery, another MMP-9 inhibitor, e.g., a small molecule inhibitor, another anti-MMP-9 Fab or IgG (e.g., another Fab or IgG described herein), an anti-MMP-9/-2 binding protein (e.g., IgG or Fab, e.g., 539A-M0237-D02 or a protein containing one or more heavy and/or light chains CDRs thereof), an anti-MMP14 binding protein (e.g., IgG or Fab, e.g., DX-2400, or a protein described in U.S. Pub. App. No. 2007-0217997), peptide inhibitor, or small molecule inhibitor. Examples of other MMP-9 inhibitors that can be used in combination therapy with an MMP-9 binding protein described herein are provided herein.

One or more small-molecule MMP inhibitors can be used in combination with one or more MMP-9 binding proteins described herein. For example, the combination can result in a lower dose of the small-molecule inhibitor being needed, such that side effects are reduced.

The MMP-9 binding proteins described herein can be administered in combination with one or more of the other therapies for treating cancers, including, but not limited to: surgery; radiation therapy, and chemotherapy. For example, proteins that inhibit MMP-9 or that inhibit a downstream event of MMP-9 activity (e.g., cleavage of pro-MMP-2 to MMP-2) can also be used in combination with other anti-cancer therapies, such as radiation therapy, chemotherapy, surgery, or administration of a second agent. For example, the second agent can be a Tie-1 inhibitor (e.g., Tie-1 binding proteins; see e.g., U.S. Ser. No. 11/199,739 and PCT/US2005/0284, both filed August 9, 2005). As another example, the second agent can be an anti-MMP14 binding protein (e.g., IgG or Fab, e.g., DX-2400, or a protein described in U.S. Pub. App. No. 2007-0217997). As another example, the second agent can be one that targets or negatively regulates the VEGF signaling pathway. Examples of this latter class include VEGF antagonists (e.g., anti-VEGF antibodies such as bevacizumab) and VEGF receptor antagonists (e.g., anti-VEGF receptor antibodies). One particularly preferred combination includes bevacizumab. As a further example, the second agent is an inhibitor of plasmin, such as a kunitz domain-containing protein or polypeptide (e.g., a plasmin-inhibiting kunitz domain disclosed in U.S. Patent No. 6,010,880, such as a protein or polypeptide comprising the amino acid sequence MHSFCAFKAE TGPCRARFDRWFFNIFTRQCEEFIYGGCEGNQNR FESLEECKKMCTRD (SEQ ID NO:1)). As another example, the second agent is an agent that binds to Her2, such as a Her2-binding antibody (e.g., trastuzumab). The combination can further include 5-FU and leucovorin, and/or irinotecan.

Inhibitors of MMP-9 (e.g., the MMP-9 binding proteins disclosed herein) can potentiate the activity of an agent that targets Her2 (e.g., a Her2-binding antibody such as trastuzumab). Accordingly, in one combination therapy for the treatment of breast cancer, the second therapy is an agent that binds Her2, such as a Her2-binding antibody (e.g., trastuzumab). When an MMP-9 binding protein is used in a combination therapy with a Her2 binding agent, the dose of the Her2 binding agent may be reduced from the dose of the Her2 binding agent when administered not in combination with an MMP-9 binding protein (e.g., is at least 10%, 25%, 40%, or 50% less than the dose of the Her2 binding agent when administered not in combination with a MMP-9 binding protein). For example, the dose of trastuzumab, when administered in a combination therapy with an MMP-9 binding protein is less than about 4.0, 3.6, 3.0, 2.4, or 2 mg/kg as an initial (loading) dose, and less than about 2.0, 1.8, 1.5, 1.2, or 1 mg/kg in subsequent doses.

The MMP-9 binding proteins described herein can also be administered in combination with one or more other therapies for treating ocular disorders, such as surgical or medical (e.g., administration of a second agent) therapies. For example, in treatment of age-related macular degeneration (e.g., wet age-related macular degeneration), an MMP-9 binding protein may be administered in conjunction with (e.g., before, during, or after) laser surgery (laser photocoagulation or photocoagulation therapy). As another example, the MMP-9 binding protein can be administered in combination with a second agent, such as a VEGF antagonist (e.g., an anti-VEGF antibody such as bevacizumab or ranibizumab) or a VEGF receptor antagonist (e.g., anti-VEGF receptor antibodies).

The term “combination” refers to the use of the two or more agents or therapies to treat the same patient, wherein the use or action of the agents or therapies overlap in time. The agents or therapies can be administered at the same time (e.g., as a single formulation that is administered to a patient or as two separate formulations administered concurrently) or sequentially in any order. Sequential administrations are administrations that are given at different times. The time between administration of the one agent and another agent can be minutes, hours, days, or weeks. The use of an MMP-9 binding protein described herein can also be used to reduce the dosage of another therapy, e.g., to reduce the side-effects associated with another agent that is being administered, e.g., to reduce the side-effects of an anti-VEGF antibody such as bevacizumab. Accordingly, a combination can include administering a second

agent at a dosage at least 10, 20, 30, or 50% lower than would be used in the absence of the MMP-9 binding protein.

In addition, a subject can be treated for an angiogenesis-associated disorder, e.g., a cancer, by administering to the subject a first and second agent. For example, the first agent modulates early stage angiogenesis and the second agent modulates a subsequent stage of angiogenesis or also modulates early stage angiogenesis. The first and second agents can be administered using a single pharmaceutical composition or can be administered separately. In one embodiment, the first agent is a VEGF pathway antagonist (e.g., an inhibitor of a VEGF (e.g., VEGF-A, -B, or -C) or a VEGF receptor (e.g., KDR or VEGF receptor III (Flt4)) or a bFGF pathway antagonist (e.g., an antibody that binds to bFGF or a bFGF receptor). Other VEGF pathway antagonists are also described, herein and elsewhere. In one embodiment, the second agent inhibits or decreases the mobility or invasiveness of tumor cells. For example, the second agent comprises an MMP-9 binding protein. For example, the second agent is an MMP-9 binding protein described herein.

Once a tumor reaches a certain size (e.g., ~ 1-2 mm), the tumor requires new vasculature prior to increasing its mass. An early stage of tumor angiogenesis can include a signal from the tumor, e.g., secretion of VEGF, to stimulate the growth of new blood vessels from the host and infiltration of the tumor by the vessels. VEGF can, for example, stimulate proliferation of endothelial cells that are then assembled into blood vessels. A late stage of tumor growth can include metastasis, mobility and invasiveness of tumor cells. This mobility and invasiveness may involve the action of matrix metalloproteinases, e.g., MMP-9, MMP-16, or MMP-24. Thus, an effective therapy to treat angiogenesis-related disorders can involve a combination of an agent that modulates an early stage angiogenesis (e.g., VEGF pathway antagonists, e.g., anti-VEGF (e.g., bevacizumab) or anti-VEGF receptor (e.g., anti-KDR) antibodies; or antagonists of other pro-angiogenic pathways, e.g., anti-bFGF antibodies or anti-bFGF receptor (e.g., anti-bFGF receptor-1, -2, -3) antibodies) and an agent that modulates a late stage of tumor growth can include metastasis, mobility and invasiveness of tumor cells (e.g., antagonists of MMP-9 (e.g., anti-MMP-9 antibodies (e.g., an antibody disclosed herein)). One or more of these agents can be used in combination. One or more of these agents may also be used in combination with other anti-cancer therapies, such as radiation therapy or chemotherapy.

Exemplary VEGF receptor antagonists include inhibitors of a VEGF (e.g., VEGF-A, -B, or -C, for example bevacizumab), modulators of VEGF expression (e.g., INGN-241, oral tetrathiomolybdate, 2-methoxyestradiol, 2-methoxyestradiol nanocrystal dispersion, bevasiranib sodium, PTC-299, Veglin), inhibitors of a VEGF receptor (e.g., KDR or VEGF receptor III (Flt4), for example anti-KDR antibodies, VEGFR2 antibodies such as CDP-791, IMC-1121B, VEGFR2 blockers such as CT-322), VEGFR3 antibodies such as mF4-31C1 from Imclone Systems, modulators of VEGFR expression (e.g., VEGFR1 expression modulator Sirna-027) or inhibitors of VEGF receptor downstream signaling.

Exemplary inhibitors of VEGF include bevacizumab, pegaptanib, ranibizumab, NEOVASTAT[®], AE-941, VEGF Trap, and PI-88.

Exemplary VEGF receptor antagonists include inhibitors of VEGF receptor tyrosine kinase activity. 4-[4-(1-Amino-1-methylethyl)phenyl]-2-[4-(2-morpholin-4-yl-ethyl)phenylamino]pyrimidine-5-carbonitrile (JNJ-17029259) is one of a structural class of 5-cyanopyrimidines that are orally available, selective, nanomolar inhibitors of the vascular endothelial growth factor receptor-2 (VEGF-R2). Additional examples include: PTK-787/ZK222584 (Astra-Zeneca), SU5416, SU11248 (Pfizer), and ZD6474 ([N-(4-bromo-2-fluorophenyl)-6-methoxy-7-[(1-methylpiperidin-4-yl)methoxy]quinazolin-4-amine]), vandetanib, cediranib, AG-013958, CP-547632, E-7080, XL-184, L-21649, and ZK-304709. Other VEGF antagonist agents are broad specificity tyrosine kinase inhibitors, e.g., SU6668 (see, e.g., Bergers, B. et al., 2003 J. Clin. Invest. 111:1287-95), sorafenib, sunitinib, pazopanib, vatalanib, AEE-788, AMG-706, axitinib, BIBF-1120, SU-14813, XL-647, XL-999, ABT-869, BAY-57-9352, BAY-73-4506, BMS-582664, CEP-7055, CHIR-265, OSI-930, and TKI-258. Also useful are agents that down regulate VEGF receptors on the cell surface, such as fenretinide, and agents which inhibit VEGF receptor downstream signaling, such as squalamine

The second agent or therapy can also be another anti-cancer agent or therapy. Non-limiting examples of anti-cancer agents include, e.g., anti-microtubule agents, topoisomerase inhibitors, antimetabolites, mitotic inhibitors, alkylating agents, intercalating agents, agents capable of interfering with a signal transduction pathway, agents that promote apoptosis, radiation, and antibodies against other tumor-associated antigens (including naked antibodies, immunotoxins and radioconjugates). Examples of the particular classes of anti-cancer agents are provided in detail as follows: antitubulin/antimicrotubule, e.g., paclitaxel, vincristine,

vinblastine, vindesine, vinorelbin, taxotere; topoisomerase I inhibitors, e.g., irinotecan, topotecan, camptothecin, doxorubicin, etoposide, mitoxantrone, daunorubicin, idarubicin, teniposide, amsacrine, epirubicin, merbarone, piroxantrone hydrochloride; antimetabolites, e.g., 5 fluorouracil (5 FU), methotrexate, 6 mercaptopurine, 6 thioguanine, fludarabine phosphate, cytarabine/Ara C, trimetrexate, gemcitabine, acivicin, alanosine, pyrazofurin, N-Phosphoracetyl-L-Asparate=PALA, pentostatin, 5 azacitidine, 5 Aza 2' deoxycytidine, ara A, cladribine, 5 fluorouridine, FUDR, tiazofurin, N-[5-[N-(3,4-dihydro-2-methyl-4-oxoquinazolin-6-ylmethyl)-N-methylamino]-2-thenoyl]-L-glutamic acid; alkylating agents, e.g., cisplatin, carboplatin, mitomycin C, BCNU=Carmustine, melphalan, thiotepa, busulfan, chlorambucil, plicamycin, dacarbazine, ifosfamide phosphate, cyclophosphamide, nitrogen mustard, uracil mustard, pipobroman, 4 ipomeanol; agents acting via other mechanisms of action, e.g., dihydrolenperone, spiromustine, and desipeptide; biological response modifiers, e.g., to enhance anti-tumor responses, such as interferon; apoptotic agents, such as actinomycin D; and anti-hormones, for example anti-estrogens such as tamoxifen or, for example antiandrogens such as 4'-cyano-3-(4-fluorophenylsulphonyl)-2-hydroxy-2-methyl-3'-(trifluoromethyl) propionanilide.

A combination therapy can include administering an agent that reduces the side effects of other therapies. The agent can be an agent that reduces the side effects of anti-cancer treatments. For example, the agent can be leucovorin.

Combination therapies that include administering an MMP-9 binding protein or other binding protein described herein can also be used to treat a subject having or at risk for another angiogenesis related disorder (e.g., a disorder other than cancer, e.g., disorders that include undesired endothelial cell proliferation or undesirable inflammation, e.g., rheumatoid arthritis).

Diagnostic Uses

Proteins that bind to MMP-9 and identified by the method described herein and/or detailed herein have *in vitro* and *in vivo* diagnostic utilities. The MMP-9 binding proteins described herein (e.g., the proteins that bind and inhibit, or the proteins that bind but do not inhibit MMP-9) can be used, e.g., for *in vivo* imaging, e.g., during a course of treatment for a disease or condition in which MMP-9 is active, e.g., a disease or condition described herein, or in diagnosing a disease or condition described herein.

In one aspect, the disclosure provides a diagnostic method for detecting the presence of an MMP-9, *in vitro* or *in vivo* (e.g., *in vivo* imaging in a subject). The method can include localizing MMP-9 within a subject or within a sample from a subject. With respect to sample evaluation, the method can include, for example: (i) contacting a sample with MMP-9 binding
5 protein; and (ii) detecting location of the MMP-9 binding protein in the sample.

An MMP-9 binding protein can also be used to determine the qualitative or quantitative level of expression of MMP-9 in a sample. The method can also include contacting a reference sample (e.g., a control sample) with the binding protein, and determining a corresponding assessment of the reference sample. A change, e.g., a statistically significant change, in the
10 formation of the complex in the sample or subject relative to the control sample or subject can be indicative of the presence of MMP-9 in the sample. In one embodiment, the MMP-9 binding protein does not cross react with another metalloproteinase.

The MMP-9 binding proteins are also useful for *in vivo* tumor imaging. Better clinical endpoints are needed to monitor the efficacy of drugs, such as MMP-inhibitors, that are designed
15 to block enzymatic function (Zucker et al, 2001, Nature Medicine 7:655- 656). Imaging of tumors *in vivo* by using labeled MMP-9 binding proteins could be of help to target the delivery of the binding protein to tumors for cancer diagnosis, intraoperative tumor detection, and for investigations of drug delivery and tumor physiology. MMP-9 binding proteins can be used to monitor native enzymatic activity *in vivo* at invasive sites. Another exemplary method includes:
20 (i) administering the MMP-9 binding protein to a subject; and (iii) detecting location of the MMP-9 binding protein in the subject. The detecting can include determining location or time of formation of the complex.

The MMP-9 binding protein can be directly or indirectly labeled with a detectable substance to facilitate detection of the bound or unbound antibody. Suitable detectable
25 substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials and radioactive materials.

Complex formation between the MMP-9 binding protein and MMP-9 can be detected by evaluating the binding protein bound to the MMP-9 or unbound binding protein. Conventional detection assays can be used, e.g., an enzyme-linked immunosorbent assays (ELISA), a
30 radioimmunoassay (RIA) or tissue immunohistochemistry. Further to labeling the MMP-9 binding protein, the presence of MMP-9 can be assayed in a sample by a competition

immunoassay utilizing standards labeled with a detectable substance and an unlabeled MMP-9 binding protein. In one example of this assay, the biological sample, the labeled standards, and the MMP-9 binding protein are combined and the amount of labeled standard bound to the unlabeled binding protein is determined. The amount of MMP-9 in the sample is inversely
5 proportional to the amount of labeled standard bound to the MMP-9 binding protein.

Fluorophore and chromophore labeled proteins can be prepared. Because antibodies and other proteins absorb light having wavelengths up to about 310 nm, the fluorescent moieties should be selected to have substantial absorption at wavelengths above 310 nm and preferably above 400 nm. A variety of suitable fluorescers and chromophores are described by Stryer, 1968,
10 *Science* 162:526 and Brand, L. et al., 1972, *Annu. Rev. Biochem.* 41:843 868. The proteins can be labeled with fluorescent chromophore groups by conventional procedures such as those disclosed in U.S. Patent Nos. 3,940,475, 4,289,747, and 4,376,110. One group of fluorescers having a number of the desirable properties described above is the xanthene dyes, which include the fluoresceins and rhodamines. Another group of fluorescent compounds are the
15 naphthylamines. Once labeled with a fluorophore or chromophore, the protein can be used to detect the presence or localization of the MMP-9 in a sample, e.g., using fluorescent microscopy (such as confocal or deconvolution microscopy).

Histological Analysis. Immunohistochemistry can be performed using the proteins described herein. For example, in the case of an antibody, the antibody can be synthesized with
20 a label (such as a purification or epitope tag), or can be detectably labeled, e.g., by conjugating a label or label-binding group. For example, a chelator can be attached to the antibody. The antibody is then contacted to a histological preparation, e.g., a fixed section of tissue that is on a microscope slide. After an incubation for binding, the preparation is washed to remove unbound antibody. The preparation is then analyzed, e.g., using microscopy, to identify if the antibody
25 bound to the preparation.

Of course, the antibody (or other polypeptide or peptide) can be unlabeled at the time of binding. After binding and washing, the antibody is labeled in order to render it detectable.

Protein Arrays. The MMP-9 binding protein can also be immobilized on a protein array. The protein array can be used as a diagnostic tool, e.g., to screen medical samples (such
30 as isolated cells, blood, sera, biopsies, and the like). Of course, the protein array can also include other binding proteins, e.g., that bind to MMP-9 or to other target molecules.

Methods of producing polypeptide arrays are described, e.g., in De Wildt et al., 2000, *Nat. Biotechnol.* 18:989-994; Lueking et al., 1999, *Anal. Biochem.* 270:103-111; Ge, 2000, *Nucleic Acids Res.* 28, e3, I-VII; MacBeath and Schreiber, 2000, *Science* 289:1760-1763; WO 01/40803 and WO 99/51773A1. Polypeptides for the array can be spotted at high speed, e.g.,
5 using commercially available robotic apparatus, e.g., from Genetic Microsystems or BioRobotics. The array substrate can be, for example, nitrocellulose, plastic, glass, e.g., surface-modified glass. The array can also include a porous matrix, e.g., acrylamide, agarose, or another polymer.

For example, the array can be an array of antibodies, e.g., as described in De Wildt, *supra*. Cells that produce the proteins can be grown on a filter in an arrayed format. Polypeptide
10 production is induced, and the expressed polypeptides are immobilized to the filter at the location of the cell. A protein array can be contacted with a labeled target to determine the extent of binding of the target to each immobilized polypeptide. Information about the extent of binding at each address of the array can be stored as a profile, e.g., in a computer database. The protein array can be produced in replicates and used to compare binding profiles, e.g., of a target
15 and a non-target.

FACS (Fluorescence Activated Cell Sorting). The MMP-9 binding protein can be used to label cells, e.g., cells in a sample (e.g., a patient sample). The binding protein is also attached (or attachable) to a fluorescent compound. The cells can then be sorted using fluorescence
activated cell sorter (e.g., using a sorter available from Becton Dickinson Immunocytometry
20 Systems, San Jose CA; see also U.S. Patent Nos. 5,627,037; 5,030,002; and 5,137,809). As cells pass through the sorter, a laser beam excites the fluorescent compound while a detector counts cells that pass through and determines whether a fluorescent compound is attached to the cell by detecting fluorescence. The amount of label bound to each cell can be quantified and analyzed to characterize the sample.

25 The sorter can also deflect the cell and separate cells bound by the binding protein from those cells not bound by the binding protein. The separated cells can be cultured and/or characterized.

In Vivo Imaging. Also featured is a method for detecting the presence of a MMP-9 expressing tissues *in vivo*. The method includes (i) administering to a subject (e.g., a patient
30 having, e.g., a cancer (e.g., metastatic cancer, e.g., metastatic breast cancer), an inflammatory disease (e.g., chronic obstructive pulmonary disease (COPD), asthma, rhinitis (e.g., allergic

rhinitis), inflammatory bowel disease, synovitis, rheumatoid arthritis), heart failure, septic shock, neuropathic pain, osteoarthritis, or an ocular condition (e.g., macular degeneration)) an anti-MMP-9 antibody, conjugated to a detectable marker; (ii) exposing the subject to a means for detecting said detectable marker to the MMP-9 expressing tissues or cells. For example, the
 5 subject is imaged, e.g., by NMR or other tomographic means.

Examples of labels useful for diagnostic imaging include radiolabels such as ^{131}I , ^{111}In , ^{123}I , $^{99\text{m}}\text{Tc}$, ^{32}P , ^{125}I , ^3H , ^{14}C , and ^{188}Rh , fluorescent labels such as fluorescein and rhodamine, nuclear magnetic resonance active labels, positron emitting isotopes detectable by a positron emission tomography ("PET") scanner, chemiluminescers such as luciferin, and enzymatic
 10 markers such as peroxidase or phosphatase. Short range radiation emitters, such as isotopes detectable by short range detector probes can also be employed. The protein can be labeled with such reagents; for example, see Wensel and Meares, 1983, *Radioimmunoimaging and Radioimmunotherapy*, Elsevier, New York for techniques relating to the radiolabeling of antibodies and D. Colcher et al., 1986, *Meth. Enzymol.* 121: 802 816.

15 The binding protein can be labeled with a radioactive isotope (such as ^{14}C , ^3H , ^{35}S , ^{125}I , ^{32}P , ^{131}I). A radiolabeled binding protein can be used for diagnostic tests, e.g., an in vitro assay. The specific activity of a isotopically-labeled binding protein depends upon the half life, the isotopic purity of the radioactive label, and how the label is incorporated into the antibody.

In the case of a radiolabeled binding protein, the binding protein is administered to the
 20 patient, is localized to cells bearing the antigen with which the binding protein reacts, and is detected or "imaged" in vivo using known techniques such as radionuclear scanning using e.g., a gamma camera or emission tomography. See e.g., A.R. Bradwell et al., "Developments in Antibody Imaging", *Monoclonal Antibodies for Cancer Detection and Therapy*, R.W. Baldwin et al., (eds.), pp 65 85 (Academic Press 1985). Alternatively, a positron emission transaxial
 25 tomography scanner, such as designated Pet VI located at Brookhaven National Laboratory, can be used where the radiolabel emits positrons (e.g., ^{11}C , ^{18}F , ^{15}O , and ^{13}N).

MRI Contrast Agents. Magnetic Resonance Imaging (MRI) uses NMR to visualize internal features of living subject, and is useful for prognosis, diagnosis, treatment, and surgery. MRI can be used without radioactive tracer compounds for obvious benefit. Some MRI
 30 techniques are summarized in EP-A-0 502 814. Generally, the differences related to relaxation

time constants T1 and T2 of water protons in different environments is used to generate an image. However, these differences can be insufficient to provide sharp high resolution images.

The differences in these relaxation time constants can be enhanced by contrast agents. Examples of such contrast agents include a number of magnetic agents paramagnetic agents (which primarily alter T1) and ferromagnetic or superparamagnetic (which primarily alter T2 response). Chelates (e.g., EDTA, DTPA and NTA chelates) can be used to attach (and reduce toxicity) of some paramagnetic substances (e.g., Fe^{+3} , Mn^{+2} , Gd^{+3}). Other agents can be in the form of particles, e.g., less than 10 μm to about 10 nm in diameter). Particles can have ferromagnetic, antiferromagnetic, or superparamagnetic properties. Particles can include, e.g., magnetite (Fe_3O_4), $\gamma\text{-Fe}_2\text{O}_3$, ferrites, and other magnetic mineral compounds of transition elements. Magnetic particles may include: one or more magnetic crystals with and without nonmagnetic material. The nonmagnetic material can include synthetic or natural polymers (such as sepharose, dextran, dextrin, starch and the like.

The MMP-9 binding protein can also be labeled with an indicating group containing of the NMR active ^{19}F atom, or a plurality of such atoms inasmuch as (i) substantially all of naturally abundant fluorine atoms are the ^{19}F isotope and, thus, substantially all fluorine containing compounds are NMR active; (ii) many chemically active polyfluorinated compounds such as trifluoroacetic anhydride are commercially available at relatively low cost; and (iii) many fluorinated compounds have been found medically acceptable for use in humans such as the perfluorinated polyethers utilized to carry oxygen as hemoglobin replacements. After permitting such time for incubation, a whole body MRI is carried out using an apparatus such as one of those described by Pykett, 1982, *Sci. Am.* 246:78 88 to locate and image tissues expressing MMP-9.

The contents of all references, pending patent applications and published patents, cited throughout this application are hereby expressly incorporated by reference. The following examples provide further illustrate and are not limiting.

EXAMPLES

Example 1: Selection and Screening of Anti-MMP-9 Fabs and IgGs

Two strategies were employed to identify anti-MMP-9 antibodies:

(1) Capture of a non-biotinylated form of MMP-9 (PMA-activated) by a biotinylated binding but not inhibiting Fab with the subsequent immobilization of the biotinylated entity on a streptavidin coated surface; and

(2) MMP-9 (PMA activated) in solution. Phage, suitably depleted (e.g., previous contact
5 with streptavidin) were allowed to interact with the target, unbound phage washed away and the output sampled and/or amplified for the next round of selection. This was repeated until the output phage in ELISA analysis indicate a high percentage of binders. 128/2076 unique sFabs were identified by ELISA and sequencing.

After sequencing analysis, the phage display were converted into sFabs and then into
10 IgG1s. Their ability to inhibit MMP-9 and other MMPs (1, 3, 7, 8, 9, 10, 12, 13, 14) was determined by usual means. Compounds were initially screened at 1 μ M against MMP-9 and those compounds that inhibited MMP-9 >80% were subjected to additional screens against purified recombinant human MMPs. For these additional screens, an IC_{50} value was determined.

Example 2: Exemplary Clone Identified

15 539A-M0166-F10 is a selective inhibitor of human MMP-9 ($IC_{50}=1.8 \pm 0.3$ nM). 539A-M0166-F10 does not inhibit activity of mouse MMP-9. 539A-M0166-F10 potently inhibits activity of hMMP-9 on tumor sections.

Example 3: CDR Amino Acid Sequences of MMP-9 Binding Anti-MMP-9 Binding Fabs

CDR sequences of MMP-9 binding proteins are summarized in **Table 3**.

Table 3. CDR Sequences of MMP-9 Binding Proteins

Isolate	Initial Name	Groups	LV-CDR1	LV-CDR2	LV-CDR3	HV-CDR1	HV-CDR2	HV-CDR3
539A-R0017-A02	539A-M0071-A05	1	RASQGISNYLA	AASN1QS	QQYKTYPFT	PYRMH	YIGSSGGPTAYADSVKG	ARAGTFFDS
539A-R0017-A03	539A-M0071-A06	2	RSSQSLVSSNGNTYLN	YKVSNRDS	MQGTHWPYT	MYRMM	YIGSSGGMTSYADSVKG	DSVFRGERDAFDI
539A-R0017-A04	539A-M0071-D03	3	RASQSISSSFLA	GASSRAI	QQTYSTPLT	KYSMV	VISP SGGYTG YADSVKG	MRVPA AIGGWLDP
539A-R0017-A05	539A-M0071-D11	4	RASQNI GKFLA	GASTLQL	QKYDSALWT	GYGMW	SISP SGGWTF YADSVKG	VKVRHGGGFY
539A-R0017-A06	539A-M0071-E02	5	KSSQNVLLSSNSKNYLA	WASTRES	QQYYSIPWS	NYRMS	SIGSSGGQTM YADSVKG	SHEVSGGVFDF
539A-R0017-A07	539A-M0071-E03	6	RASQGISSWLA	YATSSLQS	QQSKSF PPT	RYRMN	YIGSSGGNTAYADSVKG	RRIGVGAKGGGTFDI
539A-R0017-A08	539A-M0071-E10	7	RASQSVSSYLA	DASNRAT	QQRSNWPLT	HYRMY	YIGSSGGMTSYADSVKG	SDRS GDNYYGMDY
539A-R0017-A09	539A-M0071-E12	8	RASQSISSDLN	AASSLQS	QQSYSTPVT	DYRMF	SISSSGGFTNYADSVKG	DQGGTVVVVATADY
539A-R0017-A11	539A-M0071-F10	9	RASQSISSWLA	KASSLES	QQYNSYPWT	KYKMF	SIGSSGGATSYADSVKG	GGFWSGYGY
539A-R0017-A12	539A-M0071-G11	10	RASETVRYGQVA	DASKRAT	QQRSNWPLT	LYRMN	YIGSSGGATAYADSVKG	SMRGGH LDS
539A-R0017-B01	539A-M0071-H05	11	SGDKLGDKYAS	QDRKRPS	QAWDSNTVV	HYDMW	RIVP SGGLT TYADSVKG	HSEWSGY YGAFDI
539A-R0017-B02	539A-M0071-H10	12	RASQGISSWLA	AASTLQS	QPTYSTSWT	TYSMV	RIGSSGGDTFYADSVKG	DRADTVV TAGDYYYYYGM DV
539A-R0017-B03	539A-M0072-B02	13	RASQSISSWLA	KASSLES	QQYNSYPWT	NYKMH	SIGSSGGMTSYADSVKG	RDWQHLAGDAFDF
539A-R0017-B04	539A-M0072-C04	14	RASQGIRNDLG	AASSLQS	QQLNSYPPT	PYRMH	RIGSSGGATSYADSVKG	DGIAVAGIAFDI
539A-R0017-B05	539A-M0072-C12	15	RASQDIRSSLA	AASSLQS	QQANSFPPT	SYRMQ	YIGSSGGMTSYADSVKG	GSWRGGSQYFDY
539A-R0017-B06	539A-M0072-F02	16	RASQSISSYLN	AASSLQS	QQSYSTPRT	HYVMS	SIGSSGGDTHYADSVKG	VWISGSYLD AFDI
539A-R0017-B07	539A-M0072-F05	17	RASQSISSHLA	GASNRAT	QQRSNWPPT	AYRMQ	YIGSSGGQTSYADSVKG	DPVGAKYYGMDV
539A-R0017-B08	539A-M0072-G08	18	RASQSVSSYLA	DASNRAT	QQRSNWP IT	AYGMV	VIRSSGGPTSYADSVKG	AGGGTYLDY
539A-R0017-B09	539A-M0072-H07	19	RASQSVSSNLA	GASTRAT	HQYNDWPLT	PYKMY	YIGSSGGMTSYADSVKG	RGYSSG PLRY
539A-R0017-B10	539A-M0072-H08	20	RASQSISS TITYLN	AASNRAT	QQRSNWPPT	DYKMW	SIRSSGGP IGYADSVKG	ETNQMGMDV
539A-R0017-B11	539A-M0072-H10	21	RASQSVSSYLA	DASNRAT	QQRGNWP IT	PYRMS	SIGSSGGQTSYADSVKG	EPPGY YFDS
539A-R0017-B12	539A-M0073-C11	22	RASQSVSSSYLA	DASNRAT	QQRSNWP IT	NYRMH	WISSSGGPTSYADSVKG	GGSYRHNNVFDI
539A-R0014-A05	539A-M0073-G10	23	RASQTVSRNYLA	DASKRAT	QQRSNWPPT	LYRMV	SFGP SGGPTMYADSVKG	RGYTVDVNAFDI
539A-R0017-C02	539A-M0073-G12	24	RASQSVSSNLA	GASTRAT	QQYNKWPQT	IYRMH	YIGSSGGNTSYADSVKG	EWVGSSAALDY
539A-R0017-C03	539A-M0074-D05	25	KSSQSVLYSSNNKNYLA	WASTRES	QQSYSTPLT	AYRMH	YIGSSGGMTTYADSVKG	STVTTL DY
539A-R0017-C04	539A-M0074-D09	26	RASQSVRSYLA	DVSNRAT	QQRSNWPLT	MYRMI	WIGSSGGQTSYADSVKG	GLWCDN
539A-R0017-C05	539A-M0074-E11	27	RASQSVSSSYLA	GASSRAI	QQYGSSTRT	QYRMF	YIGSSGGMTSYADSVKG	SMGYGDAFDI
539A-R0017-C06	539A-M0074-G03	28	RASQTISSYLA	GASSRAA	QQYGVSPFYS	YNNMV	VISP SGGWTP YADSVKG	EVGGSGWL GDAFDI
539A-R0017-C07	539A-M0075-A07	29	RASQGISSALA	DASSLES	QQFHTYPFT	TYRMV	YIGSSGGQTA YADSVKG	HNRAIGT EY
539A-R0017-C08	539A-M0075-B09	30	KSSQSILYSSNNRN YLA	WASTRES	QHY YTA PYT	GYSMH	SIWPSGGYTR YADSVKG	GNDSDSFA YRF
539A-R0017-C09	539A-M0075-D06	31	RTSQSVSDSLA	DASNRAT	QQRGSWP IT	NYRMM	YIGSSGGMTSYADSVKG	ETNWN DLGRYFDY
539A-R0017-C10	539A-M0075-D11	32	RASHSVGGGYLA	DAFN RAT	QQRSEWPWT	RYKMS	YIGSSGGMTSYADSVKG	DLTATGYFDY

539A-R0017-C11	539A-M0075-D12	33	RASQGISSWLA	GASSLES	QQANFPPT	DYRMT	WIGSSGGQTSYADSVKG	GTPRVASYFDY
539A-R0017-C12	539A-M0075-F03	34	RASQSVGSDYLA	AASTRAT	QQRSSWPPT	KYIMV	YISPSSGGTTYADSVKG	NYDSSSGTRGAFDI
539A-R0017-D01	539A-M0075-G09	35	KSSQSVLYSSNNKNYLA	WASTRES	QQYYSTPLT	EYRMT	YIGSSGGMTTYADSVKG	GSGSGYDS
539A-R0017-D02	539A-M0075-G12	36	RASQGIRNDLG	AASSLQS	QQTITFPPLT	SYRMM	WISSSGGSTGYADSVKG	TTVTRVGSFYFDL
539A-R0017-D03	539A-M0075-H05	37	TGTSSDVGYINYVS	DVSARPS	CSYAGSYTYV	MYMQ	SIRSSGGFTSYADSVKG	GLRLDM
539A-R0017-D04	539A-M0076-D03	38	RASQGIRNDLD	SASNLOS	LQHNSFPPLT	LYRMN	YIGSSGGATAYADSVKG	GAWYLDs
539A-R0017-D05	539A-M0076-D07	39	RASQSVSTFLA	DASNRAT	QQYASPPRT	GYMS	SISPSSGNTETAEsVKG	DSGQTFYYAFDI
539A-R0017-D06	539A-M0076-E11	40	RASQGISRWLA	DASNRAT	QQRSNWPPRLT	FYHMS	SIGPSSGGWINYADSVKG	DGGLGMDV
539A-R0017-D07	539A-M0076-H03	41	RA SQGVSNYLA	AASTLQS	QKYNSAPYT	NYSMG	GIYSSGGYTQYADSVKG	GHYVWDsGWYSAFDI
539A-R0017-D08	539A-M0078-G07	42	RASQSVSSDLA	GVSTKAT	QQYHNWPPPLT	SYTME	WISPSSGGYTFYADSVKG	GYSYGSIDL
539A-R0017-D09	539A-M0081-B03	43	RASQGISSWLA	AASSLQS	QQANSFPYLT	TYMMM	SIWSSGGSTFYADSVKG	GVVVPALDY
539A-R0017-D10	539A-M0081-D05	44	RASESISRNLA	GAATRVA	QQANTFPFT	MYRMS	YIGSSGGPTAYADSVKG	EGDARVPAAIGY
539A-R0017-D11	539A-M0081-E01	45	RA SQSISSYLN	AASSLQS	QQSYSTPRT	HYVMS	SIGSSGGDTHYADSVKG	VWISGSYLDAFDI
539A-R0017-D12	539A-M0081-G03	46	RTSHNVANFLA	DAYNRAT	QQRANWPPLS	RYPME	YISSSGGWTsYADSVKG	DGLELFGGWLES
539A-R0017-E01	539A-M0082-F03	47	RASQSTSNLS	AASRLQS	QQSWRTPLT	QYWMT	GIGPSSGGPTTYADSVKG	HSTTVTTNFDY
539A-R0017-E03	539A-M0082-G08	48	RASQSISSYLN	AASSLQS	QQSYSTPRT	MYMY	SIRSSGGETQYADSVKG	VWISGSYLDAFDI
539A-R0017-E04	539A-M0082-G09	49	RATQYISNYVN	AASSLQS	QQANFPPT	AYSMH	RLGSSGGPTSYADSVKG	RSSYGRGFDY
539A-R0017-E05	539A-M0083-A05	50	RASQSISSYLN	AASSLQS	QQSYSTPRT	HYPMS	YIYSSGGDTEYADSVKG	YGSGGWMTYGLDV
539A-R0017-E06	539A-M0084-E03	51	RASQSIDTYIN	AASKLED	QQSYSSPGIT	HYDMS	SIWPSSGGVTWYADSVKG	GGYNNYYYALDV
539A-R0017-E08	539A-M0085-H01	52	RASQNIAGLLA	KASTLES	QQYSFNSGT	KYHMH	SISPSSGGVTsYADSVKG	DACSGGTCQLDY

Example 4: CDR Amino Acid Sequences of MMP-9 Binding Anti-MMP-9 Binding Fabs

Unique Fab on phage sequences SC-014 SR-001 539A-M00166, SC-015 SR-001 539A-M0167, SC-016 SR-001 539A-M0168

1. SC-014 SR-001 **539A-M00166** (phage was depleted on biotinylated Fab M0076-D03 immobilized streptavidin beads were selected against MMP-9 captured by Fab D03 immobilized on streptavidin beads)

42 intact clones (both LV and HV); all clones unique to this selection arm, except for 1 clone that was found also in SC-015 SR-001 (plate M0167)

2. SC-015 SR-001 **539A-M0167** (a similar procedure as above was followed but the Fab used during depletion and selection was M0078-G07)

24 intact clones (both LV and HV); all clones unique to this selection arm, except for 1 clone described above and second clone found in previous selection attempts (more on that later)

3. SC-016 SR-001 **539A-M0168** (phage depleted on D03 streptavidin beads were incubated with MMP-9 in solution and subsequently the MMP-9 with or without phage captured onto D03 streptavidin beads)

18 intact clones (both LV and HV); all clones unique to this selection arm.

CDRs of clones with complete sequence summarized in **Table 4**.

Table 4. Unique Fab on phage sequences SC-014 SR-001 539A-M00166, SC-015 SR-001 539A-M0167, SC-016 SR-001

539A-M0168

Isolate	Initial Name	Groups	LV-CDR1	LV-CDR2	LV-CDR3	HV- CDR1	HV-CDR2	HV-CDR3
539A-R0027-A02	539A-M0166-A02	1	RASQSVSSSYLA	GASSRAT	QQYGSSPLT	MYGMP	VISPSGGSTTYADSVKG	GTPYYYDSSYNGGRAFDI
539A-R0027-A06	539A-M0166-A06	2	RASQSISSYLN	AASSLQS	QQTYITPPIT	PYLMH	YIVPSGGNTFYADSVKG	GIGVASGLGSRYLDY
539A-R0027-A07	539A-M0166-A07	3	KSSQSVLYSSNNKNYLA	WASTRES	QQYISTPPT	PYMMMA	RIGSWTNYADSVKG	RSRDGYKGGFDY
539A-R0027-A10	539A-M0166-A10	4	QGDSLRSYYAS	GKNNRPS	QAWDSSTVV	IYWMM	YISPSGGMTSYADSVKG	GIYCSSTSCYDYFDY
539A-R0027-B01	539A-M0166-B01	5	RASQSVSSSYLA	GASSRAP	QQYGSSYT	VYMMP	YISSSGGKTEYADSVKG	DGAAAGPWDYYYYYGLDV
539A-R0027-B03	539A-M0166-B03	6	TGTSSDVGGYNYVS	EVSKRPS	SSYAGTNNFV	HYWMK	SIVPSGGVTYYADSVKG	DLTNMAFDI
539A-R0027-B06	539A-M0166-B06	7	SGSSSNIGSNTVN	SNNQRPS	AAWDDSVSGVV	RYKMS	YIYSSGGLTMVADSVKG	DGGVVEAEDLFDY
539A-R0027-B08	539A-M0166-B08	8	RASQTINNWL	KAFNLES	QQYDTYSWT	WYGMS	SIWSSGGYTGADSVKG	SGSGYIAY
539A-R0027-B11	539A-M0166-B11	9	RASQGISSWLA	GATSLES	QQSNSFPIT	PYAMR	SIDPSGGPTYADSVKG	RGRYYYDSYDAFDI
539A-R0027-C01	539A-M0166-C01	10	RASQSVTGNYLA	GVSSRAT	QQYGSAPFA	YYYMY	YIYPSGGFTSYADSVKG	LLGGTVPPPDY
539A-R0027-C03	539A-M0166-C03	11	RASEDIRSALA	GASSLES	LQHSNYPAT	LYLMM	GIYPSGGYTQYADSVKG	DKGRWDL LGWYFDL
539A-R0027-C04	539A-M0166-C04	12	RSSQSLHSGNYNILD	LGSNRAS	MQARQTPWT	VYFMP	YIYPSGGRTFYADSVKG	QDSSGWYYFDY
539A-R0027-C05	539A-M0166-C05	13	TGTSSDVGGYNFVS	DVSNRPS	SSYTRSSSTVI	MYNMY	YIVPSDGWTPYADSVKG	EDPSISGYINAFDI
539A-R0027-C07	539A-M0166-C07	14	RTSLSISSNLA	DASTRAT	QQYETLPLT	WYEMF	SIYPSGGLTPYADSVKG	DIHAIFGPFYYYYYGM DV
539A-R0027-C09	539A-M0166-C09	15	RASQAIRHDLG	EVSNLQS	QQLNSYPRT	QYLMW	YIVPSGGYTLYADSVKG	SQALRFLESPGAFDI
539A-R0027-D02	539A-M0166-D02	17	KSSQSVLYSSNNKNYLA	WASTRES	QQYISTPPT	MYYMD	GISSGGFTAYADSVKG	EGGYCSSTSCYVDY
539A-R0027-D04	539A-M0166-D04	18	TGTRDVGGYDYVS	EVNNRPS	NSVAGSNKLI	VYPMP	VISPSGGHTTYADSVKG	SVPLYYFDY
539A-R0027-D05	539A-M0166-D05	19	RASHIIIRYLN	SASTLQG	QQSYSSPLT	PYSMN	RIVPSGGFTLYADSVKG	VGSSSWYLPYFDY
539A-R0027-D06	539A-M0166-D06	20	RASQSVSSSYLA	GASSRAT	QQYGSSVT	NYPMW	YIVSSGGTMYADSVKG	CSSGWYVNYYYYYYGM DV
539A-R0027-D09	539A-M0166-D09	21	SGDELGFGSVC	YEDNRRPS	QAWATTIVI	KYMMQ	VIVSSGGFTWYADSVKG	HLWYYYGM DV
539A-R0027-D11	539A-M0166-D11	23	TGTSSDVGGYNYVS	EVSNRPS	SSYTSRSTPYV	QYR	SIYPSGGPTGYADSVKG	GYSTGFYNSGGYFDY
539A-R0027-E03	539A-M0166-E03	24	SGSSSNIGSNYVS	NNNQRPS	AAWDDSLSSAV	TYMMN	SIVSSGGYTEYADSVKG	DGLPVVAATFNYYYYYMDV
539A-R0027-E11	539A-M0166-E11	25	RASQSISSYLN	AASSLQS	QQSYSTPLT	KYFMG	VISPSGGYTYADSVKG	WGSSWYYFDY
539A-R0027-E12	539A-M0166-E12	26	QATQDISNYLN	DASILET	LQHNRYPWT	SYGMP	VIYPSGGNTPYADSVKG	GYDILTGYYGPNWFDP
539A-R0027-F01	539A-M0166-F01	27	GGINIGSKSVH	YFDSDRPS	QVWDSRSDQYV	DYQME	VIRPSGGKTA YADSVKG	AE LGYCSGGSCYFDGAWFDP
539A-R0027-F02	539A-M0166-F02	28	RASQSVTSSYVA	GASSRAT	QQYEDSTHS	TYNMP	RIYSSGGYTPYADSVNG	QGLDDD IWTDYRDF
539A-R0027-F09	539A-M0166-F09	29	SGDKLGDKFAS	QDRKRPS	QVWDITSDHRGV	DYIMW	RIYSSGGFTN YADSVKG	DLGGLSFADY
539A-R0027-F10	539A-M0166-F10	30	SGSSSNIGSNTVT	NNYERPS	ATWDDSLIAN YV	PYLMN	SIYSSGGTG YADSVKG	IYHSSSGPFFYGM DV
539A-R0027-G02	539A-M0166-G02	31	RASQSVSSGSLA	ATSSRAS	QQCGDSPRT	WYRMP	YIGPSGGDTVYADSVKG	RGYEFDF
539A-R0027-G04	539A-M0166-G04	32	RASQGISNWL	GASSLQS	QQDNSFPIT	PYRMP	YIYPSGGNTGYADSVKG	SYDFWSGYWFYD
539A-R0027-G10	539A-M0166-G10	34	SGNNLGNKFVY	QDTKRPS	QAWDSSTAV	DYIM	WISSSGGTT YADSVKG	VSPYSSGWYPYNWFDP
539A-R0027-H01	539A-M0166-H01	35	SGSSYNI GYDVY	TNNQRPS	AAWDDSLSGSWM	DYWMY	YIYSSGGFTGYADSVKG	KVADSGMNWFDP

WO 2009/111450

PCT/US2009/035840

539A-R0027-H02	539A-M0166-H02	36	SQSVSSSYLA	GASSRAT	QQYGSRT	FYGMN	GIGSSGYTPYADSVKG	AYDFWSGYQEILDY
539A-R0027-H03	539A-M0166-H03	37	TGTSSDVGGYNVVS	EVNNRPS	SSYTHRNSFV	VYGMF	WIYSSGGKTEYVDSVKG	DPVRFLEWLWGIDY
539A-R0027-H04	539A-M0166-H04	38	TRSSGSITSNFVQ	EDKRRPS	QSYDFTNQI	QYVMP	YIVPSGGETDYADSVKG	LDDSSGWYSFDY
539A-R0027-H08	539A-M0166-H08	39	SGDKLGDKYAC	QDSKRPS	QAWDSMSVV	LYYMW	WIYPSGGYTPYADSVKG	GIHSGSYSQGQDY
539A-R0027-H10	539A-M0166-H10	40	RASQSVSSNLA	GASTRAT	QHVDRYPLT	FYPMV	WIGPSGGTTKYADSVKG	DWGYYYDSGSRLDY
539A-R0027-H11	539A-M0166-H11	41	QASQDINTYLN	DASNLET	QQYDNLRT	WYYMR	RIVSSGGDTPYADSVKG	EVGPRSFDS
539A-R0028-A03	539A-M0167-A03	42	RASQSVTSTFLA	GASSRAT	QOCGSSPFA	FYYMW	SIGSSGGFTEYADSVKG	EDYDYVWGSYRSPFFDY
539A-R0028-A08	539A-M0167-A08	43	RASQSVSSNLA	GASTRAT	QORSVWPWT	DYSMD	SISPSGGWTIYADSVKG	SSGDFWSGYYPYMDV
539A-R0028-B01	539A-M0167-B01	44	QASQDISNYLN	DASNLET	QQYDNLFP	LTLWF	GISPSGGKTIYADSVKG	DWYCGGSCFDWYFDL
539A-R0028-B03	539A-M0167-B03	45	RASQISISVSLH	GASSLQS	QQSYRIPPT	KYFME	SIWSSGGYTIYADSVKG	SPSDDFWSGYHGGAFDI
539A-R0028-B10	539A-M0167-B10	47	GGDNIGGRSVQ	DDGDRPL	QAWDSSRDHPV	GYMP	WIGPSGGNTLYADSVKG	ASYIVATIPQYFDY
539A-R0028-C11	539A-M0167-C11	48	RASQSVSSSYLA	GASSRAT	QQYGSPLT	KYDME	SIVPSGGFTDYADSVKG	DSSSWYKRFDP
539A-R0028-D01	539A-M0167-D01	49	KTSHRISSSYLA	GTSHRAT	HQRSNPQT	VYNML	YIYSSGGHTIYADSVKG	QAGVGWQLEPDNWFDP
539A-R0028-D02	539A-M0167-D02	50	TGTGSDVGDYNYVS	DVSNRPS	SSYTNSSVI	PYMAA	RIYPSGGETTYADSVKG	GQSYCSSTSCYPYYYYYGMVDV
539A-R0028-D03	539A-M0167-D03	51	RASQISISSYLN	AASSLQS	QQSYSTPF	PYVMP	YIGPSGGNTRYADSVKG	DLLSGDYYYYYYPLDV
539A-R0028-D08	539A-M0167-D08	52	TGATSDIGTYDLVS	EVTNRPS	SSYTRTNTVI	PYKMF	YIRSSGGKTHYADSVKG	DSNAPYYYYDSSGYDAFDI
539A-R0028-D12	539A-M0167-D12	53	RASQISISSYLN	AASSLQS	QQSYSTPLT	TYGMT	SISPSGGATRYVDSVKG	EDL
539A-R0028-E01	539A-M0167-E01	54	RASQISISSYLN	AASSLQS	QQSYSTPLT	KYFMG	VISPSGGYTYADSVKG	WGSSWYFDY
539A-R0028-E04	539A-M0167-E04	55	RASQISISSYLN	AASTLQS	QHLNTYPIT	PYMME	SYIGSSGGYTKYADSVKG	ILGGDYFDY
539A-R0028-E05	539A-M0167-E05	56	KSSQNVLSSNSKNYLA	WASTRES	QQYYSIPWS	NYRMS	SIGSSGGQTMYADSVKG	SHPVSGGVFDF
539A-R0028-E06	539A-M0167-E06	57	RASQISISSWLA	KASSLES	QQYDTYPLT	SYWMH	GIYPSGGNTNYADSVKG	VIYDFWSGYFFDY
539A-R0028-E08	539A-M0167-E08	58	RASESISSYVA	GASNRAT	QQYGSPPPLT	RYTMM	YIGSSGGVTSYADSVKG	DPRDYSDYRGGYWYFDL
539A-R0028-F01	539A-M0167-F01	59	TLSSGHSNYAIA	KLFSDDRHNKGD	QTWVAGIVV	RYLMM	YIYPSGGSTTYADSVKG	DRVVVAATPLTGFDY
539A-R0028-F02	539A-M0167-F02	60	RASQSISSWLA	KASSLES	QQYDTYPLT	SYWMH	GIYPSGGNTNYADSVKG	VIYDFWSGYFFDY
539A-R0028-F04	539A-M0167-F04	61	RASQPVSSTYLA	DTSKRAT	QQYGRSPYT	PYIMK	SISSSGGPTNYADSVKG	SYSNYPRRFFDY
539A-R0028-F06	539A-M0167-F06	62	RASQSIATYLN	AATSLQS	QQTKIEPTWT	AYLMD	VIYSSGGPTMYADSVKG	WCSSGWYPQCHN
539A-R0028-F07	539A-M0167-F07	63	RASHRVGTGYLN	ATSTVQS	QQSYSAFR	DYIMP	RIYPSGGPTWYADSVKG	DTTSGDYFDL
539A-R0028-F10	539A-M0167-F10	64	TGTSSDVGAYNYVS	DVSNRPS	SSYTSSSTRV	LYVMF	RIRPSGGVTDYADSVKG	DTRYDYDFWSGYTGFDDI
539A-R0028-G10	539A-M0167-G10	65	SGETLGGQFAS	QNTKRPS	QAWDTNTVV	KYWMQ	WIYPSGGNTPYADSVKG	SGSRPSYYYYYGMVDV
539A-R0029-A01	539A-M0168-A01	66	TGTSSDVGAYNYVS	EVSNRPS	NSYTTSATLV	LYYMY	GIVPSGGRTDYADSVKG	GLLRFLEWLLYPFDY
539A-R0029-A11	539A-M0168-A11	67	SGSSSNIQTNTLN	GNNQRPS	ATWDDSLIGPV	PYSME	SIRPSGGLTAYADSVKG	WLGFDILTGYFDY
539A-R0029-B02	539A-M0168-B02	68	SGDKLGDKFVS	QDSKRPS	QAWDSSTFYV	FTL	GIYSSGGLTWYADSVKG	DGVLYYSYYGMEV
539A-R0029-B05	539A-M0168-B05	69	RASQNIRSFILA	KTSNLQS	QQYTYTSWT	GYWMK	SIYPSGGKTPYADSVKG	WFTSDYGGKYWFDP
539A-R0029-C01	539A-M0168-C01	70	SSQSLHSDGKTYLY	EASNRFS	MQSIELPRT	PYYMQ	RISSSGGPTNYADSVKG	GYGHGLDY
539A-R0029-C03	539A-M0168-C03	71	TLSSGYSNYAIA	RVNSDGSBSKGD	QTWGMGILVV	QYRMP	WIWPSGGWTQYADSVKG	GDSSGYPYFFDY
539A-R0029-C07	539A-M0168-C07	72	SGSSSNIQTNTLN	ANNQRPS	AAWDDSLSGL	FYTMR	SIGSSGGYTGADSVKG	RHYGNSPYFFDY

WO 2009/111450

PCT/US2009/035840

539A-R0029-C08	539A-M0168-C08	73	TGTSNDVGGYNYVS	EVSNRPS	NSYTSRTWV	PYEMN	GIVPSGGITMYADSVKG	DNRNFMVMIDY
539A-R0029-C09	539A-M0168-C09	74	RASQGISNYLA	DASSLES	QQFNSYPLT	HYMP	SIYSSGGVTWYADSVKG	VSYDSSGYYPFDY
539A-R0029-D04	539A-M0168-D04	75	TGTSSDVGGYNYVS	EVSKRPS	SSYAGSNNLGV	MYML	SIYSSGGMTMYADSVKG	VGIAVAGRALDY
539A-R0029-D09	539A-M0168-D09	76	RASQVISWLA	AASSLQS	QQYNSYPWA	KYIMM	WIYSSGGNTNYADSVKG	EGAYSGSYGGDAFDI
539A-R0029-D12	539A-M0168-D12	77	TGNTTDVGGYNYVA	DVSNRPS	SSEFTRSTHV	HYPMP	YIYPSGGVTPYADSVKG	DPFYDFWSGYYTS
539A-R0029-E01	539A-M0168-E01	78	RTSQDVRNWVA	MASTLQS	QQADTFPWT	MYSMN	SIVSSGGDTRYADSVKG	DISGYPPYFDY
539A-R0029-F02	539A-M0168-F02	79	RASQNIHSYLH	AASTLQR	HQSYMSPPT	WYMMG	VIYPSGGHTPYADSVKG	DHIRTASGAFWFDP
539A-R0029-F07	539A-M0168-F07	80	RASQSVSSNYLA	HADNR	QQYGTSPGVT	IYIME	RISPSGGDTIYADSVKG	TKGVDCSGGSCYRAGIDY
539A-R0029-H01	539A-M0168-H01	81	TGTSSDVGAYNYVS	DVSDRPS	CSVARASTFSYV	MYWVG	SIVSSGGWTQYADSVKG	DHDSSGYWFDD
539A-R0029-H02	539A-M0168-H02	82	QASQDINIYLN	DASNLEP	QREFDDLTY	VYGMY	RIGPSGGMTYYADSVKG	ERLPYGDHQHYFDY
539A-R0029-H03	539A-M0168-H03	83	QASQDIDNYLN	DASNLET	QQYDDDLPRDT	VYWML	YIYSSGGWTVYADSVKG	VVFE.SGDFWSGYPPYYFDY
539A-R0029-H07	539A-M0168-H07	84	SGSSNIENNTVN	GDTERPS	ATWDDTLDGYV	VYIMG	SIYSSGGSTNYADSVKG	RGDWGSVGFDP

Example 5: CDR Sequences of MMP-9 Binding Fabs

Unique Fab on phage sequences SC-017 SR-001 539A-M00166, SC-018 SR-001 539A-M0167, SC-019 SR-001 539A-M0168: Results are summarized in **Table 5**.

Table 5. Unique sequence clones found in the SC17-19 phage screening.

Isolate	Initial Name	L-Info	Project Reps	LV-CDR1	LV-CDR2	LV-CDR3	HV-CDR1	HV-CDR2	HV-CDR3
539A-R0031-A04	539A-M0186-B05	L.KU	3	RASQSISGWLA	KASTLES	QQYDSYPYT	YMN	VISPSGGTTNYADSVKG	GSSIAARPLDY
539A-R0031-B10	539A-M0186-E08	L.KU	1	RASQSIGSYLN	AVSSLQS	QQSYSNPIS	DYTME	GISPSGGYTDYADSVKG	NLITMIVVGEFDY
539A-R0031-C12	539A-M0186-G11	L.LJ	2	SGSSSNIGGNRVN	STNQRPS	AAWDDTLNGPV	NYDMM	SIGSSGGITFYADSVKG	EYSSGWPLDY
539A-R0031-D10	539A-M0196-A01	L.KU	1	RASQSISTFLN	AASSLQS	QQSYSTPPIT	RYDML	GISPSGGFTTYADSVKG	PALYYYGSGRLKAFDI
539A-R0031-E07	539A-M0196-B03	L.KU	1	RSSQSLLSHNGYNYLD	LGSYRAS	MQALQTPIT	RYQMG	SISPSGGGTYYADSVKG	NYYIMDV
539A-R0031-E09	539A-M0196-B05	L.KU	1	RASQGIRNDLG	AASSLQS	LQHNSYPFT	AYRMQ	YIGSSGGQTSYADSVKG	AKPGRPFDF
539A-R0031-E12	539A-M0196-B08	L.KU	1	RASQSISSYLN	AASSLQS	QQSYSTPHT	HYVMS	SIGSSGGDTHYADSVKG	VWISGSYLDAFDI
539A-R0031-F07	539A-M0196-C07	L.KU	1	RSSQSLLSHNGYNYLD	LGSHRAS	MQALQTPVIT	KYMMF	SIYPSGGWTYYADSVKG	LGYPFY
539A-R0031-F08	539A-M0196-C09	L.KU	9	RASQSISSWLA	KASFLKS	QQYNSYPFT	HYIMF	GIYPSGGFTYYADSVKG	GHDAFDI
539A-R0031-G07	539A-M0196-D11	L.KU	1	RASQSVGSQLA	DASTRAT	HQYDNWPHT	FYRMS	WIGSSGGFTSYADSVKG	SGGVAGTFGY
539A-R0031-G08	539A-M0196-D12	L.KU	1	RASQSIHWLA	KASSLQS	QQYDSYPFT	PYYMS	VISPSGGVTHYADSVKG	SSSSSWYAFDY

Table 5 continued

539A-R0031-G11	539A-M0196-E03	L.KU	1	RASQFISHWLA	KSSTLKS	QQYDSVPYT	YYGML	YISP SGGFTKYADSVKG	DLSSGGFDY
539A-R0031-H09	539A-M0196-F04	L.KU	1	RASQTISSWLA	RASTLKS	QQYDSYRYT	KYYMG	YIGSSGGYTNYADSVKG	PQLAFDI
539A-R0032-A02	539A-M0196-G03	L.LU	2	AGSSSNIGNSVY	SNNKRPS	AAWDDSLRSVV	RYGML	VIYPSGGVTWYADSVKG	PATMVRY
539A-R0032-B05	539A-M0206-C04	L.KU	1	RVSQSVSSSYLA	GASSRAT	QQRSNWPPIT	VYAMH	SIVPSGGVTLYADSVKG	SSSSFLYYYYGMDV
539A-R0032-B09	539A-M0206-E10	L.LU	1	TGTSSDVGGYNYVS	EVGNRPS	SSYTSSSTWV	LYVMQ	VIVPSGGDTYYADSVKG	GYCTGGVCYLGFDG
539A-R0032-B11	539A-M0206-F05	L.KU	1	RSSQSLHSDGYNLYD	LGSNRAS	MQALQTPLT	WYTMA	SIWSSGGQTQYADSVKG	PGLPIAGSFHGDFFL
539A-R0032-B12	539A-M0206-F08	L.KU	2	RANQVISTWLS	TASTLQS	QQANSFPIT	HYPMI	SIRPSGGDTKYADSVKG	METGYDILTGYIIRWRYFDY
539A-R0032-C01	539A-M0206-F10	L.LU	1	SGSSSNIGSNYVY	INDHRPS	AVWDDSLSGWV	DYFMY	SIGPSGGWTWYADSVKG	GTGSFDY
539A-R0032-C06	539A-M0206-G09	L.LU	1	SGSSSNIGSNYVY	TNNQRPS	ATWDDDLGPFV	KYAMY	SIVSSGGETHYADSVKG	GGQWLPHYFDS
539A-R0032-C08	539A-M0206-H09	L.KU	1	RASQSVSTNLA	GASTRAT	QQYGSSQLT	NYRMI	RISSSGGNTQYADSVKG	AGGYSYGPPTYYYYGMDV

Example 6: Affinity Ranking of MMP-9 Binding Fabs

Affinity ranking of 24 Fabs from the R0017 plate was performed by BIACORE® Flexchip.

5 Results are summarized in **Table 6**.

Table 6. Affinity ranking by Flexchip

Difference from reference	spot content	ka	kd	KD	rmax average
1794.8151	Anti-MMP-9	No Binding *	No Binding *	---	
13678.9169	anti-His	No Binding *	No Binding *	---	
314.5676	PBS	No Binding *	No Binding *	---	
845.8099	TIMP-1	No Binding *	No Binding *	---	
1746.5973	539A-M0081-D05	4.10E+04	2.04E-04	4.97E-09	9.20E+01
1725.9598	539A-M0076-D03	5.97E+04	3.51E-04	5.88E-09	3.68E+02
1454.5212	539A-M0072-H07	1.42E+05	1.01E-03	7.13E-09	3.59E+01
1744.5526	539A-M0075-D12	6.77E+04	6.53E-04	9.65E-09	2.88E+02
1889.4882	539A-M0075-B09	1.38E+05	1.57E-03	1.14E-08	1.90E+02
1539.5203	539A-M0075-A07	1.18E+05	1.61E-03	1.36E-08	1.93E+02
1756.4697	539A-M0076-D07	5.78E+04	1.02E-03	1.76E-08	3.03E+02
1527.4429	539A-M0081-G03	5.93E+04	1.13E-03	1.91E-08	9.80E+01
1661.5067	539A-M0072-F02	5.30E+04	1.24E-03	2.33E-08	3.23E+02
1428.5431	539A-M0071-E12	9.11E+04	2.18E-03	2.40E-08	9.71E+01
2059.7763	539A-M0082-G08	4.36E+04	1.13E-03	2.60E-08	2.82E+02
1952.6749	539A-M0076-E11	6.55E+04	1.79E-03	2.73E-08	8.38E+01
1561.9125	539A-M0072-H10	2.32E+05	6.96E-03	3.00E-08	8.78E+01

1673.6221	539A-M0072-C04	1.42E+05	5.07E-03	3.58E-08	7.42E+01
2073.5321	539A-M0084-E03	1.10E+05	4.60E-03	4.18E-08	2.82E+02
2062.6144	539A-M0082-G09	1.62E+05	7.54E-03	4.66E-08	9.33E+01
1911.293	539A-M0073-C11	9.54E+04	4.49E-03	4.71E-08	1.76E+02
1840.1489	539A-M0072-G08	7.42E+04	3.82E-03	5.15E-08	3.22E+02
1774.4522	539A-M0071-E02	4.82E+04	2.69E-03	5.58E-08	2.37E+02
1804.1611	539A-M0075-F03	9.17E+04	5.64E-03	6.15E-08	1.37E+02
2037.2009	539A-M0081-E01	3.33E+04	2.17E-03	6.52E-08	3.34E+02
1598.526	539A-M0071-D03	6.46E+04	5.30E-03	8.20E-08	2.62E+02
1989.357	539A-M0075-G12	1.51E+05	1.30E-02	8.63E-08	1.20E+02

No Binding * an air bubble was introduced to the flowcell during the injection.

Example 7: Competition Experiments

5 Results of the competition experiments are summarized in **Table 7**.

Isolate	M0078- G07	M0081- D05	M0076- D03	M0072- H07	M0075- D12
KD (nM)	3.1	5.0	5.9	7.1	9.6
epitope	B	A	A	A	A
t1/2 (min)	25	57	33	11	18

R0025-B12_M0131-F06 used for competition.

Example 8:

As shown in FIG. 1A, antibody 539A-M0166-F10 has an IC₅₀ of 4.3 ± 1.9 nM on human
 10 MMP-9 activity. The IC₅₀ is ~ 33nM for the 539A-M0166-F10 Fab.

FIG. 1B shows that 539A-M0166-F10 is specific for human MMP-9 (hMMP-9) as compared to the other human (h) and murine (m) MMPs tested.

The residual enzyme activity was measured in the presence of 1 μ M antibody (Fab or hIgG-1, as indicated in FIG. 1B). The human MMP-1, -2, -3, -7, -8, -9, -10, -12, -13, and -14 were obtained from BIOMOL (Human MMP-9: SE-244, BIOMOL; Human MMP-14: SE-259, BIOMOL; Human MMP-1, -2, -8, -13: MMP MultiPack-1 from BIOMOL; Human MMP-3, -7, -10, -12: MMP MultiPack-2 from BIOMOL). The mouse MMP-2 and -9 were from R&D (Mouse MMP-9: 909-MM, R&D Mouse MMP-2: 924-MP, R&D). The substrate was Mca-Pro-Lys-Pro-Leu-Ala-Leu-Dap(Dnp)-Ala-Arg-NH₂ (M-2225, Bachem) for human MMP-3, and Mca-Lys-Pro-Leu-Gly-Leu-Dap(Dnp)-Ala-Arg-NH₂ (M-2350, Bachem) for all the other enzymes. The substrate concentration in the assay was 10 μ M.

10 Example 9:

539A-M0166-F10 also decreases MMP-9 activity in MCF-7 and Colo205 tumors, as measured by in situ zymography (data not shown).

Example 10:

The DNA and amino acid sequences of variable regions of 539A-M0166-F10 sFAB are as follows:

539A-M0166-F10 (phage/SFAB) VL leader +VL

SEQ ID NO: 2:

TTCTATTCTCACAGTGCACAGAGCGAATTGACTCAGCCACCGTCAGCGTCTGCGGCCCCCGGGCAGAGGGTCACCATCTCTT
 20 GTTCTGGAAGCAGCTCCAACATCGGAAGTAACACTGTAACCTGGTACCAGAAGCTCCCAGGAACGGCCCCCAAGCTCCTCAT
 TTACAATAATTATGAGCGGCCCTCAGGGGTCCCTGCCGATTCTCTGGCTCCAAGTCTGGCACCTCAGCCTCCCTGGCCATC
 AGTGGGCTCCAGTCTGAGGATGAGGCTGATTATTACTGTGCAACATGGGATGACAGCCTGATTGCCAATTACGTCTTCGGAA
 GTGGGACCAAGGTCACCGTCCTAGGTCAGCCCAAGGCCAACCCC

25 SEQ ID NO: 3:

FYSHSAQSELTQPPSASAAPGQRVTISCSGSSSNIGSNTVTWYQKLPGTAPKLLIYNNYERPSGVPARFSGSKSGTSASLAI
 SGLQSEDEADYYCATWDDSLIANYVFGSGTKVTVLGQPKANP

539A-M0166-F10 (phage/SFAB) VH leader + VH

SEQ ID NO: 4:

ATGAAGAAGCTCCTCTTTGCTATCCCGCTCGTCGTTCTTTTGTGGCCCAGCCGGCCATGGCCGAAGTTCAATTGTTAGAGT
 5 CTGGTGGCGGTCTTGTTTCAGCCTGGTGGTTCTTTACGTCTTTCTTGCGCTGCTTCCGGATTCACTTTCTCTCCTTACCTTAT
 GAATTGGGTTTCGCCAAGCTCCTGGTAAAGGTTTGGAGTGGGTTTCTTCTATCTATTCTTCTGGTGGCGGTACTGGTTATGCT
 GACTCCGTTAAAGGTCGCTTCACTATCTCTAGAGACAACCTCTAAGAATACTCTCTACTTGCAGATGAACAGCTTAAGGGCTG
 AGGACACGGCCGTGTATTACTGTGCGAGAATATACCATAGCAGCAGTGGACCTTTCTACGGTATGGACGTCTGGGGCCAAGG
 GACCACGGTCACCGTCTCAAGCGCCTCCACCAAGGGCCCATCGGTCTTCCCGCTAGCACCTCCTCCAAGAGC

10

SEQ ID NO: 5:

MKKLLFAIPLVVPFVAQPAMAEVQLLES GGGLVQPGGSLRLSCAASGFTFSPYLMNWVRQAPGKGLEWVSSIYSSGGGTGYA
 DSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARIYHSSSGPFYGM DVWGQGTTVTVSSASTKGPSVFPLAPSSKS

15 Example 11:

Experiments were performed to characterize the interaction of the M0166-F10 hIgG1 with human MMP-9. The K_i was measured and the inhibition mechanism was determined. The results show that inhibition of human MMP-9 by M0166-F10 appears to follow a competitive model, with a K_i value equal to 0.3 ± 0.5 nM.

20 The experiments were performed as follows:

Materials :

- Substrate : Mca -KPLGL-Dap(Dnp)-AR-NH₂ (M-2350) from BACHEM (521575). A 10mM stock solution was prepared in DMSO.
- Human MMP-9 catalytic domain (BIOMOL, SE-244), stock solution at 0.24 mg/ml.
- 25 - M0166-F10 hIgG1: 2551-095. Dialysed against TCN. Stock solution at 0.226 mg/ml.
- Experiments were performed in TCNB: 50 mM Tris/HCl, 10 mM CaCl₂, 150 mM NaCl, 0.05% Brij 35, pH 7.5.
- 96-well black plates from Perkin Elmer (6005270).
- Spectramax M2e to measure fluorescence emission of the substrate upon hydrolysis
- 30 (temperature control set at 30°C; λ_{exc} =328 nm and λ_{em} =393 nm).

Procedure :

- 90 µl of the enzyme (final concentration = 0.6 nM) was preincubated with 90 µl of various concentrations (0-100 nM final) of M0166-F10 for 1.5 h at 30°C. 20 µl the substrate was then added to a final concentration ranging from 3 to 15µM, and initial rates were recorded.
- Each data point was measured in triplicate, and initial rates were averaged.
- Averaged initial rates were plotted against the M0166-F10 concentration for each substrate concentration, and IC₅₀'s were calculated using the following equation :

$$y = \frac{Range}{1 + \left(\frac{x}{IC_{50}} \right)^s}$$

- The IC₅₀ values were then plotted against the substrate concentration.

Results :

The plot of the measured IC₅₀ (nM) vs. the substrate concentration (µM) is shown in FIG. 2. The IC₅₀ increases linearly with the substrate concentration, which indicates that M0166-F10 behaves as a competitive inhibitor of the human MMP-9.

For a competitive inhibition model, the following equation applies:

$$IC_{50} = K_i + \frac{E}{2} + \frac{K_i}{K_m} [S]$$

and therefore the value of the K_i can be calculated from the intercept. Here, K_i = 0.3 ± 0.5 nM.

The IC₅₀ measurements for M0166-F10 at various concentrations of substrate Mca -KPLGL-Dap(Dnp)-AR-NH₂ from BACHEM (M-2350) (3 µM, 5 µM, 7.5 µM, 10 µM, 12.5 µM, and 15 µM) are shown in FIG. 3.

FIG. 4 shows the IC₅₀ measurements for an MMP-9 binding protein (539A-M0240-B03) at 10 mM concentration of human MMP-9 (top) or mouse MMP-9 (bottom).

The results in FIG. 5 show that an MMP-9 binding protein (539A-M0240-B03) inhibits human and mouse MMP-9 but not human MMP-1, -2, -3, -7, -8, -10, -12, and -14.

The results in FIGS. 6A and 6B show IC₅₀ (nM) versus substrate concentration (µM) of an MMP-9/-2 binding protein (539A-M0237-D02). Human MMP-9 (FIG. 6A) and mouse MMP-9 (FIG. 6B) were used as substrates.

Example 12: Exemplary Clone Identified

539A-M0240-B03 is a selective inhibitor of MMP-9. 539A-M0240-B03 can decrease or inhibit the activity of human and mouse MMP-9.

The sequences of the complementarity determining regions (CDRs) of 539A-M0240-B03 light chain (LC) and heavy chain (HC) are as follows:

LC CDR1: TGTSSDVGGYNYVS

LC CDR2: DVSKRPS

LC CDR3: CSYAGSYTLV

HC CDR1: TYQMV

HC CDR2: VIYPSGGPTVYADSVKG

HC CDR3: GEDYYDSSGPGAFDI

Example 13: Additional MMP-9 binding proteins

A protein containing the HC CDR sequences of 539A-M0240-B03 and the light chain sequence shown below can be used in the methods described herein. A protein containing the LC CDRs shown below and the HC CDRs of 539A-M0240-B03, or a protein containing the LC variable region (light V gene) shown below and the 539A-M0240-B03 HC CDRs can also be used in the methods described herein. The protein can include a constant region sequence, such as the constant region (LC- lambda1) shown below.

Light V gene = VL2_2e; J gene = JL3

FR1-L	CDR1-L	FR2-L	CDR2-L
QSALTQPRSVSGSPGQSVTISC	TGTSSDVGGYNYVS	WYQQHPGKAPKLMY	DVSKRPS GVPD

FR3-L	CDR3-L	FR4-L
RFSGSKSGNTASLTISGLQAEDEADYYC	CSYAGSYTLV	FGGGTKLTVL

LC-lambda1

GQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSHRSYS
CQVTHEGSTVEKTVAPTECS

CDR regions are in bold.

The amino acid and nucleic acid sequences for another exemplary protein that can be used in the methods described herein are provided below. A protein containing the LC and HC CDRs shown below, or a protein containing the light chain and heavy chain variable regions (LV and HV, respectively) shown below can also be used in the methods described herein.

5 **539A-M0240-B03:** Parental isolate (sFab; IgG-pBh1(f)).

539A-X0034-C02 (GS clone): DX-2802: Germlined, sequence optimized. The entire antibody fragment, containing the signal sequence, variable region and constant region of both the light and heavy chains were sequenced. The sequence data is available in 539A-R0108-A01 (539A-X0034-C02).

Light Chain

Light V gene = VL2_2e 2e.2.2/V1-3/DPL12
Light J gene = J13

5

FR1-L FR2-L CDR1-L FR2-L CDR2-L
539A-M0240-B03: QYELTQPRSVSGSPGQSVTISC TGISSDYGGYNYVS WYQQHPGKAPKLMY DVSKRPS GVPD
FR3-L CDR3-L FR4-L
539A-M0240-B03: RFSGSKSGNTASLTISGLQAEDEADYYC CSYAGSYTLV FGGGTKLTVL

10

Heavy Chain

Heavy V gene: VH3_3-23 DP-47/V3-23
Heavy J gene: JH3

15

FR1-H FR2-H CDR1-H FR2-H CDR2-H
539A-M0240-B03: EVQLLESGGGLVQPGGSLRLSCAASGFTFS TQQMV WVRQAPGKGLEWVS VIYPSSGGPTIVYADSVKQ
FR3-H CDR3-H FR4-H
539A-M0240-B03: RFTISRDN SKNTLYLQMNSLRAEDTAVYYCAR GEDIYDSSGPGAFDE WQGTMVTVSS

20

Light Variable
539A-M0240-B03-Light: Parental clone (sFab; IgG in pBh1(f)) light variable

25

Q Y E L T Q P R S V S G S P G Q S V T I
539A-M0240-B03 : CAGTACGAATTGACTCAGCCCTCGCTCAGTGTCGGGTCTCCTGGACAGTCAGTCACCATC
S C T G T S S D V G G Y N Y V S W Y Q Q
539A-M0240-B03 : TCCTGCACTGGAAACCAGCAGTGAATGTTGGTGTATTAACATAIGTCTCCGTGGTACCAACAG
H P G K A P K L M I Y D V S K R P S G V
539A-M0240-B03 : CACCCAGGCAAAGCCCCCAACTCATGATTATGAATGACGTAAGCGGCCCTCAGGGGTC
P D R F S G S K S G N T A S L T I S G L
539A-M0240-B03 : CCTGATCGCTTCTCTGGCTCCAAGTCTGGCAACACGGCCCTCCCTGACCATCTCTGGGCTC

30

35

539A-M0240-B03 : Q A E D E A D Y Y C C S Y A G S Y I L V
: CAGGCTGAGGATGAGGCTGATTATTACTGCTGCTCAATAICCAAGGACCTACACTTTGGTG
F G G T K L T V L
539A-M0240-B03 : TTCGGCGGAGGGACCAAGCTGACCGTCCTA
Heavy Variable
539A-M0240-B03-Heavy: Parental clone (sFab; IgG in pBh1(f)) Heavy variable
10 539A-M0240-B03 : E V Q L L E S G G L V Q P G S L R L
: GAAGTTCAATTGTTAGAGTCTGGTGGGCTTTGTTTTCAGCCTGGTGGTTCTTTACGTCCT
15 539A-M0240-B03 : S C A A S G F T F S I Y Q M V W V R Q A
: TCTTGGCTGCTTCGGATTCACTTTCTCTACTTACCAGATGGTTTGGGTTTCGCCAAGCT
539A-M0240-B03 : P G K G L E W V S V I Y P S G G P I V Y
: CCTGGTAAAGGTTTGGAGTGGGTTTCTGTTAATCAATCCCTTCTGGTGGCCCTACTGTTTAT
123 539A-M0240-B03 : A D S V K G R F T I S R D N S K N T L Y
: GCTGACTCCGTTAAAGGTCGCTTCACTATCTCTAGAGACAACTCTAAGAACTACTCTCTAC
25 539A-M0240-B03 : L Q M N S L R A E D T A V Y Y C A R G E
: TTGCAGATGAACAGCTTAAGGGCTGAGGACACGGCCGTGTATTACTGTGCGAGAGGGGAG
539A-M0240-B03 : D Y Y D S S G P G A F D I W G Q G T M V T V S S
: GACTACTAATGATAGTAGTGGCCCGGGGCTTTTIGATAATCTGGGGCCAAAGGACAAATGGTCACCGTCTCAAGC
30

The amino acid and nucleic acid sequences for another exemplary protein that can be used in the methods described herein are provided below. A protein containing the LC and HC CDRs shown below, or a protein containing the light chain and heavy chain variable regions (LV and HV, respectively) shown below can also be used in the methods described herein. A protein containing the light chain and heavy chain (designated as LV+ LC and HV + HC, respectively, below) sequences can also be used.

Light Chain

Light V gene = VL2_2e 2e.2.2/V1-3/DPL12
Light J gene = JL3

5

539A-X0034-C02 (DX-2802): QSALTQPRSVSGSPGQSVTI SC FR1-L CDR1-L FR2-L CDR2-L
FR3-L FR4-L
RFSGSKSGNTASLTISGLQAEDEADYYC CSYAGSYTLV FGGGTKLTVL WYQQHPGKAPKLMY DVSKRPQ GVPD

10

Heavy Chain

Heavy V gene: VH3_3-23 DP-47/V3-23
Heavy J gene: JH3

15

539A-X0034-C02 (DX-2802): EVQLLES GGLVQPGGSLRLSCAASGFTFS FR1-H CDR1-H FR2-H CDR2-H
FR3-H FR4-H
RFTISRDN SKNTLYIQMNSLRAEDTAVYYCAR GEDYYDS3GFGAFDI WGQGTMTVTVSS

125

Light Variable

539A-X0034-C02 (DX-2802)-Light: Germlined, codon optimized in GS vector

25

539A-X0034-C02 (DX-2802) : CAGAGCGCCCTGACCCAGCCCAAGCGTGTCGGCAGCCAGCCAGCGTGACCATC
Q S A L T Q P R S V S G S P G Q S V T I

539A-X0034-C02 (DX-2802)

: AGCTGCACCGGCACCGACGACGAGCGTGGCGGCTACAACTACGTCCTGGTATCAGCAG
S C T G T S S D V G G Y N Y V S W Y Q Q

30

539A-X0034-C02 (DX-2802)

: CACCCCGCAAGGCCCAAGCTGATCTACGACGGTGTCCAAAGAGCCCGCGCGTG
H P G K A P K L M I Y D V S K R P S G V

539A-X0034-C02 (DX-2802)

: CCCGACAGGTTCAGCGGCAGCAAGAGCGGCAACCCGCGCCTGACCATCTCCGGACTG
P D R F S G S K S G N T A S L T I S G L

35

539A-X0034-C02 (DX-2802)

: CAGGCCGAGGACGAGCGCGACTACTGCTGCAGCTACGCGGCGAGCTACACCCCTGGTG
Q A E D E A D Y Y C C S Y A G S Y T L V

539A-X0034-C02 (DX-2802) : TTCGGCGGAGGGACCAAGCTGACCGTGCTG
F G G G T K L T V L

Heavy Variable
539A-X0034-C02 (DX-2802)-Heavy: Germlined, codon optimized in GS vector

539A-X0034-C02 (DX-2802) : GAGGTGCAATTGCTGGAAAGCGGCGGAGGACTGGTGCAGCCAGGCGGCGAGCCCTGAGGCTG
E V Q L L E S G G L V Q P G G S L R L

539A-X0034-C02 (DX-2802) : TCCTGCGCGCCAGCGGCTTCACCTTCAGCACCTACCAAGATGGTGTGGGTGCGGCCAGGCC
S C A A S G F T F S I Y Q M V W V R Q A

539A-X0034-C02 (DX-2802) : CCAGGCAAGGCCCTGGAATGGGTGTCCTGATCTACCCGAGCGCGGAGCCGCGTGTAC
P G K G L E W V S V I Y P S G G P I V Y

539A-X0034-C02 (DX-2802) : GCCGACAGCGTGAAGGCGAGGTTACCATCAGCAGGGACAACAGCAAGAACACCCCTGTAC
A D S V K G R F T I S R D N S K N T L Y

539A-X0034-C02 (DX-2802) : CTGCAGATGAACAGCCCTGAGGGCCGAGGACACCGCCGTGTACTACTGCGCCAGGCGCGGAG
L Q M N S L R A E D T A V Y C A R G E

539A-X0034-C02 (DX-2802) : GACTACTACACAGCAGCGCGCGCCAGCGCGCTTCACATGCTGGGCCAGGGCACAAATGGTGACCGTGTCCAGC
D Y Y D S S G P G A F D I W G Q G T M V T V S S

>539A-X0034-C02 (DX-2802): LV+LC dna
CAGAGCGCCCTGACCCAGCCAGAGCGTGTCCGGCAGCCAGGCCAGAGCGTGACCATCAGCTGCACCCGGCACCCAGCAGCGACGTGGCGGGCTACAACTA
CGTGTCCTGGTATCAGCAGCACCCCGCAAGGCCCAAGCTGATGATCTACGACGTGTCCAAGAGGCCCAGCGCGGTGCCGACAGGTTCAGCGGACGAAGAGCG
GCAACACCGCCAGCCTGACCATCTCCGACTGCAGGCCGAGGCGGACTACTGCTGAGCTACGCCGGCAGCTACACCTGGTGTTCGGCGGAGGGACC
AAGCTGACCGTGTGGCCAGCCCAAGGCTGCCCCAGCGTGACCCCTGTTCGCCCCAGCAGGAACTGCAGGCAACAAAGGCCACACTGGTGTGCCGTGATCAG
CGACTTCTACCCAGGCGCGTGACCGTGGCCTGGAAGGCCGACAGACGCCCCGTGAAGGCCGCGTGGAGACAAACCCAGCAAGCAGCAACAAGTACG
CCGCCAGCAGCTACCTGAGCCTGACCCCCGAGCAGTGGAAGTCCCACAGGTCTCTACAGCTGCCAGGTGACCCACGAGGGCAGCACCGTGGAGAAAACCGTGGCCCCC
ACCGAGTGTAGCTGATGA

> 539A-X0034-C02 (DX-2802): HV+HC dna
GAGGTGCAATTGCTGGAAGCGGCGGAGGACTGGTGCAGCCAGCGGCGAGCCTGAGGCTGTCTCTGCGCCGCCAGCGGCTTCACCTTCAGCACCTACCAGAT
GGTGTGGGTGCGCCAGGCCCAAGGCGCTGGAATGGGTGTCCGTGATCTACCCAGCGGCGGACCCACCGTGTACGCCGACAGCGTGAAGGCGAGGTTACCA
TCAGCAGGGACACAGCAAGAACACCCCTGTACCTGCAGATGAACAGCCTGAGGGCCGAGGACACCGCCGTGTACTACTGCGCCAGGGCGGAGGACTACTACGACAGC

AGCGGCCAGGCGCCTTCGACATCTGGGGCCAGGGCACAATGGTGACCGTGTCAGCGCCAGCACCAAGGGCCCCAGCGTGTTCCCGCTAGCACCTTCCCTCCAAGTC
CACCTCTGGCGGCACCGCGCTCTGGGCTGCCCTGGTGAAGGACTACTTCCCTGAGCCTGTGACCGTGAGCTGGAACCTCTGGCGCCCTGACCTCCGGCGTGCATACCT
TCCCTGCCGTGCTGCAGTCCCTCCGGCCTGTACTCCCTGTCTCCCTCCGTGGTGACAGTGCCCTCCCTCCCTGGCACCCAGACCTACATCTGCAACGTGAACCAACAAG
CCTTCCAACACCAAGGTGGACAAGCGGGTGGAGCCTAAGTCCTGCGACAAGACCCACACTGCCCTGCCCTGAGCTGCTGGCGGACCCCTCCGTGTT
CCTGTTCCCTCCTAAGCCTAAGGACACCCCTGATGATCTCCCGACCCCTGAGGTGACCTGCGTGGTGACGTGCCACGAGGCCAGAGGTGAAGTTTAATT
GGTATGTGACGGCGTGGAGTCCACAAAGCCCAAGCAAGCTCGGGAGGAACAGTACAACCTACCGGGTGGTGTCCTGCTGCTGACCGTGTGACCCAGGAC
TGGCTGAACGGCAAGGAATACAAGTGCAAGTCTCCAACAAAGGCCCTGCTGCCCCATCGAGAAAACCAATCTCCAAGGCCAAGGGCCAGCCTCGCGAGCCTCAGGT
GTACACCCCTGCCCTCCTAGCCGGGAGGAAATGACCAAGAACCAAGGTGTCCCTGACCTGTCTGGTGAAGGGCTTCTACCCCTCCCGATATCGCCGTGGAGTGGAGTCCA
ACGGCCAGCCTGAGAACAACTACAAGACCAACCCCTCCTGTGCTGGACTCCGACGGCTCCTTCTTCTGTACTCCAAGCTGACCGTGGACAAGTCCCGGTGGCAGCAG
GGCAACGTGTTCTCCTGCTCCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCCCTGTCCCTGAGCCCTGGCAAGTGA

> 539A-X0034-C02 (DX-2802): LV+LC aa
QSALTQPRSVSGSPGQSVTISCTGTSSDVGGYNVSWYQQHPGKAPKLMYDVSKRPSGVDPDRFSGSKSGNTASLTIISGLQAEDEADYYCCSYAGSYTLVF
GGGTKLTVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETITPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEK
TVAPTECSss

> 539A-X0034-C02 (DX-2802): HV+HC aa
EVQLLESGGGLVQPGGSLRLSCAASGFTTFSTYQMVWVRQAPGKGLEWVSVIYPSGGPTVYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARGED
YYDSSGPGAFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQYICN
VNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMI SRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTV
LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYITLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPPVLDSDGSFFLYSKLTVDKS
RWQQGNVFCSCVMHEALHNHYTQKSLSLSPGKS

Example 14: Studies with colon cancer cells

The efficacies of novel antibodies DL8, DL12, DL15 and DL2 in the Colo205 colon carcinoma model were evaluated. The antibodies were tested alone or in combination. The results are shown in FIG. 7.

5

Drugs and Treatment:

Gr.	N	1 Drug/Testing Agent					2 Drug/Testing Agent				
		Agent	Vehicle	mg/kg	Route	Schedule	Agent	Vehicle	mg/kg	Route	Schedule
1 [#]	10	vehicle	PBS	-	ip	qod to end	-	-	-	-	-
2	10	paclitaxel	5%EC	30	iv	qod x 5	-	-	-	-	-
3	10	DL8	PBS	20	ip	qod to end	-	-	-	-	-
4	10	DL12	PBS	20	ip	qod to end	-	-	-	-	-
5	10	DL15	PBS	20	ip	qod to end	-	-	-	-	-
6	10	DL2	Citrate buffer	10	ip	qod to end	-	-	-	-	-
7	10	DL2	Citrate buffer	10	ip	qod to end	DL12	PBS	20	ip	qod to end

#: Control Group

Procedures:

- Set up HRLN female nu/nu mice with 1×10^6 Colo205 tumor cells in 50% Matrigel subcutaneously (sc) in flank
- Do a pair match when tumors reach an average size of 100 - 150 mg, and begin treatment
- Body Weight: daily for the first five days and then biwk to end
- Caliper Measurement: biwk to end
- Final body weights and calipers should be taken on the last day of the study.
- Endpoint TGI (tumor growth inhibition). Animals are to be monitored as a group. The endpoint of the experiment is a mean tumor weight in Control Group of 1 gms or 45 days, whichever comes first. When the endpoint is reached, all the animals are to be euthanized.

20

Study Conditions:

- Statistical analysis of the data will be performed using:

- Kruskal-Wallis with post hoc Dunn's test Groups 3-7 vs Group 1 and Group 7 vs Groups 4 and 6
- Mann-Whitney test Group 1 vs Group 2
- Clinical agent PACLITAXEL is for use as a positive control only

5

Dosing:

- Prepare dosing solutions:
 - DL2, DL8, DL12, DL15 - every week, store at 4° C
 - paclitaxel - every dose, store at room temp
- 10 • DL12 = B03 in PBS = 539A-M0240-B03 IgG1 (h/mMMP-9 antibody inhibitor) (parental) (539A-M0240-B03 listed above)
- DL8 = D02 in PBS = 539A-M0237-D02 IgG1 (MMP-9/-2 dual reactive antibody inhibitor) (parental)
- DL15 = F10 in PBS = 539A-M0166-F10 IgG1 (hMMP-9 antibody inhibitor) (parental)
- 15 • DL2 = DX-2400 in citrate buffer solution.
- paclitaxel = paclitaxel in 5% Ethanol : 5% Cremophor EL : 90% D5W
- vehicle = PBS
- Dosing volume = 10 mL/kg (0.200 mL/20 g mouse). Adjust volume accordingly for body weight.
- 20 • Save remaining compound for future use
- Discard remaining dosing solution

Sampling:

- **Sampling 1**
 - 25 ○ Timepoint: 24 hours post 5th dose of DL10 (**Day 10**)
 - All Groups, the 6 Animals closest to mean:
 - Blood Collection

- Collect full volume blood by terminal cardiac puncture under CO₂ anesthesia
- Process blood for:
 - Serum (anti-coagulant - none, preservation - freeze, shipping condition - -80° C.)

5

- **Sampling 2**

- Timepoint: 24 hours post 10th dose of DL10 (**Day 20**)
- All Groups, same animals sampled in Sampling 1:
- Blood Collection as above

10

- **Sampling 3**

- Timepoint: at endpoint (24 hrs post last DL dose)
- All Groups All Animals:
- Blood Collection
 - Collect full volume blood by terminal cardiac puncture under CO₂ anesthesia
 - Process blood for:
 - Serum (anti-coagulant - none, preservation - freeze, shipping condition - -80° C.)

15

- Organ Collection

20

- Tumor (weigh sample, divide into 2 parts)
 - Part 1: preservation - snapfreeze in a cryovial, shipping condition - -80° C.
 - Part 2: preservation - OCT, shipping condition - -80° C.

25

539A-M0166-F10: The variable domain sequences for 539A-M0166-F10 are provided above.

DX-2400: DX-2400 is an inhibitory MMP-14 binding antibody. The variable domain sequences for DX-2400 are:

VH:

5 DX-2400 FR1----- CDR1- FR2----- CDR2-----
EVQLLESGGGLVQPGGSLRLSCAASGFTFS **LYSMN** WVRQAPGKGLEWVS **SIYSSGGSTLY**

DX-2400 CDR2-- FR3----- CDR3-- FR4-----
ADSVKG RFTISRDN SKNTLYLQMNSLRAEDTAVYYCAR **GRAFDI** WGQGTMTVTVSS

10 CDR regions are in bold.

VL:

15 DX-2400 FR1----- CDR1----- FR2----- CDR2---
DIQMTQSPSSLSASVGDRVTITC **RASQSVGTYLN** WYQQKPGKAPKLLIY **ATSNLRS GVPS**

DX-2400 FR3----- CDR3----- FR4-----
RFSGSGSGTDFTLTISSLPEDFATYYC **QQSY SIPRFT** FGPGTKVDIK

CDR regions are in bold.

20 **539A-M0237-D02:** 539A-M0237-D02 is an inhibitory MMP-9/-2 dual reactive antibody.

The variable domain sequences for 539A-M0237-D02 are:

VH:

25 MKKLLFAIPLVVPFVAQPAMAEVQLLESGGGLVQPGGSLRLSCAASGFTFSQYPMWWVRQAPGKGLEWVS YIVP
SGGRITYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKDRA YGDYVGWNGFDYWGQGT LVTVSSAST
KGPSVFPLAPSSKS

VL:

30 FYSHSAQDIQMTQSPATLSLSPGERATLSCRASQSISSFLAWYQQKPGQAPRL LIYDASYRATGIPARFSG
SGSGTDFTLTISSLEPEDYAVYYCQQRG NWPITFGQGT RLEIKRTVAAPS

Example 15: Studies with colon cancer cells

The efficacies of novel antibodies DL8, DL12, and DL2 in the BxPC-3 pancreatic carcinoma model were evaluated. The antibodies were tested alone or in combination. The results are shown in FIG. 8.

35

Drugs and Treatment:

Gr.	N	1 Drug/Testing Agent					2 Drug/Testing Agent				
		Agent	Vehicle	mg/kg	Route	Schedule	Agent	Vehicle	mg/kg	Route	Schedule
1 [#]	10	vehicle	PBS	-	ip	qod to end	-	-	-	-	-
2	10	paclitaxel	5%EC	30	iv	qod x 5	-	-	-	-	-
3	10	DL8	PBS	20	ip	qod to end	-	-	-	-	-
4	10	DL12	PBS	20	ip	qod to end	-	-	-	-	-
5	10	DL2	Citrate buffer	10	ip	qod to end	-	-	-	-	-
6	10	DL2	Citrate buffer	10	ip	qod to end	DL12	PBS	20	ip	qod to end

#: Control Group

Procedures:

- Set up HRLN female nu/nu mice with 1mm³ Bx-PC3 tumor fragments sc in flank
- Do a pair match when tumors reach an average size of 80 - 120 mg, and begin treatment
- Body Weight: 5/2 then biwk to end
- Caliper Measurement: biwk to end
- Final body weights and calipers should be taken on the last day of the study.
- Endpoint TGI. Animals are to be monitored as a group. The endpoint of the experiment is a mean tumor weight in Control Group of 1 gms or 45 days, whichever comes first. When the endpoint is reached, all the animals are to be euthanized.

Study Conditions:

- Statistical analysis of the data will be performed using:
 - Kruskal-Wallis with post hoc Dunn's test Groups 3-6 vs Group 1 and Group 6 vs Group 3 and Group 5
 - Mann-Whitney test Group1 vs Group 2
- Clinical agent PACLITAXEL is for use as a positive control only

Dosing:

- Prepare dosing solutions:

- DL2, DL8, DL12 - every week, store at 4° C
- paclitaxel - every dose, store at room temp
- DL12 = B03 in PBS
- DL8 = D02 in PBS
- 5 • DL2 = DX-2400 in citrate buffer solution
- paclitaxel = paclitaxel in 5% Ethanol : 5% Cremophor EL : 90% D5W
- vehicle = PBS
- Dosing volume = 10 mL/kg (0.200 mL/20 g mouse). Adjust volume accordingly for body weight.
- 10 • Save remaining compound for future use
- Discard remaining dosing solution

Sampling:

- **Sampling 1**
 - 15 ○ Timepoint: 24 hours post 5th dose (**Day 10**)
 - All Groups 6 Animals closest to mean:
 - Blood Collection
 - Collect full volume blood by terminal cardiac puncture under CO₂ anesthesia
 - 20 ▪ Process blood for:
 - Serum (anti-coagulant - none, preservation - freeze, shipping condition - -80° C.)
- **Sampling 2**
 - Timepoint: 24 hours post 10th dose (**Day 20**)
 - 25 ○ All Groups same animals sampled in Sampling 1:
 - Blood Collection as above

- **Sampling 3**

- Timepoint: at endpoint (24 hours post last dose)

- All Groups All Animals:

- Blood Collection

5 ▪ Collect full volume blood by terminal cardiac puncture under CO₂ anesthesia

 ▪ Process blood for:

 ▪ Serum (anti-coagulant - none, preservation - freeze, shipping condition - -80° C.)

10 ○ Organ Collection

 ▪ Tumor (weigh sample, divide into 2 parts)

 ▪ Part 1: preservation – snap freeze in a cryovial, shipping condition - -80° C.

 ▪ Part 2: preservation - OCT, shipping condition - -80° C.

15 Example 16: Collagen-induced arthritis model

 ▪ Collagen-induced arthritis (CIA) is produced by the immunization of susceptible strains of rat / mice with native type II collagen.

 ▪ Collagen is emulsified in Complete Freund's Adjuvant (CFA) and injected subQ (100 µg collagen: 100 µg CFA/mouse) at the base of the tail. Ten mice are injected subQ with 0.05ml of distilled water/CFA emulsion. A booster injection of collagen in incomplete adjuvant is given IP 21 days after the initial immunization.

 ▪ Disease is due to an auto-immune response induced upon immunization with collagen.

25

Drugs and treatments

Group No.	No. Mice	Test Material	Dose	ROA (route of
-----------	----------	---------------	------	---------------

			(mg/kg)	administration)
1	10	Vehicle	N/A	IP, qod
2	10	539A-M0240-B03	20	IP, qod
3	10	Methotrexate	3	IP, QD
4	10	Vehicle	NON-sensitized mice	IP, qod

The joints were scored for severity of arthritis as follows, and the results are shown in FIG. 9.

0 = no visible effects of arthritis

1 = edema and erythema of one digit or joint

5 2 = edema and erythema of two joints

3 = edema and erythema of more than two joint

4 = severe arthritis of the entire paw and digits, accompanied by ankylosis of the ankle and deformity of the limb. The score for each limb was summed and recorded as the arthritic index (AI) for each individual animal.

10

The joints were scored for inflammation, pannus, cartilage damage and bone resorption as follows, and the results are shown in FIG. 10.

Inflammation scoring

- 15
- 0=Normal
 - 1=Minimal infiltration of inflammatory cells in synovium and periarticular tissue of affected joints.
 - 2=Mild infiltration, if paws, restricted to affected joints
 - 3=Moderate infiltration with moderate edema, if paws, restricted to affected joints.
 - 20 • 4=Marked infiltration affecting most areas with marked edema
 - 5=Severe diffuse infiltration with severe edema

Pannus

- 0=Normal
- 1=Minimal infiltration of pannus in cartilage and subchondral bone
- 2=Mild infiltration with marginal zone destruction of hard tissue in affected joints.
- 5 • 3=Moderate infiltration with moderate hard tissue destruction in affected joints.
- 4=Marked infiltration with marked destruction of joint architecture, most joints.
- 5=Severe infiltration associated with total or near total destruction of joint architecture, affects all joints.

10 *Cartilage Damage*

- 0=Normal
- 1=Minimal=minimal to mild loss of toluidine blue staining with no obvious chondrocyte loss or collagen disruption in affected joints
- 2=Mild=mild loss of toluidine blue staining with focal mild (superficial)chondrocyte loss
15 and/or collagen disruption in affected joints.
- 3=Moderate=moderate loss of toluidine blue staining with multifocal moderate (depth to middle zone) chondrocyte loss and/or collagen disruption in affected joints
- 4=Marked=marked loss of toluidine blue staining with multifocal marked (depth to deep
20 zone) chondrocyte loss and/or collagen disruption in most joints
- 5=Severe =severe diffuse loss of toluidine blue staining with multifocal severe (depth to tide mark) chondrocyte loss and/or collagen disruption in all joints.

25

Bone Resorption

- 0=Normal
- 1=Minimal=small areas of resorption, not readily apparent on low magnification, rare osteoclasts in affected joints
- 30 • 2=Mild=more numerous areas of, not readily apparent on low magnification, osteoclasts more numerous in affected joints

- 3=Moderate=obvious resorption of medullary trabecular and cortical bone without full thickness defects in cortex, loss of some medullary trabeculae, lesion apparent on low magnification, osteoclasts more numerous in affected joints
- 4=Marked=Full thickness defects in cortical bone, often with distortion of profile of remaining

5

A number of embodiments of the invention have been described. Nevertheless, it will be understood that various modifications may be made without departing from the spirit and scope of the invention. Accordingly, other embodiments are within the scope of the following claims.

WHAT IS CLAIMED IS:

1. An isolated Matrix Metalloproteinase-9 (MMP-9) binding protein, wherein the protein comprises at least one immunoglobulin variable region.

2. The protein of claim 1, wherein the protein binds human MMP-9.

3. The protein of claim 1, wherein the protein inhibits the catalytic activity of MMP-9.

4. The protein of claim 1, wherein the protein comprises an antibody.

5. The protein of claim 1, wherein the protein guides a nano-particle or toxin to a cell expressing MMP-9 on the cell surface.

6. The protein of claim 1, wherein the protein causes effector functions to kill the cell which expresses MMP-9.

7. The protein of claim 1, wherein comprises a heavy chain (HC) immunoglobulin variable domain sequence and a light chain (LC) immunoglobulin variable domain sequence.

8. The protein of claim 7, wherein the HC and LC variable domain sequences are components of the same polypeptide chain.

9. The protein of claim 7, wherein the HC and LC variable domain sequences are components of different polypeptide chains.

10. The protein of claim 1, wherein the protein binds to human MMP-9 specifically, and not to MMP-9 from another species.

30 11. The protein of claim 1, wherein the protein binds MMP-9 specifically, and not to another matrix metalloproteinase.

12. The protein of claim 1, wherein the protein comprises one or more of the following characteristics:

- 35 (a) a human CDR or human framework region;
- (b) the HC immunoglobulin variable domain sequence comprises one or more CDRs that are at least 85% identical to a CDR of a HC variable domain described herein;
- (c) the LC immunoglobulin variable domain sequence comprises one or more CDRs that are at least 85% identical to a CDR of a LC variable domain described herein;
- 40 (d) the LC immunoglobulin variable domain sequence is at least 85% identical to a LC variable domain described herein;
- (e) the HC immunoglobulin variable domain sequence is at least 85% identical to a HC variable domain described herein;
- (f) the protein binds an epitope bound by a protein described herein, or an epitope that overlaps with such epitope; and
- 45 (g) a primate CDR or primate framework region.

13. The protein of claim 1, wherein the protein also binds to MMP-16 or MMP-24.

50 14. The protein of claim 1, wherein the protein comprises an antibody comprising light and heavy chains of an antibody selected from the group of antibodies consisting of: 539A-M0240-B03, 539A-X0034-C02, M0078-G07, M0081-D05, M0076-D03, M0072-H07, M0075-D12, and M0166-F10.

55 15. The protein of claim 1, wherein the protein comprises an antibody comprising a heavy chain selected from the group of antibodies consisting of: 539A-M0240-B03, 539A-X0034-C02, M0078-G07, M0081-D05, M0076-D03, M0072-H07, M0075-D12, and M0166-F10.

60

16. The protein of claim 1, wherein the protein comprises an antibody comprising a light chain selected from the group of antibodies consisting of: 539A-M0240-B03, 539A-X0034-C02, M0078-G07, M0081-D05, M0076-D03, M0072-H07, M0075-D12, and M0166-F10.

65

17. The protein of claim 1, wherein the protein comprises an antibody comprising one or more heavy chain CDRs selected from the group of antibodies consisting of: 539A-M0240-B03, 539A-X0034-C02, M0078-G07, M0081-D05, M0076-D03, M0072-H07, M0075-D12, and M0166-F10.

70

18. The protein of claim 1, wherein the protein comprises an antibody comprising one or more light chain CDRs selected from the group of antibodies consisting of: 539A-M0240-B03, 539A-X0034-C02, M0078-G07, M0081-D05, M0076-D03, M0072-H07, M0075-D12, and M0166-F10.

75

19. The protein of claim 1, wherein the protein comprises the light and heavy chains of antibody M0166-F10.

20. The protein of claim 1, wherein the protein comprises the light and heavy chain CDRs of antibody M0166-F10.

80

21. The protein of claim 1, wherein the protein comprises the light and heavy chains of antibody 539A-M0240-B03.

22. The protein of claim 1, wherein the protein comprises the light and heavy chain CDRs of antibody 539A-M0240-B03.

85

23. The protein of claim 1, wherein the protein comprises the light and heavy chains of antibody 539A-X0034-C02.

90

24. The protein of claim 1, wherein the protein comprises the light and heavy chain CDRs of antibody 539A-X0034-C02.

95 25. The protein of claim 1, wherein the protein is a human or humanized antibody or is non-immunogenic in a human.

26. The protein of claim 1, wherein the protein is capable of binding to tumor cells expressing MMP-9.

100 27. A method of inhibiting an interaction between MMP-9 and an MMP-9 substrate, the method comprising: contacting an MMP-9 binding protein of claim 1 with MMP-9, wherein the binding protein binds to MMP-9 and thereby prevents the binding of the MMP-9 substrate to MMP-9.

105 28. An MMP-9 binding protein-drug conjugate, wherein the conjugate comprises a MMP-9 binding protein of claim 1 and a drug.

29. The conjugate of claim 28, wherein the drug is a cytotoxic or cytostatic agent.

110 30. The conjugate of claim 29, wherein the drug is a cytotoxic agent and is selected from the group consisting of an auristatin, a DNA minor groove binding agent, a DNA minor groove alkylating agent, an enediyne, a lexitropsin, a duocarmycin, a taxane, a puromycin, a dolastatin, a podophyllotoxin, a baccatin derivative, a cryptophysin, a combretastatin, a maytansinoid, and a vinca alkaloid.

115 31. The conjugate of claim 29, wherein the drug is a cytotoxic agent and is an antitubulin agent.

120 32. The conjugate of claim 28, wherein the MMP-9 binding protein is conjugated to the drug via a linker.

33. A pharmaceutical composition comprising an MMP-9 binding protein of claim 1 and a pharmaceutically acceptable carrier.

125 34. A method of detecting an MMP-9 in a sample, the method comprising:
contacting the sample with an MMP-9 binding protein of claim 1; and detecting
an interaction between the protein and the MMP-9, if present.

130 35. The method of claim 34, wherein the protein includes a detectable label.

36. A method of modulating MMP-9 activity, the method comprising:
contacting an MMP-9 with an MMP-9 binding protein of claim 1, thereby
modulating MMP-9 activity.

135 37. A method for modulating metastatic activity in a subject, the method
comprising:
administering, to a subject, an MMP-9 binding protein of claim 1 in an amount
effective to modulate metastatic activity.

140 38. A method of treating cancer, the method comprising:
administering, to a subject, an MMP-9 binding protein of claim 1 in an amount
sufficient to treat a cancer in the subject.

145 39. The method of claim 38 further comprising providing to the subject a second
therapy that is an anti-cancer therapy.

40. A method of treating heart failure, the method comprising:
administering, to a subject, an MMP-9 binding protein of claim 1 in an amount
sufficient to treat heart failure in the subject.

150 41. The method of claim 40 further comprising providing to the subject a second
therapy that is a heart failure therapy.

42. A method of treating septic shock, the method comprising:
155 administering, to a subject, an MMP-9 binding protein of claim 1 in an amount
sufficient to treat septic shock in the subject.

43. The method of claim 42 further comprising providing to the subject a second
therapy that is a therapy for septic shock.

160

44. A method of treating neuropathic pain, the method comprising:
administering, to a subject, an MMP-9 binding protein of claim 1 in an amount
sufficient to treat neuropathic pain in the subject.

165 45. The method of claim 44 further comprising providing to the subject a second
therapy that is a therapy for neuropathic pain.

46. A method of treating inflammatory pain, the method comprising:
administering, to a subject, an MMP-9 binding protein of claim 1 in an amount
170 sufficient to treat inflammatory pain in the subject.

47. The method of claim 46 further comprising providing to the subject a second
therapy that is a therapy for inflammatory pain.

175 48. A method of treating an ocular condition, the method comprising:
administering, to a subject, an MMP-9 binding protein of claim 1 in an amount
sufficient to treat the ocular condition in the subject.

49. The method of claim 48 further comprising providing to the subject a second
180 agent an agent that antagonizes signaling through a VEGF pathway.

50. A method of treating an inflammatory disease, the method comprising:

administering, to a subject, an MMP-9 binding protein of claim 1 in an amount sufficient to treat the inflammatory disease in the subject.

185

51. The method of claim 50 further comprising providing to the subject a second therapy that is an anti-inflammatory therapy.

190

52. A method of imaging a subject, the method comprising:
administering an MMP-9 binding protein of claim 1 to the subject.

53. The method of claim 52, wherein the MMP-9 binding protein comprises a detectable label.

	Fab	hIgG-1
hMMP-1	104	82
hMMP-2	126	69
hMMP-3	82	120
hMMP-7	93	82
hMMP-8	100	88
hMMP-9	8	4
hMMP-10	102	103
hMMP-12	99	80
hMMP-13	100	87
hMMP-14	118	89
mMMP-2	113	89
mMMP-9	94	112

>50% inhibition (1µM final)

FIG. 1B

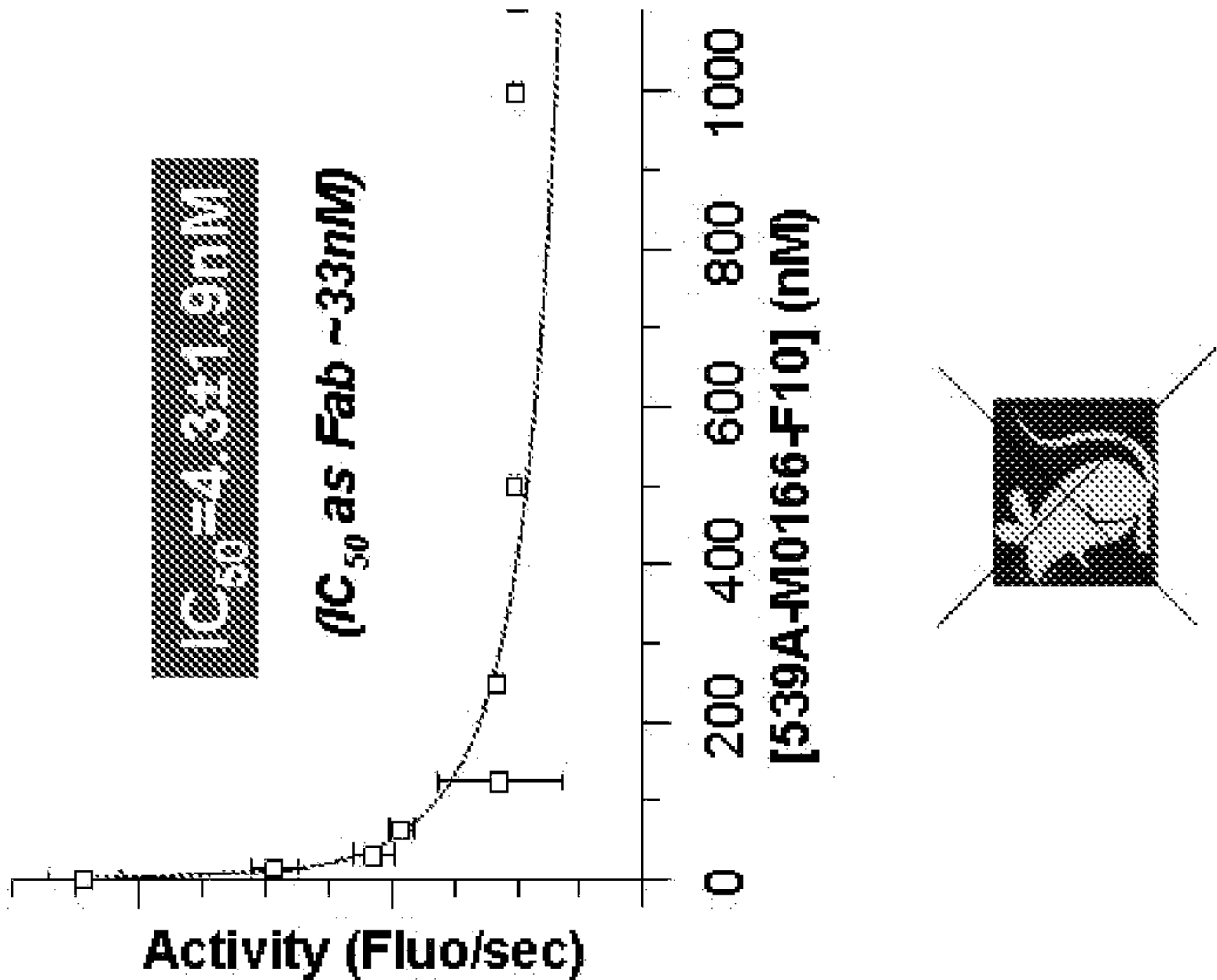


FIG. 1A

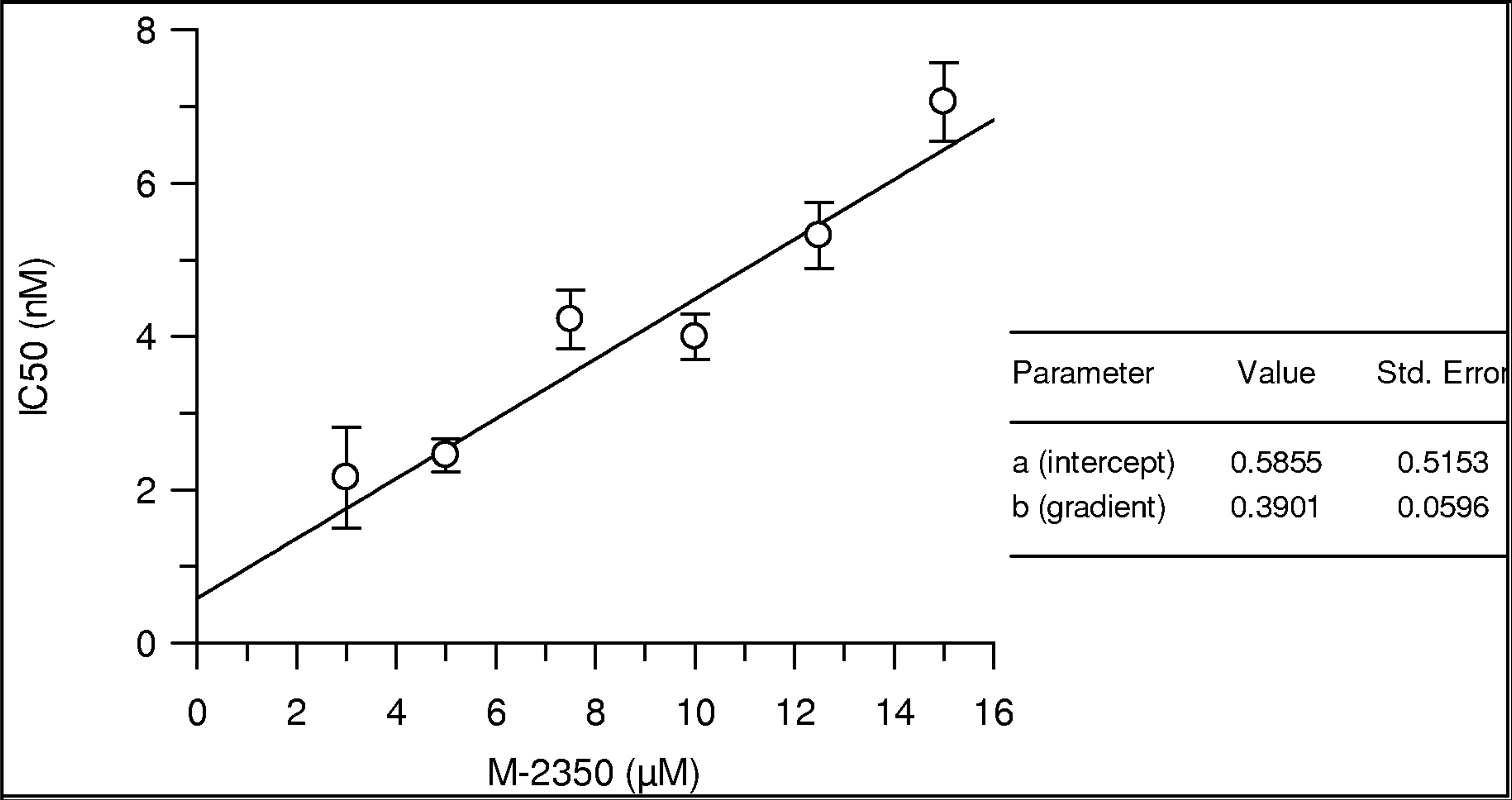


FIG. 2

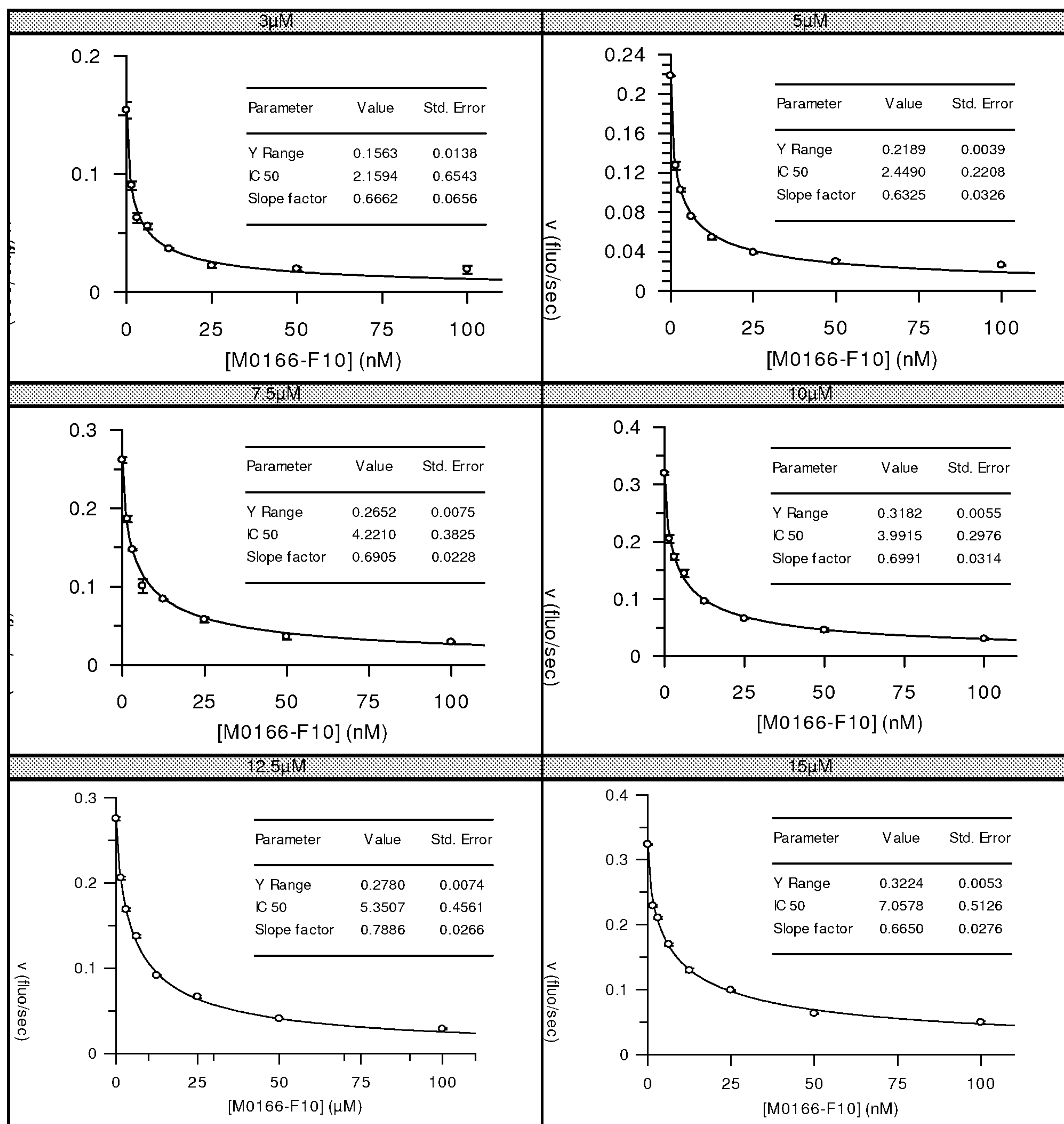
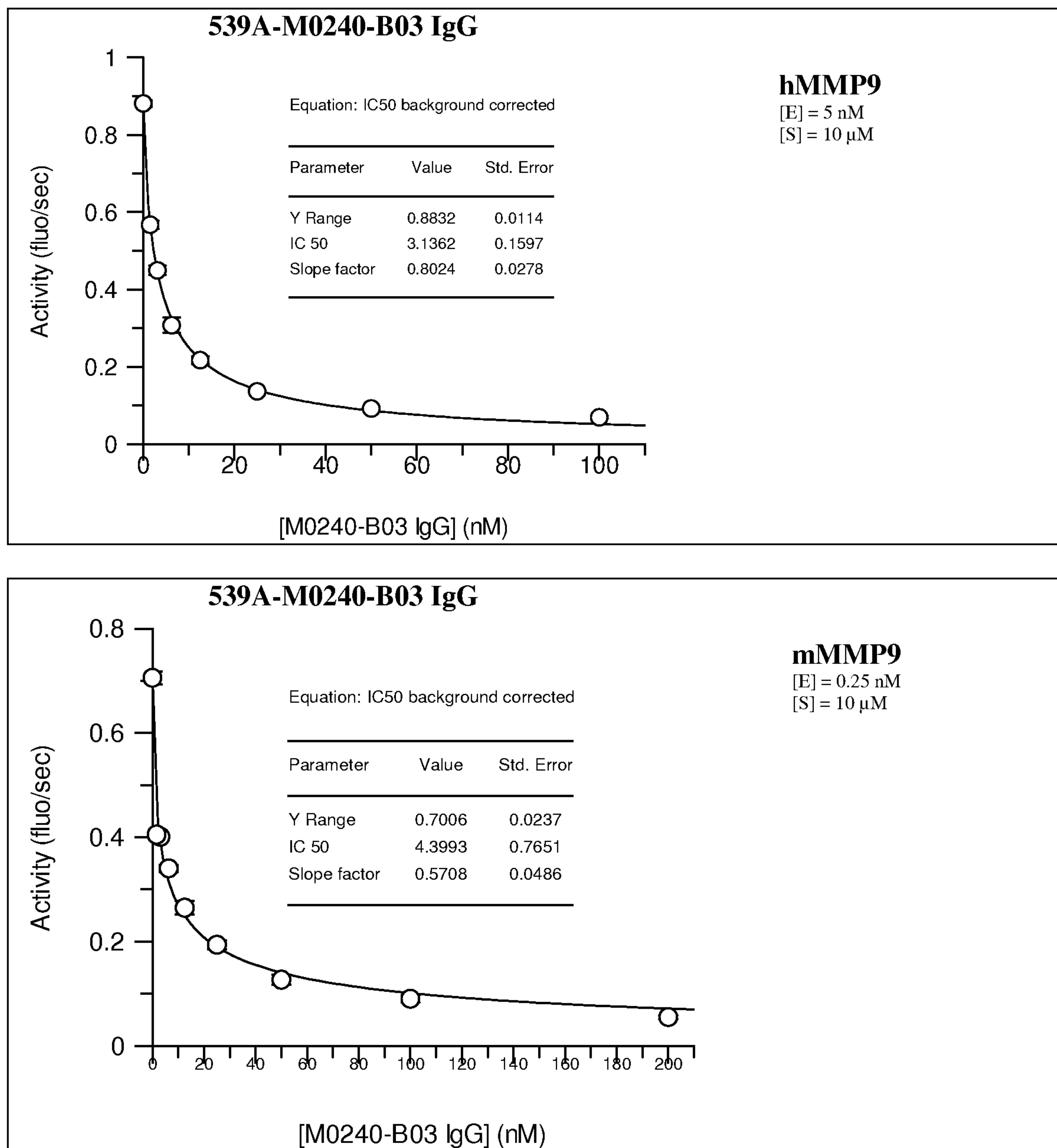


FIG. 3

4/10

**FIG. 4**

	Dilution enzyme	Substrate used	% Activity at 1µM final	% Activity at 500nM final
Hu-MMP1	1/300	M2350	108	
Hu-MMP2	1/500	M2350	107	
Hu-MMP3	1/200	M2225	112	
Hu-MMP7	1/500	M2350	111	
Hu-MMP8	1/1000	M2350	104	
Hu-MMP9	1/500	M2350	6	
Mouse-MMP9	1/20000	M2350	3	
Hu-MMP10	1/100	M2350	110	
Hu-MMP12	1/500	M2350	ND	104
Hu-MMP13	1/5000	M2350	100	
Hu-MMP14	1/1000	M2350	105	

FIG. 5

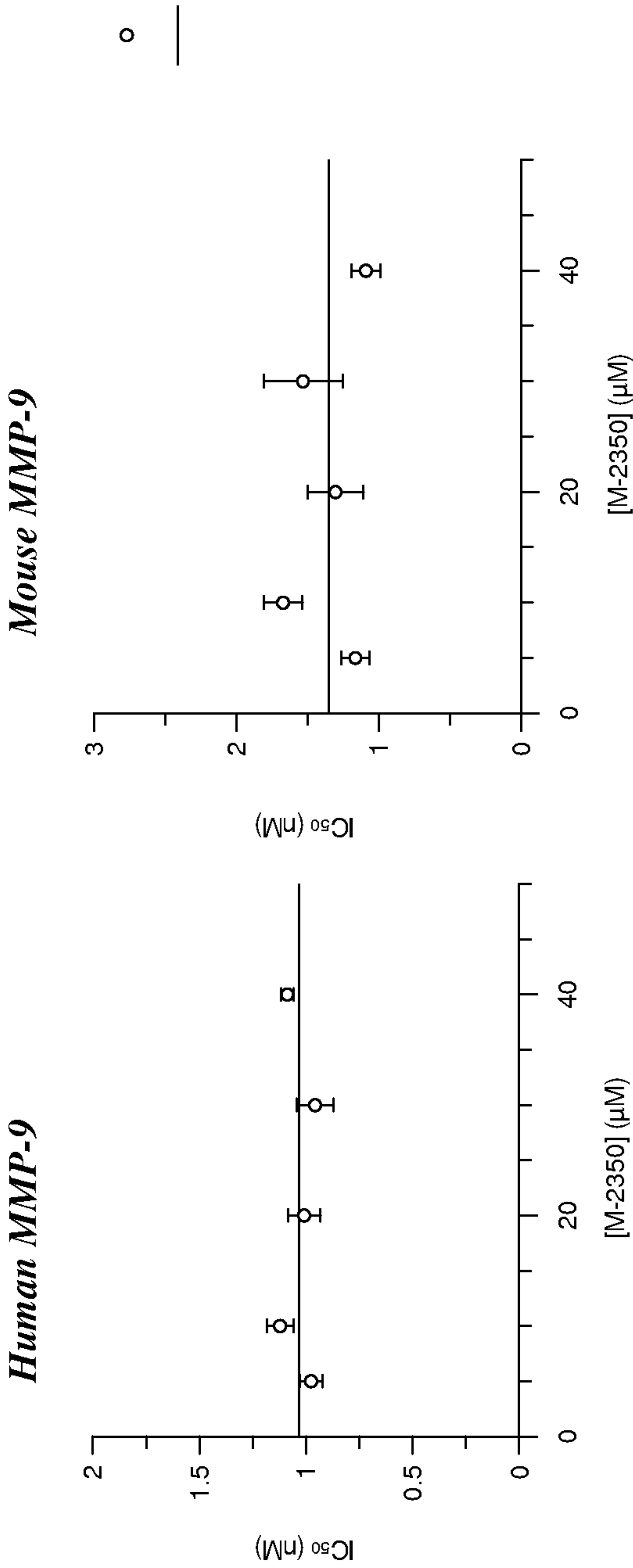


FIG. 6B

FIG. 6A

7/10

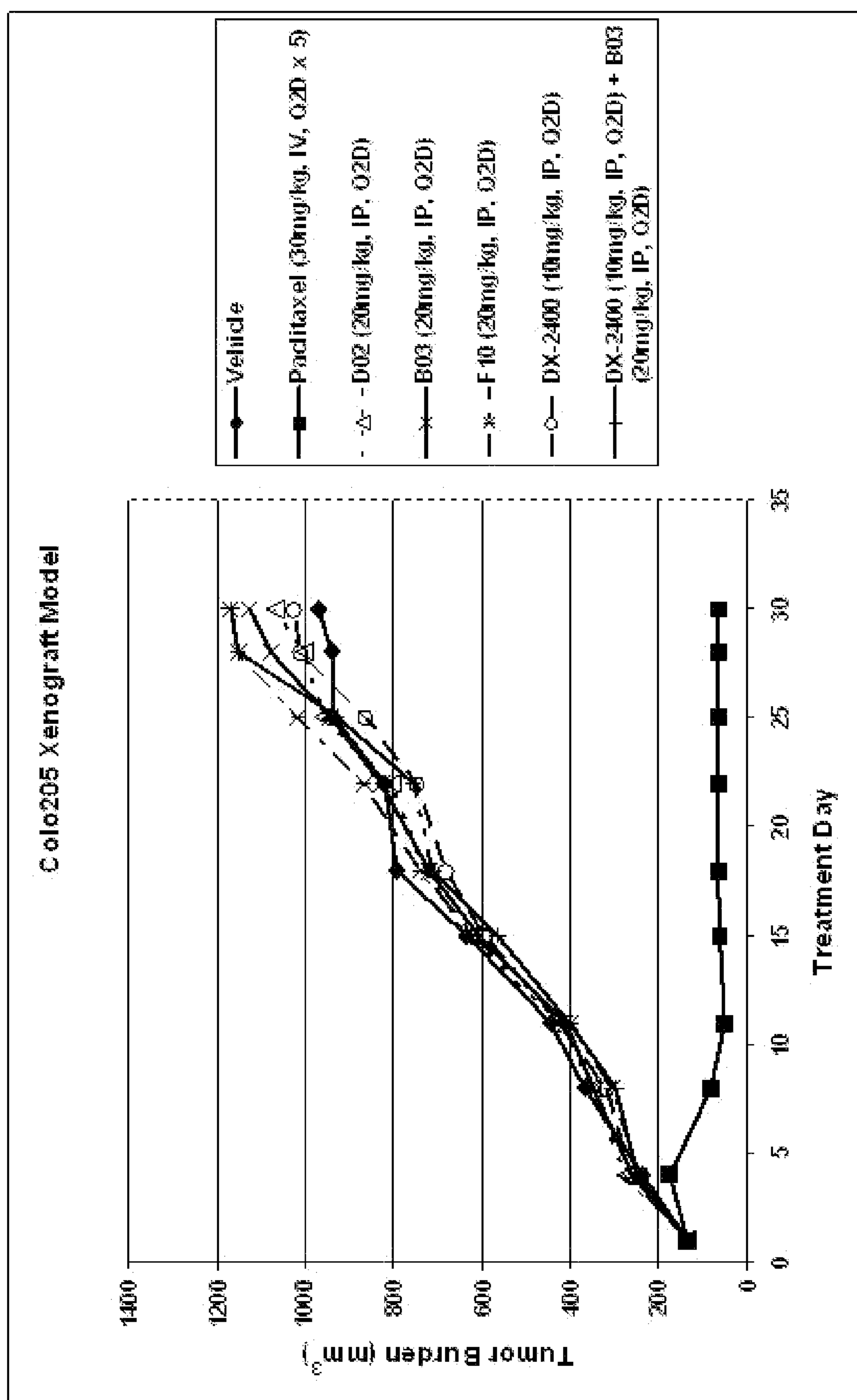


FIG. 7

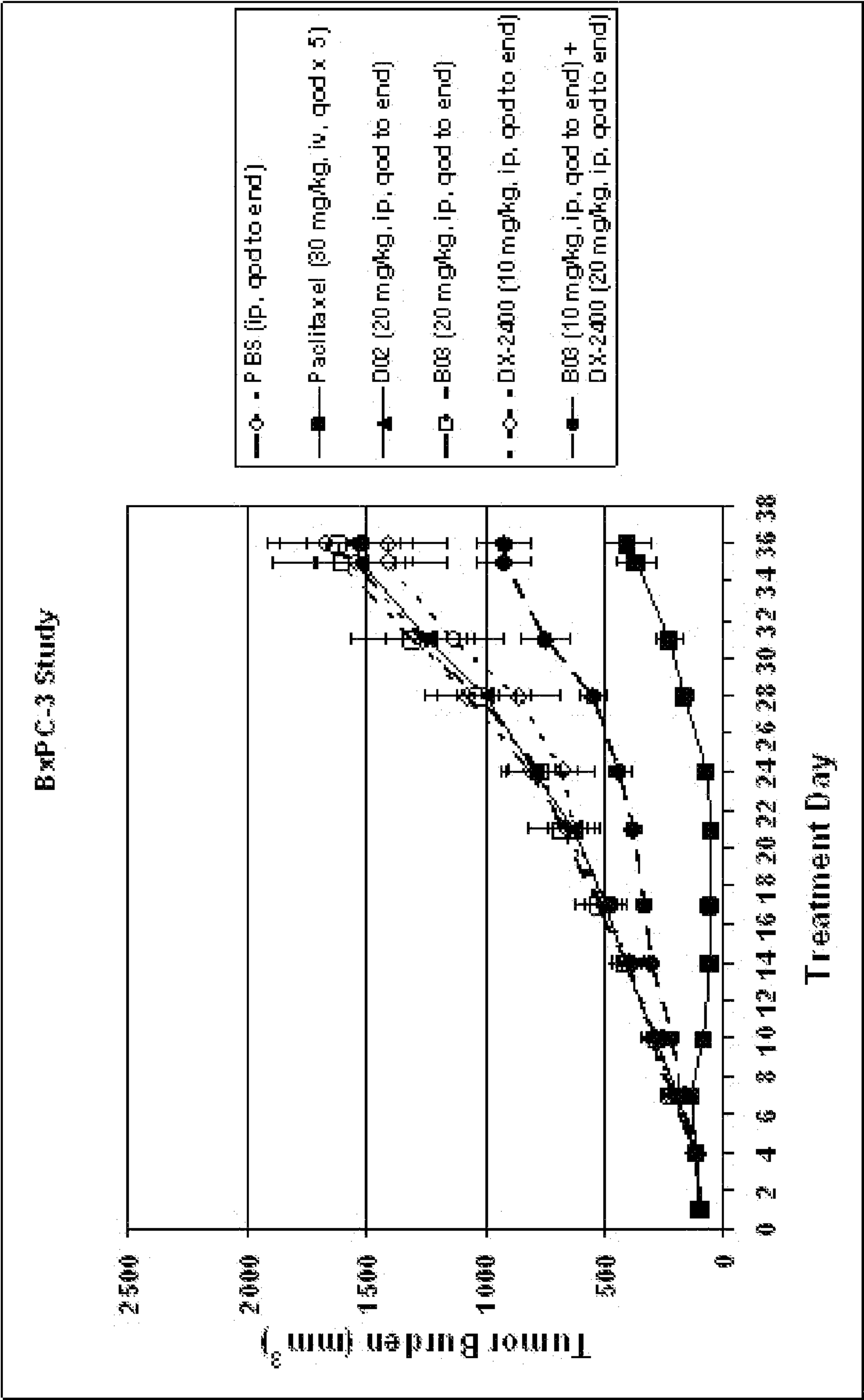
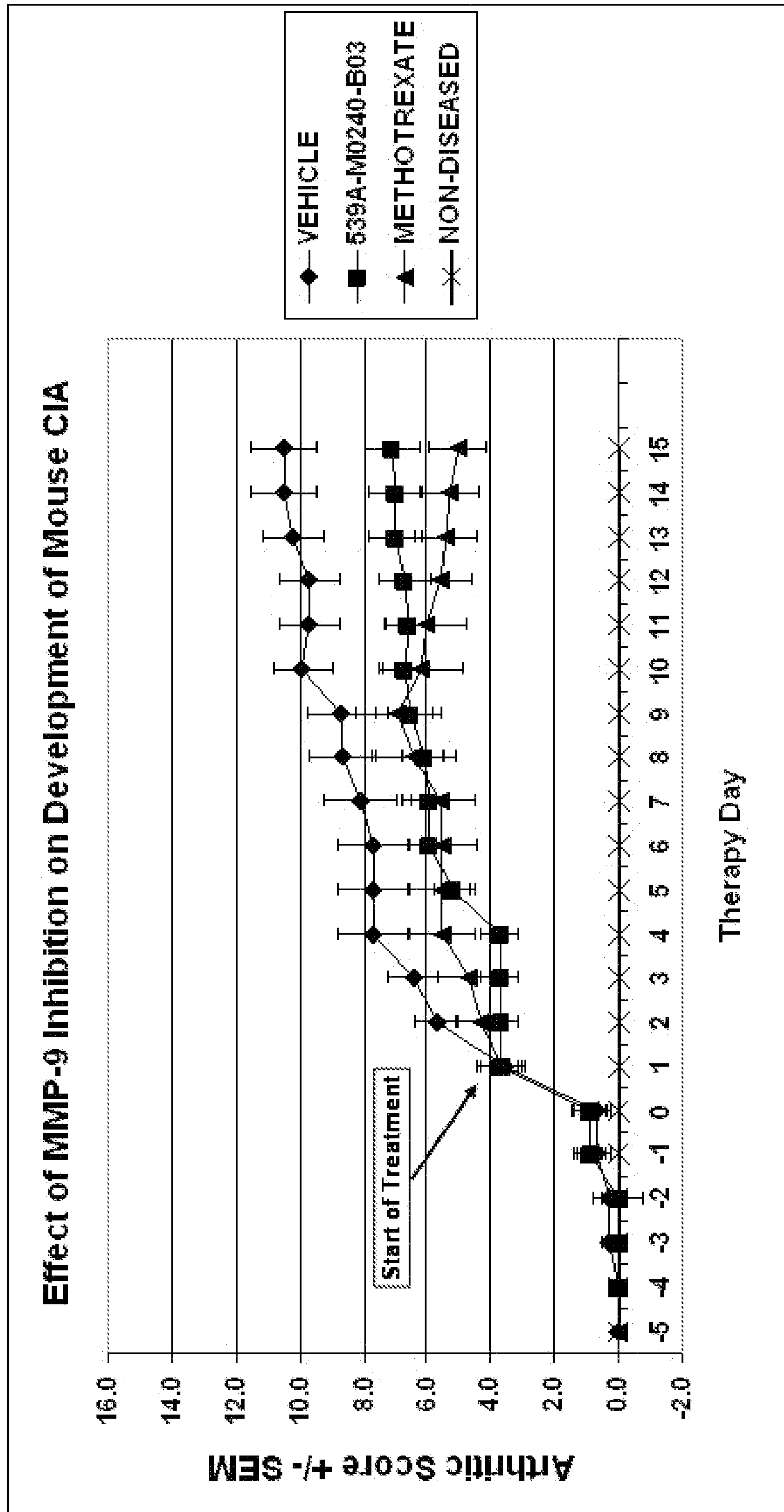
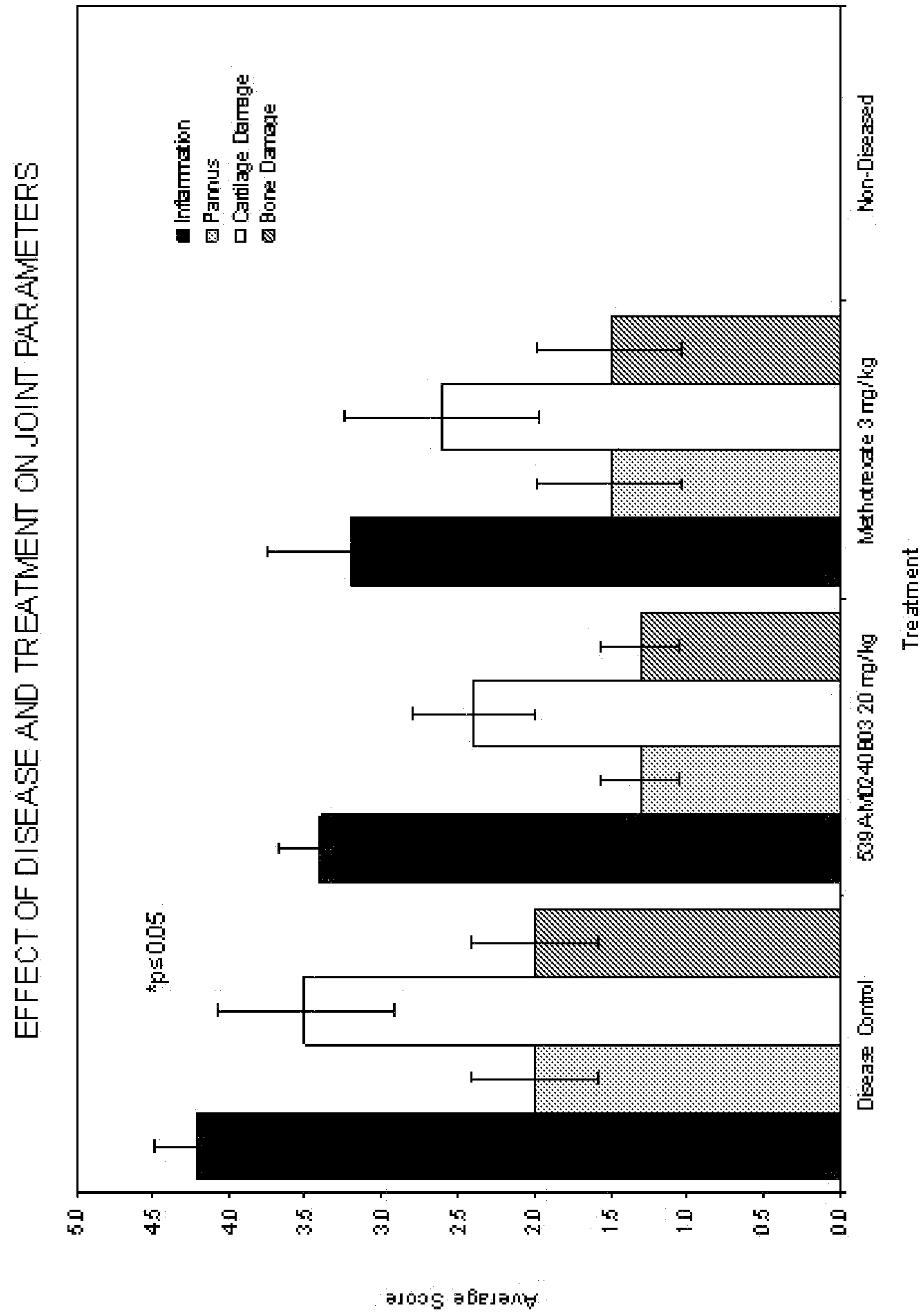


FIG. 8

9/10

**FIG. 9**

10/10

**FIG. 10**

Effect of MMP-9 Inhibition on Development of Mouse CIA

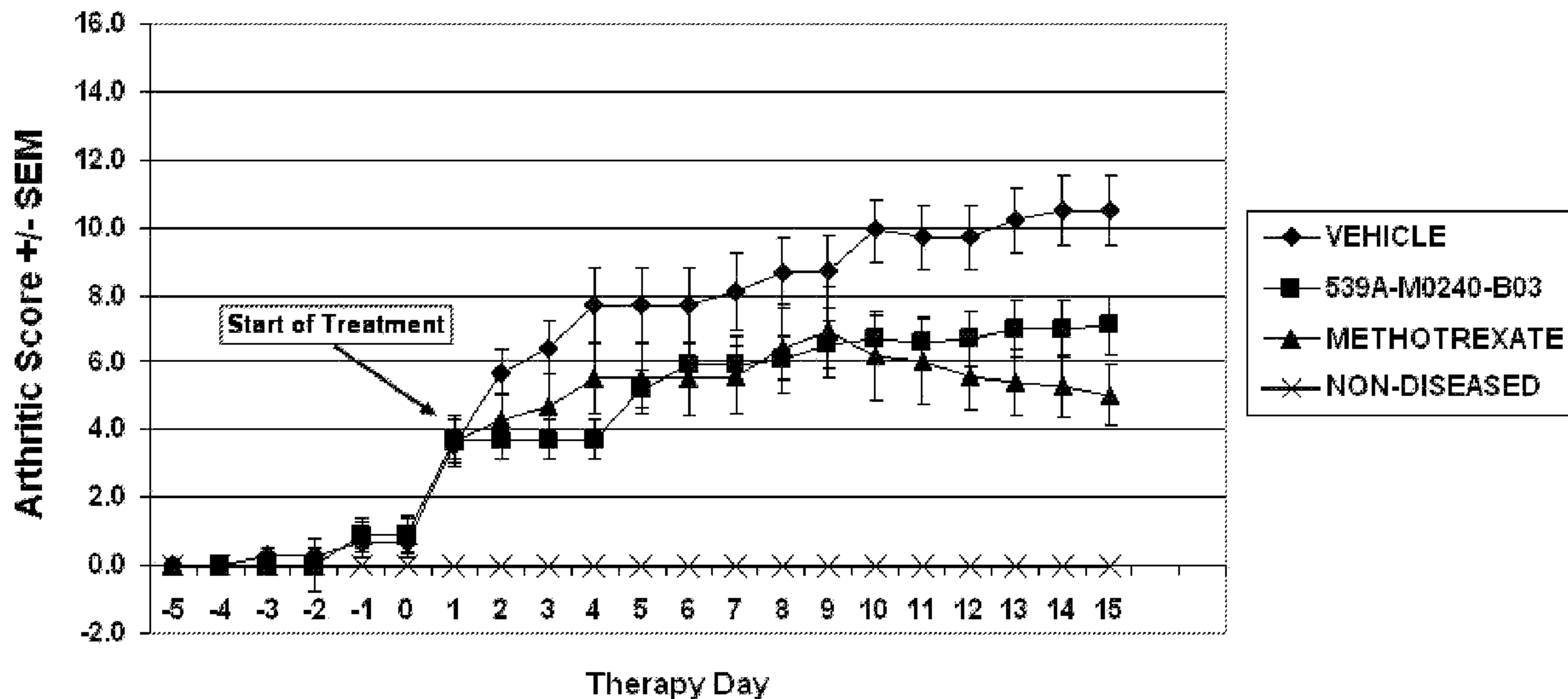


FIG. 9