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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
25 June 2009 (25.06.2009)

PCT

(10) International Publication Number
WO 2009/079585 A2

(51) International Patent Classification:
A61K 39/395 (2006.01) *A61P 19/10* (2006.01)
A61P 35/00 (2006.01)

(21) International Application Number:
PCT/US2008/087236

(22) International Filing Date:
17 December 2008 (17.12.2008)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/008,153 17 December 2007 (17.12.2007) US
61/025,032 31 January 2008 (31.01.2008) US
61/107,510 22 October 2008 (22.10.2008) US

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

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

Published:
— *without international search report and to be republished upon receipt of that report*

WO 2009/079585 A2

(54) **Title:** COMPOSITIONS AND METHODS FOR TREATING OSTEOLYTIC DISORDERS COMPRISING MMP-14 BINDING PROTEINS

(57) **Abstract:** Provided are methods and compositions for using MMP- 14 or MMP-9 binding proteins alone or in combination with other therapeutic agents to treat osteolytic disorders such as osteotropic cancer and osteoporosis.

COMPOSITIONS AND METHODS FOR TREATING OSTEOLYTIC DISORDERS COMPRISING MMP-14 BINDING PROTEINS

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Application Serial No. 61/008,153, filed on December 17, 2007, U.S. Application Serial No. 61/025,032, filed on January 31, 2008, and U.S. Application Serial No. 61/107,510, filed on October 22, 2008. The disclosures of the prior applications are considered part of (and are incorporated by reference in) the disclosure of this application.

BACKGROUND

Osteoclasts, which mediate bone resorption, are involved in normal and abnormal bone remodeling processes, including osteolytic disorders. Osteoclasts are multinucleated cells differentiating from haemopoietic cells. It is generally accepted that osteoclasts are formed by the fusion of mononuclear precursors derived from haemopoietic stem cells in the bone marrow, rather than incomplete cell division. The differentiation of osteoclast precursors into mature multinucleated osteoclasts requires different factors including hormonal and local stimuli and living bone and bone cells have been shown to play a critical role in osteoclast development. Osteoblastic or bone marrow stromal cells are also required for osteoclast differentiation. Osteoclasts are responsible for dissolving both the mineral and organic bone matrix. Osteoclasts represent terminally differentiated cells expressing a unique polarized morphology with specialized membrane areas and several membrane and cytoplasmic markers.

Several molecular mechanisms bring about cancer cells to metastasize to bone, and osteotropic cancer cells are believed to acquire bone cell-like properties which improve homing, adhesion, proliferation and survival in the bone microenvironment. Signaling pathways involved in tumor growth and development of osteolytic lesions include RANK, RANKL, osteoprotegerin (OPG), IGF and the membrane type (MT)-matrix metalloproteinases (MMPs). The initial phase of bone degradation consists of removal of the unmineralized type I collagenous layer followed by degradation of the mineralized matrix, which also comprises type I collagen. Tumor expansion in bone requires the removal of this matrix that is particularly abundant and resistant to degradation. The assistance of osteoclasts appears to be mandatory because osteoclasts are the primary cells involved in bone matrix

solubilization. The capacity of osteoclasts to degrade bone resides in their ability to secrete protons, cathepsin K and MMPs. A generalized increase in MMPs levels within the bone environment when cancer cells are present is due, in part, to production of MMPs by the cancer cells themselves.

Since osteoclasts play a major role in osteolytic bone metastases and other osteolytic diseases, there is a need in the art for new agents and methods for preventing osteoclast stimulation and function. Several therapeutic strategies targeting osteolytic disease are currently being used or under development, where efforts have mainly focused on the development of drugs to block bone resorption through inhibiting the formation or activity of osteoclasts. The bisphosphonates (BPs), pyrophosphate analogs that concentrate in bone, are to date the most effective inhibitor of bone resorption. BPs are taken up by osteoclasts, inhibiting their activity and causing the cells to undergo apoptosis, thereby inhibiting bone resorption. Advanced cancers are prone to metastasize. Effective treatments for bone metastases are not yet available – existing treatments such as bisphosphonates, chemotherapy and radiotherapy improve the quality of life with no life-prolonging benefits and have significant side effects.

SUMMARY

Disclosed herein are methods for the treatment of osteolytic disorders, in particular osteotropic cancer and osteoporosis. In one aspect, the invention provides methods for the treatment or prevention of an osteolytic disorder comprising administration of an effective amount of a MMP-14 or MMP-9 binding protein. In certain embodiments wherein the osteolytic disorder is osteotropic cancer, the methods act specifically to decrease and/or prevent the occurrence of osteolytic lesions which can occur due to metastatic spread to bone of a number of cancers including but not limited to breast, lung and prostate, by administration of a MMP-14 or MMP-9 binding protein. In other embodiments, the methods act specifically to prevent osteolytic lesions from forming in subjects having bone metastases, by administration of a MMP-14 or MMP-9 binding protein.

In one embodiment, an MMP-14 binding protein is administered in combination with an MMP-9 binding protein.In one embodiment, the MMP-14 or MMP-9 binding protein is administered in combination with an additional cancer therapeutic or treatment, such as, for example, bisphosphonates (e.g., amino and non-amino bisphosphonates), hormone-related compounds (e.g., estrogens and SERMs), RANKL antagonists, $\alpha_{\gamma}\beta_{3}$ antagonists, Src inhibitors, cathepsin K inhibitors, calcitonin, chemotherapy and radiotherapy.

In one embodiment, an MMP-14 binding protein is administered in combination with an MMP-9 binding protein and an additional cancer therapeutic or treatment, such as, for example, bisphosphonates (e.g., amino and non-amino bisphosphonates), hormone-related compounds (e.g., estrogens and SERMs), RANKL antagonists, $\alpha_\gamma\beta_3$ antagonists, Src inhibitors, cathepsin K inhibitors,
5 calcitonin, chemotherapy and radiotherapy.

In one aspect, the invention provides kits for the treatment of an osteolytic disorder. The kits include a MMP-14 and/or MMP-9 binding protein, and instructions for administering the MMP-14 and/or MMP-9 binding protein to a subject having an osteolytic disorder. In one embodiment, the kit further includes instructions for administration of an additional therapeutic for the treatment of an
10 osteolytic disorder, and may optionally contain the additional therapeutic. In one embodiment, the instructions provide a dosing regimen, dosing schedule, and/or route of administration of the MMP-14 and/or MMP-9 binding protein that differs from the dosing regimen, dosing schedule and/or route of administration for the inhibitor in the absence of the additional therapeutic.

In another aspect, provided herein is the use of a MMP-14 and/or MMP-9 binding protein for
15 the manufacture of a medicament for the treatment of an osteolytic disorder. The medicament may optionally include an additional therapeutic for the treatment of an osteolytic disorder, such as a bisphosphonate.

The MMP-14 and/or MMP-9 binding protein used in any disclosed method, kit or composition can have one or more of the characteristics described below in the Detailed Description. Preferred
20 compositions, e.g., used in any method or kit described herein, may further comprise one or more pharmaceutically acceptable buffers, carriers, and excipients, which may provide a desirable feature to the composition including, but not limited to, enhanced administration of the composition to a patient, enhanced circulating half-life of the inhibitor, enhanced compatibility of the composition with patient blood chemistry, enhanced storage of the composition, and/or enhanced efficacy of the composition
25 upon administration to a patient.

The details of one or more embodiments of the invention are set forth in the 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.

DESCRIPTION OF DRAWINGS

FIGURE 1 depicts amino acid sequences of Fab heavy chain (HC) and light chain (LC) variable regions of some exemplary MMP-14 binding proteins which may be used in the provided methods and compositions for treating osteolytic disorders. The standard numbering of the HC V domain is shown. The length of HC CDR3 varies considerably. By convention, the second cysteine is numbered 92 and the W of the conserved WG motif of FR4 is number 103. If there are more than 9 residues between C92 and W103, then residues after 102 are numbered 102a, 102b, etc. =

FIGURE 2 depicts (left) X-ray analysis of the size of osteolytic tibial lesions treated with a PBS control (top) or DX-2400 (bottom) and (right) bone histomorphometric analysis of the size of osteolytic tibial lesions treated with a PBS control or DX-2400, as indicated.

FIGURE 3 depicts amino acid sequences of Fab heavy chain (HC) and light chain (LC) variable regions of some exemplary MMP-9 binding proteins which may be used in the provided methods and compositions for treating osteolytic disorders.

DETAILED DESCRIPTION

Expression of MMP-2, MMP-9 and MMP-14 in sections from core bone biopsy specimens from patients with bone-metatstatic prostate cancer has been observed. Further, expression of RANKL, MMP-2, MMP-13 and MMP-14 has been observed to be markedly elevated in bone with metastasis of breast cancer MDA-MB-231 cells *in vivo*. The disclosure provides methods of using MMP-14 or MMP-9 binding proteins, including MMP-14 or MMP-9 binding proteins that inhibit MMP-14 or MMP-9 binding activity, in the treatment and prevention of osteolytic disorders such as osteotropic cancer and osteoporosis, as well as compositions and kits for the same.

Definitions

For convenience, before further description of the present invention, certain terms employed in the specification, examples and appended claims are defined here.

The singular forms “a”, “an”, and “the” include plural references unless the context clearly dictates otherwise.

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.

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 (Clq) 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, 92, 95, 98, 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., U.S. 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.

5 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,
10 irrespective of how the antibody was generated.

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-14, MMP-16, or MMP-24. Higher affinity binding of a binding protein to a first target relative to a
15 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
20 least 1.5, 2, 3, 4, 5, 10, 15, 20, 37.5, 50, 70, 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
25 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:

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

30 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, that 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.

5 The term “binding protein” refers to a protein or polypeptide that can interact with a target molecule. This term is used interchangeably with “ligand.” An “MMP-14 binding protein” refers to a protein that can interact with MMP-14, and includes, in particular, proteins that preferentially interact with and/or inhibit MMP-14. For example, the MMP-14 binding protein may be an antibody. An “MMP-9 binding protein” refers to a protein that can interact with MMP-9, and includes, in particular, 10 proteins that preferentially interact with and/or inhibit MMP-9. For example, the MMP-9 binding protein may be an antibody.

The terms “cancer” and “cancerous” refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. Examples of cancer include but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia. More particular examples 15 of such cancers include breast cancer, prostate cancer, colon cancer, squamous cell cancer, small-cell lung cancer, non-small cell lung cancer, gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, colorectal cancer, endometrial carcinoma, salivary gland carcinoma, kidney cancer, liver cancer, vulval cancer, thyroid cancer, hepatic carcinoma and various types of head and neck cancer.

20 The term “cognate ligand” refers to a naturally occurring ligand of an MMP-14 or MMP-9, including naturally occurring variants thereof (e.g., splice variants, naturally occurring mutants, and isoforms).

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 25 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.

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 30 (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic 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 substitutions.

5 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.

10 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 15 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 20 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 25 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 30 60%, and even more preferably at least 70%, 80%, 90%, 92%, 95%, 97%, 98%, or 100% of the length

of the reference sequence. For example, the reference sequence may be the length of the immunoglobulin variable domain sequence.

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, U.S. 6,407,213 and U.S. 5,693,762.

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 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 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 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.

An MMP-14 or 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., a 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 conservative or by the method of Bowie, et al. (1990) *Science* 247:1306-1310.

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-14 or MMP-9 protein, e.g., the MMP-14 or MMP-9 catalytic domain.

An "isolated composition" refers to a composition 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.

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.

As used herein, the phrase "metastatic cancer" is defined as a cancer that has potential to spread to other areas of the body, particularly bone. A variety of cancers can metastasize to the bone, but the most common metastasizing cancers are breast, lung, renal, multiple myeloma, thyroid and prostate. By way of example, other cancers that have the potential to metastasize to bone include but are not limited to adenocarcinoma, blood cell malignancies, including leukemia and lymphoma; head and neck cancers; gastrointestinal cancers, including esophageal cancer, stomach cancer, colon cancer, intestinal cancer, colorectal cancer, rectal cancer, pancreatic cancer, liver cancer, cancer of the bile duct or gall bladder; malignancies of the female genital tract, including ovarian carcinoma, uterine endometrial cancers, vaginal cancer, and cervical cancer; bladder cancer; brain cancer, including neuroblastoma; sarcoma, osteosarcoma; and skin cancer, including malignant melanoma and squamous cell cancer. The present invention especially contemplates prevention and treatment of tumor- induced osteolytic lesions in bone.

As used herein, an "osteolytic disorder" is any condition resulting from increased osteoclast activity. A subject at risk of an osteolytic disorder may be a subject in a group predisposed to develop an osteolytic disorder, or a subject suffering from a disease that causes or contributes to increased osteoclastic activity. In exemplary embodiments of the invention, the osteolytic disorder maybe a metabolic bone disease associated with relatively increased osteoclast activity, including an endocrinopathy (hypercortisolism, hypogonadism, primary or secondary hyperparathyroidism, hyperthyroidism), hypercalcemia, deficiency state (rickets/osteomalacia, scurvy, malnutrition), chronic disease (malabsorption syndromes, chronic renal failure (renal osteodystrophy), chronic liver disease (hepatic osteodystrophy)), drugs (glucocorticoids (glucocorticoid-induced osteoporosis), heparin, alcohol), or hereditary disease (osteogenesis imperfecta, homocystinuria), cancer, osteoporosis, osteopetrosis, inflammation of bone associated with arthritis and rheumatoid arthritis, periodontal disease, fibrous dysplasia, and/or Paget's disease. In other exemplary embodiments, the osteolytic disorder may be a metastatic cancer to bone (osteotropic cancer), wherein the metastatic cancer is breast, lung, renal, multiple myeloma, thyroid, prostate, adenocarcinoma, blood cell malignancy, including leukemia and lymphoma; head and neck cancer; gastrointestinal cancer, including esophageal cancer, stomach cancer, colon cancer, intestinal cancer, colorectal cancer, rectal cancer, pancreatic cancer, liver cancer, cancer of the bile duct or gall bladder; malignancy of the female genital tract, including ovarian carcinoma, uterine endometrial cancer, vaginal cancer, or cervical cancer; bladder cancer; brain cancer, including neuroblastoma; sarcoma, osteosarcoma; or skin cancer, including malignant melanoma or squamous cell cancer. In some embodiments, increased osteoclast activity, e.g., in a subject, refers to osteoclast activity that is increased as compared to the levels of osteoclast activity in a standard, e.g., the osteoclast activity in a cohort of subjects, e.g., a cohort of subjects without a symptom of an osteoclast disorder, or the levels of osteoclast activity of a random sampling of subjects. Osteoclast activity can increase by, e.g., about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, or more, as compared to the standard. Osteoclast activity can be measured, e.g., by tartrate resistant acid phosphatase (TRAP) staining; ELISA analysis of Receptor Activator for Nuclear Factor κB Ligand (RANKL) concentration in blood serum; and/or Alizarin Red staining of osteoblastic cells which were isolated from bone marrow. Additional methods are described, e.g., in WO 2003/031597.

The term "osteoporosis" refers to a disease in which the bones become extremely porous, are subject to fracture, and heal slowly, occurring especially in women following menopause and often leading to curvature of the spine from vertebral collapse.

The term "osteotropic cancer" refers to metastatic cancer of the bone, i.e., a secondary cancer present in bone that originates from a primary cancer, such as that of the breast, lung, or prostate.

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 term "preventing" a disease in a subject refers to subjecting the subject to a pharmaceutical treatment, e.g., the administration of a drug, such that at least one symptom of the disease is prevented, that is, administered prior to clinical manifestation of the unwanted condition (e.g., disease or other unwanted state of the host animal) so that it protects the host against developing the unwanted condition. "Preventing" a disease may also be referred to as "prophylaxis" or "prophylactic treatment."

A "prophylactically effective amount" refers to an amount effective, at dosages and for 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.

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) 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 the strand. The nucleic acids may be present in whole cells, in a cell lysate, or in a partially purified or substantially pure form.

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, "(A/W/F/N/Q)" means that alanine, tryptophan, phenylalanine, asparagine, and glutamine are allowed at that particular position.

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 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, may refer to a difference, e.g., a statistically significant difference, between the two states.

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 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, e.g., osteotropic 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. Alternatively, this property of a composition can be evaluated by examining the ability of the compound to modulate a parameter *in vitro*.

"Treating" a disease in a subject or "treating" a subject having a disease refers to subjecting the subject to a pharmaceutical treatment, e.g., the administration of a drug, such that at least one symptom of the disease is cured, alleviated or decreased.

"Tumor", as used herein, refers to all neoplastic cell growth and proliferation, whether malignant or benign, and all pre-cancerous and cancerous cells and tissues.

MMP-14 Binding Proteins

MMP-14 expression is observed in bone samples from patients with bone-metastatic prostate cancer. Any MMP-14 binding protein, e.g., an MMP-14 binding protein described herein, may be used in the methods and compositions for treating osteolytic disorders that are disclosed herein. MMP-14 is encoded by a gene designated as *MMP14*, matrix metalloproteinase-14 precursor. Synonyms for MMP-14 include matrix metalloproteinase 14 (membrane-inserted), membrane-type-1 matrix metalloproteinase, membrane-type matrix metalloproteinase 1, MMP-14, MMP14, MMP-X1, MT1MMP, MT1-MMP, MTMMP1, MT-MMP 1. MT-MMPs have similar structures, including a signal peptide, a prodomain, a catalytic domain, a hinge region, and a hemopexin domain (Wang, et al., 2004, J Biol Chem, 279:51148-55). According to SwissProt entry P50281, the signal sequence of MMP-14 precursor includes amino acid residues 1-20. The pro-peptide includes residues 21-111. Cys93 is annotated as a possible cysteine switch. Residues 112 through 582 make up the mature, active protein. The catalytic domain includes residues 112-317. The hemopexin domains includes residues 318-523. The transmembrane segment comprises residues 542 through 562.

MMP-14 can be shed from cells or found on the surface of cells, tethered by a single transmembrane amino-acid sequence. See, e.g., Osnkowski et al. (2004, J Cell Physiol, 200:2-10).

The MMP-14 binding protein may be an isolated protein (e.g., at least 70, 80, 90, 95, or 99% free of other proteins).

The MMP-14 binding protein may additionally inhibit MMP-14, e.g., human MMP-14. In one embodiment, the protein binds the catalytic domain of human MMP-14, e.g., the protein contacts residues in or near the active site of MMP-14.

In certain embodiments, proteins that bind to MMP-14 (e.g., human MMP-14) and include at least one immunoglobulin variable region are used in the methods and compositions. For example, the

MMP-14 binding protein includes a heavy chain (HC) immunoglobulin variable domain sequence and a light chain (LC) immunoglobulin variable domain sequence. A number of exemplary MMP-14 binding proteins are described herein.

MMP-14 binding proteins may also be antibodies. MMP-14 binding antibodies may have their
5 HC and LC variable domain sequences included in a single polypeptide (e.g., scFv), or on different polypeptides (e.g., IgG or Fab). For example, antibodies may be raised against any of the following sequences:

An exemplary amino acid sequence of human MMP-14 is shown in Table 1:

Table 1: Amino-acid sequence of human MMP-14

10 MSPAPRPPRCLLLPLLTLGTALASLGSAQSSSFSPEAWLQQYGYLPPGDLRTHTQRSPQSLSAAIAAM
QKFYGLQVTGKADADTMKAMRRPRCGVPDKFGAEIKANVRRKRYAIQGLKWQHNEITFCIQNYTPKVG
EYATYEAIRKAFRVWESATPLRFREVPYAYIREGHEKQADIMIFFAEGFHGDSTPFDGEGGFLAHAYF
PGPNIGGDTHFDSAEPWTVRNEDLNGNDIFLVAVHELGHALGLEHSSDPSAIMAPFYQWMDTENFVLP
DDDRRGIQQLYGGESGFPTKMPPQPRTTSRPSVPDKPKNPTYGPNICDGNFDTVAMLRGEMFVFKERW
15 FWRVRNNQVMDGYPMPIGQFWRGLPASINTAYERKDGKFVFFKGDKHWVFDEASLEPGYPKHIKELGR
GLPTDKIDAALFWMPNGKTYFFRGNKYYRFNEELRAVDSEYPKNIKVWEGIPESPRGSFMGSDEVFTY
FYKGNKYWKFNNQKLKVEPGYPKSALRDWMGCPSGGRPDEGTEEETEVIIIEVDEEGGGAVSAAAVVL
PVLLLLLVLAVGLAVFFFRRHGTPRRLLYCQRSLLDKV (SEQ ID NO:1; Genbank Accession No. CAA88372.1).

20 An exemplary amino acid sequence of mouse MMP-14 is shown in Table 2.

Table 2: Amino-acid sequence of mouse MMP-14

MSPAPRPSRSLLLPLLTLGTALASLGWAQGSNFSPEAWLQQYGYLPPGDLRTHTQRSPQSLSAAIAAMQKFYGL
QVTGKADLATMMAMRRPRCGVPDKFGTEIKANVRRKRYAIQGLKWQHNEITFCIQNYTPKVGEYATFEAIRKAF
RVWESATPLRFREVPYAYIREGHEKQADIMILFAEGFHGDSTPFDGEGGFLAHAYFPGPNIGGDTHFDSAEPWT
25 VQNEDLNGNDIFLVAVHELGHALGLEHSNDPSAIMSPFYQWMDTENFVLPDDDRRGIQQLYGSKSGSPTKMPPQ
PRTTSRPSVPDKPKNPAYGPNICDGNFDTVAMLRGEMFVFKERWFWRVRNNQVMDGYPMPIGQFWRGLPASINT
AYERKDGKFVFFKGDKHWVFDEASLEPGYPKHIKELGRGLPTDKIDAALFWMPNGKTYFFRGNKYYRFNEEFRA
VDSEYPKNIKVWEGIPESPRGSFMGSDEVFTYFYKGNKYWKFNNQKLKVEPGYPKSALRDWMGCPSGRRPDEGT
EEETEVIIIEVDEEGSGAVSAAAVVLPVLLLLLVLAVGLAVFFFRRHGTPKRLLYCQRSLLDKV
30 SEQ ID NO:2; GenBank Accession No. NP_032634.2.

An exemplary MMP-14 protein against which MMP-14 binding proteins may be developed can include the human or mouse MMP-14 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.

Exemplary MMP-14 binding proteins include M0031-C02, M0031-F01, M0033-H07, M0037-C09, M0037-D01, M0038-E06, M0038-F01, M0038-F08, M0039-H08, M0040-A06, M0040-A11, 5 and M0043-G02. The amino acid sequences of exemplary Fab heavy chain (HC) and light chain (LC) variable regions of these binding proteins are shown in FIGURE 1, and further description of them and their discovery and production is provided in pending application USSN 11/648,423 (U.S. 2007-0217997), which is hereby incorporated by reference in its entirety. Other exemplary MMP-14 binding proteins include DX-2400 and DX-2410. DX-2400 and M0038-F01 share HC and LC CDR 10 amino acid sequences. The amino acid sequences of the heavy chain and light chain variable regions of these proteins are provided in the Examples.

Other MMP-14 inhibitors known in the art include, but are not limited to, those disclosed in the following patents and patent applications: U.S. 6,114,159; U.S. 6,399,348; JP 3802560 and EP 0750672 (all in the name of Max Delbrueck Center for Molecular Medicine); U.S. 6,184,022; U.S. 15 6,825,024; EP 0685557; JP 2694604 (all in the name of Daiichi Fine Chemicals); and U.S. Provisional Application Serial Nos. 60/755,376 and 60/805,567 (both in the name of Dyax Corp.).

MMP-9 and MMP-9 Binding Entities

Any MMP-9 binding protein may be used in the methods and compositions for treating osteolytic disorders that are disclosed herein.

20 MMP-9 is encoded by a gene designated as *MMP9* with full name 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 25 amino acid sequences are also shown below. An 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 3 shows the similar genes in other organisms and the percentage of similarity with 30 human MMP-9. No similarity-to-human data found for MMP9 for: chimpanzee (*Pan 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 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. gosspyii yeast (*Ashbya gossypii*), K. lactis yeast (*Kluyveromyces lactis*), medicago trunc (*Medicago truncatula*), malaria parasite (*Plasmodium falciparum*), schistosome parasite (*Schistosoma mansoni*), sorghum (*Sorghum bicolor*), toxoplasmosis (*Toxoplasma gondii*).

cDNA and amino acid sequences of human *MMP9*

ACCESSION AK123156
VERSION AK123156.1 GI:34528630

translation="MARKGARRPRQGPGSHKWLQPGSRREKERIPQPPPPARPPRDAA
PRRVLVPAVRRVPESGHFAGRPWAPQCHPKGLRRPSAESHSVAQAGVQCHDLGSLQPP
PPSSGDSPASASRVAGITSTVPGTLSALDDCCLITELPYKPPAVLY" (SEQ ID NO:3)

1 acactttgcg ttccgcggcc ccggcccctt ggtttcctag tcctggctcc attcccctct
61 caggcctagg gctgggaccc ctccccgccc ccggtcttgg ccctgccccc ttcaacagac
121 ggtccgcccc ggcccctccc cctcgtcccg cccggccctg gcaggccccg ccccctgcgg
181 cctctacctt tgacgtcttc ccccgggagg tggcgggggt ctgcgaccga atgccggcgg
241 gactctgggt cagggcttct ggcgggccct gcgggggca gcgaggtgac cgtgaacctg
301 cggctcatgg cgcggaaagg agccaggcgg ccgcggcaag gtccgggatc gcacaagtgg
361 ctgcaaccag gctctaggag ggagaaagag cggatccccc aaccccctcc gcccgcccgc
421 cccccgcgag acgcggcgcc gcgcagggtc ctagtgcccg ctgtgcgaag ggttcctgaa
481 tctggccact tcgctgggag gccctgggct ccccagtgcc acccgaaggg cctgaggagg
541 ccatctgcag aatctcactc tgtcgcccag gccggagtgc agtgtcatga tcttggctca
601 ctgcaacctc cgcctcccag ttcaggagat tctcctgcct cagcctcccg ggtggctggg
661 attacaagca cagtgcctgg cacattatcg gcacttgatg actgttgtct aataactgag
721 cttccataca aaccacctgc cgtcctgtac tgaaggagaa agagcttcca gccggggagg
781 caggaaatct gggtcctggt cttggttgca tccctgactt cctaaatgac ctggagaagg
841 cctctgcctc tgctgggatc ttgtctgtgc tggggcattt gtttccattt ccaagggctt
901 tttcttcctc gctcagaatt tgaccactca ctaagaggag cttagtgtgg tgtctcacga
961 agggatcctc ctcagccctc acctcggtac tggaagacgt cgtgcgtgtc caaaggcacc
1021 ccggggaaca tccggtccac ctcgctggcg ctccggggat ccaccatctg cgccttcacg
1081 tcgaacctgc gggcaggcgc ggaggagaca ggtgctgagc cggctagcgg acggaccgac

1141 ggcgcccggg ctccccctgc cggcggccgc ggcggcgctc acctccagag gcgccgcccg
1201 ctgaacagca gcatcttccc cctgccactc cggagggccc cggtcacctg ggccacgtcg
1261 gcgcccaggc ccagcttgtc cagacgcctc gggcccagca ccgacgcgcc tgtgtacacc
1321 cacacctggc gccctgcagg ggaggagggt cacgtcggtt tgggggcgca gagggagcac
5 1381 gtactcctag aacgcgagga gggagattcc ggcgaggcct ttcctagccc gcgtgcccgc
1441 agtccctgca acccaggggc agaggcgctg ggtagagcga cgcgagggcg tggagaggag
1501 ggggcagaaa ctcagccgcc cctacgtttg ctaaactgcg tccgccaggg ggcgtatttt
1561 tctaaaacgc acaagacgtt tcgtgggtta tcgatggtct cttgagcctc cttgactgat
1621 ggggattgac cgggcggggg agggaaagta ggtaactaac cagagaagaa gaaaagcttc
10 1681 ttggagagcg gctcctcaaa gaccgagtcc agcttgcggg gcagcgcggg ccacttgtcg
1741 gcgataagga aggggccctg cggccggctc cccctgccct cagagaatcg ccagtacttc
1801 ctgagaaagc gaggagggaa aggacgggct ctaagccttg gacacagggc cagtgggcgg
1861 gaagggacgg gcagcccctc cgcaaagccc cctcccgcat ccacacaacc ccgcctcctc
1921 acccatcctt gaacaaatac agctggttcc caatc (SEQ ID NO:4)

15

cDNA and amino acid sequences of mouse *MMP9*

ACCESSION NM_013599

VERSION NM_013599.2 GI:31560795

translation="MSPWQPLLLALLAFGCSSAAPYQRQPTFVVFPKDLKTSNLTDTQ
20 LAEAYLYRYGYTRAAQMMGEKQSLRPALLMLQKQLSLPQTGELDSQTLKAIRTPRCGV
PDVGRFQTFKGLKWDHHNITYWIQNYSEDLPRDMIDDAFARAFAVWGEVAPLTFTRVY
GPEADIVIQFGVAEHGDGYPFDGKDGLLAHAFPPGAGVQGDAHFDDDELWSLGKGVVI
PTYYGNSNGAPCHFPFTFEGRSYSACTTDGRNDGTPWCSTTADYDKDGKFGFCPSERL
YTEHGNGEGKPCVFPFIFEGRSYSACTTKGRSDGYRWCATTANYDQDKLYGFCPTRVD
25 ATVVGGNSAGELCVFPFVFLGKQYSSCTSDGRRDGRLWCATTSNFDTDKKWGFCPDQG
YSLFLVAAHEFGHALGLDHSSVPEALMYPLYSYLEGFPLNKDDIDGIQYLYGRGSKPD
PRPPATTTTEPQPTAPPTMCPTIPPTAYPTVGPTVGPTGAPSPGPTSSPSPGPTGAPS
PGPTAPPTAGSSEASTESLSPADNPCNVDVFDAIAEIQGALHFFKDGWYWKFLNHRGS
PLQGPFLTARTWPALPATLDSAFEDPQTKRVFFFSGRQMWVYTGKTVLGPRSLDKLGL
30 GPEVTHVSGLLPRRLGKALLFSKGRVWRFDLKSQKVDPQSVIRVDKEFSGVPWNSHDI
FQYQDKAYFCHGKFFWRVSFQNEVNKVDHEVNQVDDVGYVTYDLLQCP" (SEQ ID NO:5)

1 ctcaccatga gtccctggca gcccctgctc ctggctctcc tggctttcgg ctgcagctct
61 gctgcccctt accagcgcca gccgactttt gtggtcttcc ccaaagacct gaaaacctcc
35 121 aacctcacgg acacccagct ggcagaggca tacttgtacc gctatggtta cacccgggcc
181 gcccagatga tgggagagaa gcagtctcta cggccggctt tgctgatgct tcagaagcag
241 ctctccctgc cccagactgg tgagctggac agccagacac taaaggccat tcgaacacca
301 cgctgtggtg tcccagacgt gggtcgattc caaaccttca aggcctcaa gtgggaccat

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361 cataacatca catactggat ccaaaactac tctgaagact tgccgcgaga catgatcgat
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481 gtgtacggac ccgaagcgga cattgtcatc cagtttggtg tcgcggagca cggagacggg
541 tatcccttcg acggcaagga cggccttctg gcacacgcct ttccccctgg cgccggcgtt
601 cagggagatg cccatttcga cgacgacgag ttgtggtcgc tgggcaaagg cgtcgtgatc
661 cccacttact atggaaactc aaatggtgcc ccatgtcact ttcccttcac cttcgaggga
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781 acagctgact acgataagga cggcaaattt ggtttctgcc ctagtgagag actctacacg
841 gagcacggca acggagaagg caaaccctgt gtgttcccgt tcatctttga gggccgctcc
901 tactctgcct gcaccactaa aggccgctcg gatggttacc gctggtgcgc caccacagcc
961 aactatgacc aggataaact gtatggcttc tgccctaccc gagtggacgc gaccgtagtt
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1081 tcttcctgta ccagcgacgg ccgcagggat gggcgcctct ggtgtgcgac cacatcgaac
1141 ttcgacactg acaagaagtg gggtttctgt ccagaccaag ggtacagcct gttcctggtg
1201 gcagcgcacg agttcggcca tgcactgggc ttagatcatt ccagcgtgcc ggaagcgctc
1261 atgtacccgc tgtatagcta cctcgagggc ttccctctga ataaagacga catagacggc
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1381 actgaaccac agccgacagc acctcccact atgtgtccca ctatacctcc cacggcctat
1441 cccacagtgg gccccacggt tggccctaca ggcgcccccт cacctggccc cacaagcagc
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1621 tttgatgcta ttgctgagat ccagggcgct ctgcatttct tcaaggacgg ttggtactgg
1681 aagttcctga atcatagagg aagcccatta cagggcccct tccttactgc ccgcacgtgg
1741 ccagccctgc ctgcaacgct ggactccgcc tttgaggatc cgcagaccaa gagggttttc
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1861 ctggataagt tgggtctagg cccagaggta acccacgtca gcgggcttct cccgcgtcgt
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1981 aaggtggatc cccagagcgt cattcgcgtg gataaggagt tctctggtgt gccctggaac
2041 tcacacgaca tcttccagta ccaagacaaa gcctatttct gccatggcaa attcttctgg
2101 cgtgtgagtt tccaaaatga ggtgaacaag gtggaccatg aggtgaacca ggtggacgac
2161 gtgggctacg tgacctacga cctcctgcag tgcccttgaa ctagggctcc ttctttgctt
2221 caaccgtgca gtgcaagtct ctagagacca ccaccaccac caccacacac aaaccccatc
2281 cgagggaaag gtgctagctg gccaggtaca gactggtgat ctcttctaga gactgggaag
2341 gagtggaggc aggcagggct ctctctgccc accgtccttt cttgttggac tgtttctaat
2401 aaacacggat ccccaacctt ttccagctac tttagtcaat cagcttatct gtagttgcag
2461 atgcatccga gcaagaagac aactttgtag ggtggattct gaccttttat tttgtgtgg
2521 cgtctgagaa ttgaatcagc tggcttttgt gacaggcact tcaccggcta aaccacctct
2581 cccgactcca gcccttttat ttattatgta tgaggttatg ttcacatgca tgtatttaac
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2641 ccacagaatg cttactgtgt gtcgggcgcg gctccaaccg ctgcataaat attaaggtat
2701 tcagttgccc ctactggaag gtattatgta actatttctc tcttacattg gagaacacca
2761 ccgagctatc cactcatcaa acatttattg agagcatccc tagggagcca ggctctctac
2821 tgggcgttag ggacagaaat gttggttctt ccttcaagga ttgctcagag attctccgtg
5 2881 tcctgtaaat ctgctgaaac cagaccccag actcctctct ctcccgagag tccaactcac
2941 tcactgtggt tgctggcagc tgcagcatgc gtatacagca tgtgtgctag agaggtagag
3001 ggggtctgtg cgttatggtt caggtcagac tgtgtcctcc aggtgagatg acccctcagc
3061 tggaactgat ccaggaagga taaccaagtg tcttcctggc agtctttttt aaataaatga
3121 ataaatgaat atttacttaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa
10 3181 aaaaa (SEQ ID NO:6)

ACCESSION NP_038627
VERSION NP_038627.1 GI:7305277
1 mspwqpllla llafgcssaa pyqrqptfvv fpkdlktsnl tdtqlaeayl yrygytraaq
15 61 mmgekqslrp allmlqkqls lpqtgeldsq tlkairtprc gvpdvgrfqt fkglkwdhhn
121 itywiqnyse dlprdmidda farafavwge vapltftrvy gpeadiviqf gvaehgdgyp
181 fdgkdgllah afppgagvqg dahfdddelw slgkgvvipt yygnsngapc hfpftfegrs
241 ysacttdgrn dgtpwcstta dydkdgkfgf cpserlyteh gngegkpcvf pfifegrsys
301 acttkgrsdg yrwcattany dqdklygfcp trvdatvvgg nsagelcvfp fvflgkqyss
20 361 ctsdgrrdgr lwcattsnfd tdkkwgfcpd qgyslflvaa hefghalgld hssvpealmy
421 plysylegfp lnkddidgiq ylygrgskpd prppatttte pqptapptmc ptipptaypt
481 vgptvgptga pspgptssps pgptgapspg ptapptagss eastesl spa dnpcnvdvfd
541 aiaeiqgalh ffkdgwywkf lnhrgsplqg pfltartwpa lpatldsafe dpqtkrvfff
601 sgrqmwvytg ktvlgprsld klglgpevth vsgllprrlg kallfskgrv wrfdlksqkv
25 661 dpqsvirvdk efsgvpwnsh difqyqdkay fchgkffwrv sfqnevnkvd hevnqvddvg
721 yvtydllqcp (SEQ ID NO:7)

Table 3. MMP-9 orthologs from nine species

Organism	Gene	Locus	Description	Human Similarity	s
dog (Canis familiaris)	MMP9[1]	--	matrix metallopeptidase 9 (gelatinase B, 92kDa gelatinase	85.46*(n)* 80.97*(a)*	403885 NM_001003219.1 NP_001003219.1
rat (Rattus norvegicus)	Mmp9[1]	--	matrix metallopeptidase 9	79.15*(n)* 74.89*(a)*	81687 NM_031055.1 NP_112317.1

mouse (Mus musculus)	Mmp9[1, 4]	2 (96.00 cM)[4]	matrix metallopeptidase 9[1, 4]	78.69*(n)*[1] 75*(a)*[1]	17395[1] NM 013599.2[1] NP 038627.1[1] AK004651[4] AK142787[4] (see all 16)
chicken (Gallus gallus)	LOC395387[1]	--	matrix metallopeptidase 9 (gelatinase B, 92kDa gelatinase	66.96*(n)* 62.54*(a)*	395387 NM 204667.1 NP 989998.1
zebrafish (Danio rerio)	wufb02g06[1~]	--	Danio rerio cDNA clone MGC64165 IMAGE6797338, complete	70.96*(n)*	BC053292.1
African clawed frog (Xenopus laevis)	MGC69080[1~]	--	hypothetical protein MGC69080	72.25*(n)*	BC057745.1
rainbow trout (Oncorhynchus mykiss)	Omy.10476[1~]	--	Oncorhynchus mykiss mRNA for matrix metalloproteinase	74.67*(n)*	AJ320533.1
thale cress (Arabidopsis thaliana)	MMP[1]	--	MMP (MATRIX METALLOPROTEINASE); metalloendopeptidase/	53*(n)* 46.85*(a)*	843353 NM 105685.3 NP 177174.1
rice (Oryza sativa)	P0516G10.18[1]	--	putative zinc metalloproteinase	51.98*(n)* 41.81*(a)*	3063368 XM 467714.1 XP 467714.1

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
5 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 4**).

Table 4. MMP-9 domains

FT	SIGNAL	1	19	
5	FT	PROPEP	20	93	Activation peptide.
	FT	CHAIN	94	?	67 kDa matrix metalloproteinase-9.
	FT	CHAIN	107	707	82 kDa matrix metalloproteinase-9.
	FT	PROPEP	?	707	Removed in 64 kDa matrix
	FT				metalloproteinase-9 and 67 kDa matrix
10	FT				metalloproteinase-9.
	FT	DOMAIN	225	273	Fibronectin type-II 1.
	FT	DOMAIN	283	331	Fibronectin type-II 2.
	FT	DOMAIN	342	390	Fibronectin type-II 3.
	FT	DOMAIN	513	707	Hemopexin-like.
15	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).
	FT	METAL	177	177	Zinc 1 (structural).
20	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).
	FT	METAL	190	190	Zinc 1 (structural).
25	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).
	FT	METAL	205	205	Calcium 3.
30	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).
	FT	METAL	405	405	Zinc 2 (catalytic).
35	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).
	FT	CARBOHYD	38	38	N-linked (GlcNAc...) (Potential).
40	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.
	FT	DISULFID	288	314	By similarity.
45	FT	DISULFID	302	329	By similarity.
	FT	DISULFID	347	373	By similarity.
	FT	DISULFID	361	388	By similarity.
	FT	DISULFID	516	704	
	FT	VARIANT	20	20	A -> V (in dbSNP:rs1805088).
50	FT	VARIANT	82	82	E -> K (in dbSNP:rs1805089).
	FT	VARIANT	127	127	N -> K (in dbSNP:rs3918252).
	FT	VARIANT	239	239	R -> H.

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FT   VARIANT     279    279       R -> Q (common polymorphism;
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
FT   HELIX        41     51
FT   TURN         52     53
FT   HELIX        68     78
FT   TURN         79     79
FT   HELIX        88     94
FT   TURN         95     95
FT   STRAND      103    105
FT   STRAND      119    125
FT   STRAND      130    132
FT   HELIX       134    149
FT   TURN        150    150
FT   STRAND      151    153
FT   STRAND      155    158
FT   TURN        162    163
FT   STRAND      164    171
FT   STRAND      176    178
FT   STRAND      183    186
FT   STRAND      189    191
FT   STRAND      194    196
FT   TURN        197    200
FT   STRAND      202    205
FT   TURN        206    207
FT   STRAND      213    219
FT   HELIX       220    231
FT   TURN        232    233
FT   TURN        240    241
FT   TURN        243    244
FT   STRAND      245    247
FT   STRAND      255    261
FT   HELIX       262    265
FT   STRAND      268    270
FT   TURN        274    276
FT   STRAND      279    283
FT   TURN        284    285
FT   STRAND      290    294
FT   TURN        295    296
FT   STRAND      297    301
FT   TURN        305    306
FT   STRAND      313    319
FT   HELIX       320    323
FT   STRAND      326    328
FT   HELIX       333    335
FT   TURN        340    344
FT   STRAND      349    353
FT   TURN        354    355
FT   STRAND      356    358
FT   TURN        364    365
FT   STRAND      372    378
FT   HELIX       379    382
FT   STRAND      385    387
FT   HELIX       395    406
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FT	TURN	407	408
FT	TURN	415	416
FT	TURN	418	419
FT	HELIX	433	442
FT	STRAND	512	517
FT	HELIX	515	517
FT	STRAND	522	527
FT	TURN	528	529
FT	STRAND	530	535
FT	TURN	536	537
FT	STRAND	538	542
FT	STRAND	545	547
FT	STRAND	551	555
FT	HELIX	556	559
FT	TURN	561	562
FT	STRAND	568	572
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
FT	TURN	605	605
FT	TURN	608	609
FT	STRAND	615	618
FT	TURN	621	622
FT	STRAND	623	628
FT	TURN	629	630
FT	STRAND	631	636
FT	TURN	637	640
FT	HELIX	644	646
FT	HELIX	650	653
FT	TURN	655	656
FT	STRAND	662	667
FT	TURN	668	669
FT	STRAND	670	675
FT	TURN	676	677
FT	STRAND	678	683
FT	TURN	686	687
FT	STRAND	690	696
FT	TURN	697	700
FT	TURN	702	703

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 hetrodimeric 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, peptide, Kunitz domain) 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 decscribed herein.

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 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., 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:

MMP-9 siRNA
5'- GACUUGCCGCGAGACAUGAtt -3' (SEQ ID NO:8)
3'- ttCUGAACGGCGCUCUGUACU -5' (SEQ ID NO:9)

Control RNA (mismatch)
5'- GACUU**C**GCG**G**GA**C**ACAUGAtt -3' (SEQ ID NO:10)
3'- ttCUGAA**G**CGC**C**CU**G**UGUACU -5' (SEQ ID NO:11)

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 decscribed herein.

MMP-9 Binding Proteins

5 Provided also are proteins that bind to MMP-9 (e.g., human MMP-9) and are either peptides, polypeptides that include at least one immunoglobin variable region, or Kunitz domains. Methods for discovering and selecting and improving such binding proteins are described further below. MMP-9 expression is observed in bone samples from patients with bone-metastatic prostate cancer. An MMP-9 binding protein, e.g, an MMP-9 binding protein described herein, can be used in the methods 10 described herein, e.g., to treat an osteolytic disorder.

In a preferred embodiment, the MMP-9 binding protein includes at least one immunoglobulin variable domain. For example, the MMP-9 binding protein includes a 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. MMP-9 binding 15 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).

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

The MMP-9 binding protein may additionally inhibit MMP-9, e.g., human MMP-9. The 20 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 steric change in MMP-9 that affects (e.g., inhibits) its activity.

25 The protein can bind to MMP-9, e.g., human MMP-9, 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 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-12 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 Ki of less than 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9}, and 10^{-10} M. The protein can 30 have, for example, an IC50 of less than 100 nM, 10 nM or 1 nM. In some embodiments, the protein has an IC50 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 t1/2 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., 25 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.

Exemplary MMP-9 binding proteins include antibodies with a heavy chain (HC) and/or light chain (LC), and in some embodiments, an HC and/or LC variable domain, that is selected from the group of antibodies consisting of: 539A-M0240-B03, 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, M0078-G07, M0081-D05, M0076-D03, M0072-H07, M0075-D12, and M0166-F10. These MMP-9 binding proteins are further described in USSN 61/033,075, filed March 3, 2008 and USSN 61/054,938, filed May 21, 2008, the content of which applications are hereby incorporated by reference in their entireties. Amino acid sequences for these and additional binding proteins are also provided in FIGURE 3 and the Examples herein.

The protein, if an antibody, 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 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 HC variable domain described above; (c) the LC immunoglobulin variable domain sequence comprises one or more 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 above; (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 above; (e) 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 above; (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.

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 IgG4. The protein can be a soluble Fab (sFab). In other implementations the protein includes a Fab_2', 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, Fab_2, Fab_2', scFv, PEGylated Fab, PEGylated scFv, PEGylated Fab_2, VH::CH1::HSA+LC, 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*), gibons, 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).

MMP-9/MMP-2 Binding Proteins

MMP-9/2 binding proteins are binding proteins that bind to MMP-9 (e.g., human MMP-9) and MMP-2 (e.g., human MMP-2) and are either peptides, polypeptides that nclude at least one immunoglobin variable region, or Kunitz domains. Methods for discovering and selecting and improving such binding proteins are described further below. Both MMP-9 and MMP-2 expression is observed in bone samples from patients with bone-metastatic prostate cancer. An MMP-9/MMP-2 binding protein, e.g, an MMP-9/MMP-2 binding proteins described herein, can be used in the methods described herein, e.g., to treat an osteolytic disorder.

In a preferred embodiment, the MMP-9/2 binding protein includes at least one immunoglobin variable region. For example, the MMP-9/MMP-2 binding protein includes a heavy chain (HC)

immunoglobulin variable domain sequence and a light chain (LC) immunoglobulin variable domain sequence. MMP-9/MMP-2 binding proteins may be antibodies. MMP-9/MMP-2 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).

5 The MMP-9/MMP-2 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).

The MMP-9/MMP-2 binding protein may additionally inhibit MMP-9, e.g., human MMP-9 and/or MMP-2, e.g., human MMP-2. The binding protein can inhibit the catalytic activity of MMP-9 (e.g., human MMP-9) and/or MMP-2 (e.g., human MMP-2). In one embodiment, the protein binds the 10 catalytic domain of human MMP-9, e.g., the protein contacts residues in or near the active site of MMP-9 and/or the protein binds the catalytic domain of human MMP-2, e.g., the protein contacts residues in or near the active site of MMP-2. 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 other embodiments, the protein does not 15 contact residues in or near the active site of MMP-2 but instead binds elsewhere on MMP-2 and causes a steric change in MMP-2 that affects (e.g., inhibits) its activity.

The protein can bind to MMP-9 and/or MMP-2 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 one embodiment, the protein binds to MMP-9 and/or MMP-2 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-12 20 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 and/or MMP-2 activity, e.g., with a Ki of less than 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9}, and 10^{-10} M. The protein can have, for example, an IC50 of less than 100 nM, 10 nM or 1 nM. In some embodiments, the protein has an IC50 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), 25 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 t1/2 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., 25 minutes), at least about 35 minutes (e.g., 33 minutes), or at least about 60 minutes (e.g., 57 minutes).

30 An exemplary MMP-9/2 binding protein includes an antibody with a heavy chain (HC) and/or light chain (LC), and in some embodiments, an HC and/or LC variable domain, that is selected from

the group of antibodies consisting of: M0237-D02. Such MMP-9/2 binding proteins are further described in USSN 61/033,068, filed on March 3, 2008, USSN 61/033,075, filed March 3, 2008 and USSN 61/054,938, filed May 21, 2008, the content of which applications are hereby incorporated by reference in their entireties. Amino acid sequences are also provided in the Examples herein.

5 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 IgG4. The protein can be a soluble Fab (sFab). In other implementations the protein includes a Fab_2', scFv, minibody, scFv::Fc fusion, Fab::HSA fusion, HSA::Fab fusion, Fab::HSA::Fab fusion, or other molecule that 10 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, Fab_2, Fab_2', scFv, PEGylated Fab, PEGylated scFv, PEGylated Fab_2, VH::CH1::HSA+LC, 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 15 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 20 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*), gibons, 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 25 murine or rabbit antibody).

Methods for Discovering MMP-14 or MMP-9 Binding Proteins

MMP-14 or MMP-9 binding proteins may be discovered by any method of ligand discovery known in the art. In certain embodiments, MMP-14 or MMP-9 binding proteins may be discovered by screening a library. In certain embodiments, the library is a display library. A display library is a 30 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. 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 a soluble Fab (sFab). In one exemplary implementation, a display library can be used to identify proteins that bind to MMP-14 or MMP-9. In a selection, the polypeptide component of each member of the library is probed with MMP-14 or MMP-9 (e.g., the catalytic domain of MMP-14 or MMP-9 or other fragment) and if the polypeptide component binds to the MMP-14 or MMP-9, the display library member is identified, typically by retention on a support.

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-14 or MMP-9, or for binding to another protein, e.g., another metalloproteinase. Each candidate library member can be subjected to one or more secondary screening assays. The assay can be 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 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 ELISAs, homogenous binding assays, surface plasmon resonance (SPR) and cellular assays, the practice of which are well-known to those of skill in the art.

In addition to the use of display libraries, other methods can be used to obtain a MMP-14 or MMP-9 binding antibody. For example, MMP-14 or MMP-9 protein or a region thereof can be used as an antigen in a non-human animal, e.g., a rodent. 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 Morrison, S. L., 1985, *Science* 229:1202-1207, by Oi et al., 1986, *BioTechniques* 4:214, and by Queen et al. U.S. Patent Nos. 5,585,089, 5,693,761 and 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 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.

5 Immunoglobin MMP-14 or MMP-9 binding proteins (e.g., IgG or Fab MMP-14 or MMP-9 binding proteins) may be modified to reduce immunogenicity. Reduced immunogenicity is desirable in MMP-14 or 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-14 or MMP-9 binding proteins include deletion/modification of 10 potential human T-cell epitopes and 'germlining' of sequences outside of the CDRs (e.g., framework and Fc).

An MMP-14 or 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 15 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 20 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. 25 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 30 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 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-14 or MMP-9 binding antibodies are "germlined" by reverting one or more non-germline 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-14 or 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 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 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 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 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.

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 include 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.

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).

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, 90 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.

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

5 Germline sequences of human immunoglobin 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).

10 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 15 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, 20 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.

In one embodiment, an MMP-14 or MMP-9 binding protein is physically associated with a moiety 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-14 or MMP-9 binding 25 protein can be associated with a polymer, e.g., a substantially non-antigenic polymers, 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 about 1,000 to about 15,000, and 2,000 to about 12,500) can be used. For example, an MMP-14 or MMP-9 binding protein can be conjugated to a water soluble polymer, e.g., hydrophilic polyvinyl polymers, 30 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 that the water solubility of the block copolymers is maintained.

An MMP-14 or 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-14 or MMP-9 binding protein.

Pharmaceutical Compositions of MMP-14 or MMP-9 Binding Proteins

In another aspect, the disclosure provides compositions, e.g., pharmaceutically acceptable compositions or pharmaceutical compositions, which include an MMP-14 or MMP-9-binding protein, e.g., an antibody molecule, other polypeptide or peptide identified as binding to MMP-14 or MMP-9 described herein. The MMP-14 or MMP-9 binding protein can be formulated together with a pharmaceutically acceptable carrier and/or pharmaceutically acceptable salt. Pharmaceutical compositions include therapeutic compositions and diagnostic compositions, e.g., compositions that include labeled MMP-14 or 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-14 or 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, chloroprocaine, 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-14 or MMP-9 binding protein is administered by intravenous infusion or injection. In another preferred embodiment, the MMP-14 or MMP-9 binding protein is administered by intramuscular or subcutaneous injection.

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-14 or 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-14 or 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/m^2 or 7 to 25 mg/m^2. 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
5 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-14 or 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
10 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
15 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
20 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.

25 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-14 or 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/m^2 or about 5 to 30 mg/m^2. For binding proteins smaller in molecular weight than an antibody, appropriate amounts can be proportionally less.
30 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-14 or MMP-9 binding protein disclosed herein.

Methods of Treating Osteolytic Disorders

Proteins that bind to MMP-14 or 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 osteolytic disorders. In certain embodiments, the MMP-14 or MMP-9 binding proteins are administered to a subject, or even to osteotropic cancer cells in culture, e.g. *in vitro* or *ex vivo*, to treat or prevent osteotropic cancer. In other embodiments, the MMP-14 or MMP-9 binding proteins are administered to a subject to treat or prevent osteoporosis. Treating includes administering an amount effective to alleviate, relieve, alter, remedy, ameliorate, improve or affect the disorder, at least one symptom of the disorder or the predisposition toward the disorder, whereas preventing includes administering an amount effective to stop or slow the manifestation of the disorder, e.g., as compared to what is expected in the absence of the treatment. The treatment may also delay onset, e.g., prevent onset, or prevent deterioration of the osteolytic disorder, e.g., as compared to what is expected in the absence of the treatment.

As used herein, an amount of a MMP-14 or MMP-9 binding protein effective to prevent an osteolytic disorder, such as osteotropic cancer or osteoporosis, or a prophylactically effective amount of the MMP-14 or MMP-9 binding protein, refers to an amount of a MMP-14 or MMP-9 binding protein, e.g., an anti-MMP-14 or 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 the osteolytic disorder. Stated another way, a therapeutically effective amount of an MMP-14 or 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 are 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.

Guidance for determination of a therapeutically effective amount for treatment of an osteolytic disorder may be obtained by reference to *in vivo* models of the particular osteolytic disorder. For example, for osteotropic cancer, the amount of a MMP-14 or 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. Cancer cell lines such as PC-3 or the human breast cancer cell line, MDA-MB-231, with either a high potential to cause bone metastasis (MDA-231#16) or a low potential (MDA-MB-231#17), may be used in the preparation of such animal models, or may be used on their own as models.

A MMP-14 or MMP-9 binding protein described herein can be used to reduce an osteolytic disorder in a subject, e.g., to treat an osteotropic cancer (e.g., a solid tumor or lesion, or to kill circulating cancer cells) or osteoporosis (e.g., to reduce the porosity of the bones). The method includes administering the MMP-14 or MMP-9 binding protein to the subject, e.g., in an amount effective to modulate the osteolytic disorder (e.g., for osteotropic cancer, a tumor or lesion size), a symptom of the disorder, or progression of the disorder. The MMP-14 or MMP-9 binding protein may be administered multiple times (e.g., at least two, three, five, or ten times) before a therapeutically effective amount is attained. In one embodiment, the MMP-14 or MMP-9 binding proteins are used to inhibit an activity (e.g., inhibit at least one activity, 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-14 or MMP-9 binding proteins that do not substantially inhibit MMP-14 or MMP-9 may be used to deliver nanoparticles containing agents, such as toxins, to MMP-14 or MMP-9 associated cells or tissues, e.g., tumors.

Accordingly, the disclosure provides methods of treating (e.g., slowing, eliminating, or reversing tumor growth or bone porosity, 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, e.g., relative to a standard, e.g., as compared to what is expected in the absence of treatment or as compared to the condition of a subject (or cohort of subjects) with an osteolytic disorder that was not treated for the disease) an osteolytic disorder such as osteotropic cancer or osteoporosis by administering an effective amount of an MMP-14 or MMP-9 binding protein (e.g., an anti-MMP-14 or MMP-9 IgG or Fab). In some embodiments, the MMP-14 or MMP-9 binding protein inhibits MMP-14 or MMP-9 activity. In certain embodiments, the MMP-14 or MMP-9 binding protein is administered as a single agent treatment. In other embodiments, the MMP-14 or 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 an osteolytic disorder, by administering an effective amount of an MMP-14 or MMP-9 binding protein to a subject at risk of developing an osteolytic disorder, thereby reducing the subject's risk of developing the osteolytic disorder. For example, MMP-14 or MMP-9 binding proteins may be administered to prevent osteolytic lesions in a subject having osteotropic cancer, e.g., bone metastasis. As another example, to prevent or reduce the risk of developing an osteolytic disorder, an MMP-14 or MMP-9 binding protein may be administered to a subject who has been diagnosed with a cancer (e.g., breast, lung or prostate cancer) that has the potential to metastasize to bone.

The disclosure further provides methods of modulating (e.g., reducing or preventing) osteotropic cancer at a tumor site by administering an effective amount of an MMP-14 or MMP-9 binding protein, thereby reducing or preventing the tumor size or growth. The MMP-14 or MMP-9 binding protein may be administered to the tumor site 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-14 or MMP-9 binding protein to a subject, thereby reducing ECM degradation by a tumor in the subject.

Methods of administering MMP-14 or 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-14 or MMP-9. The dose of the MMP-14 or MMP-9 binding protein can be the amount sufficient to block 90%, 95%, 99%, or 99.9% of the activity of MMP-14 or 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 μM, and 1.8 μM of binding sites for a 5 L blood volume.

Because the MMP-14 or MMP-9 binding proteins recognize MMP-14 or MMP-9-expressing cells and can bind to cells that are associated with (e.g., in proximity of or intermingled with) osteotropic cancer cells, MMP-14 or 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 the progression of the osteolytic disorder. Reducing MMP-14 or 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-14 or 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 cancers cells. Thus, the MMP-14 or 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 MMP-14 or MMP-9 binding proteins may be used to deliver or aid or enhance the delivery of an agent (e.g., any of a variety of cytotoxic and therapeutic drugs) to cells and tissues where MMP-14 or 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 toxins or short range radiation emitters, e.g., short range, high energy α–emitters.

To target MMP-14 or MMP-9 expressing osteotropic cancer 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 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-14 or 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 cytotoxicity (ADCC). The binding proteins described herein can include a 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, 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.

The MMP-14 or 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 those tissues expressing the MMP-14 or MMP-9.

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.

Alternatively, the MMP-14 or 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 a emitters, such as ^{212}Bi, ^{213}Bi, and ^{211}At, and b emitters, such as ^{186}Re and ^{90}Y. Moreover, ^{177} Lu may also be used as both an imaging and 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 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-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. Oncol.* 65: 94-101).

Combination Therapies

The MMP-14 or MMP-9 binding proteins described herein, e.g., anti-MMP-14 or MMP-9 Fabs or IgGs, can be administered in combination with one or more of the other therapies for treating the particular osteolytic disorder of interest. For example, an MMP-14 or MMP-9 binding protein can be used therapeutically or prophylactically with surgery, another MMP-14 or MMP-9 inhibitor, e.g., a small molecule inhibitor, another anti-MMP-14 or MMP-9 Fab or IgG (e.g., another Fab or IgG described herein), peptide inhibitor, or small molecule inhibitor. Examples of MMP-14 or MMP-9 inhibitors that can be used in combination therapy with an MMP-14 or MMP-9 binding protein described herein include neovastat, marimastat, BAY 12-9566 and prinomastat. One or more small-molecule MMP inhibitors can be used in combination with one or more MMP-14 or 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 combination may result in enhanced delivery and efficacy of one or both agents.

In certain embodiments, the MMP-14 or MMP-9 binding proteins described herein can be administered in combination with one or more of the other therapies for treating osteotropic cancer, including, but not limited to: surgery; radiation therapy, chemotherapy, and other anti-cancer

therapeutic agents. For example, proteins that inhibit MMP-14 or MMP-9 or that inhibit a downstream event of MMP-14 or 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 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. The combination can further include 5-FU and leucovorin, and/or irinotecan. Other additional cancer therapeutic or treatments, include bisphosphonates (e.g., amino and non-amino bisphosphonates), hormone-related compounds (e.g., estrogens and SERMs), RANKL antagonists, RANKL pathway inhibitors, $\alpha_\gamma\beta_3$ antagonists, Src inhibitors, cathepsin K inhibitors and calcitonin.

In other embodiments, the MMP-14 or MMP-9 binding proteins described herein can be administered in combination with one or more of the other therapies for treating osteoporosis, including, but not limited to, bisphosphonates (e.g., amino and non-amino bisphosphonates), hormone-related compounds (e.g., estrogens and SERMs), calcitonin, Teriparatide (FORTEO™), tamoxifen and RANKL pathway inhibitors.

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-14 or 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-14 or MMP-9 binding protein.

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 anti-androgens such as 4'-cyano-3-(4-fluorophenylsulphonyl)-2-hydroxy-2-methyl-3'-(trifluoromethyl) propionanilide.

Other additional cancer therapeutic or treatments that may be used in treating metastatic bone cancers include bisphosphonates (e.g., amino and non-amino bisphosphonates), hormone-related compounds (e.g., estrogens and SERMs), RANKL antagonists, $\alpha_v\beta_3$ antagonists, Src inhibitors, cathepsin K inhibitors and calcitonin. All of these therapeutics may serve as bone resorption inhibitors, in addition to having other activities.

Bisphosphonates (also called: diphosphonates) are a class of drugs that inhibits osteoclast action and the resorption of bone. Their uses include the prevention and treatment of osteoporosis, osteitis deformans ("Paget's disease of bone"), bone metastasis (with or without hypercalcemia), multiple myeloma and other conditions that feature bone fragility. Exemplary bisphosphonates (also known as diphosphonates) include both amino and non-amino bisphosphonates. Specific examples of bisphosphonates that may be used in the disclosed methods include, but are not limited to, non-amino bisphosphonates such as Etidronate (DIDRONEL®), Clodronate (BONEFOS®, LORON®) and Tiludronate (SKELID®); and amino bisphosphonates such as Pamidronate (APD,AREDIA®),

Neridronate, Olpadronate, Alendronate (FOSAMAX®), Ibandronate (BONIVA®), Risedronate (ACTONEL®) and Zoledronate (ZOMETA®).

Hormone-related compounds include, but are not limited to, estrogens, selective estrogen receptor modulators (SERMs) and LH-RH agonists such as Leuprolide (LUPRON®, VIADUR®, ELIGARD®), Goserelin (Zoladex®), Raloxifene (EVISTA®) and Triptorelin (TRELSTAR®).

RANKL antagonists may be used to block RANK-RANKL interactions. Exemplary RANKL antagonists include, but are not limited to, TRANCE-Fc, OPG and OPG-Fc.

Exemplary RANKL pathway inhibitors include, but are not limited to, Denosumab (Body, et al. (2006) Clin. Cancer Res. 12:1221-1228).

$\alpha_\gamma\beta_3$ antagonists may be used to block osteoclast adhesion to bone. Exemplary $\alpha_\gamma\beta_3$ antagonists include small molecule and peptide antagonists, examples of which include but are not limited to, Vitaxin, Cilengitide, (S)-3-Oxo-8-[2-[6-(methylamino)-pyridin-2-yl]-1-ethoxy]-2-(2,2,2-trifluoroethyl)-2,3,4,5-tetrahydro-1H-2-benzazepine-4-acetic acid and 3-[2-oxo-3-[3-(5,6,7,8-tetrahydro-[1,8]naphthyridin-2-yl) propyl]-imidazolidin-1-yl]-3(S)-(6-methoxy-pyridin-3-yl) propionic acid.

Src inhibitors may be used to block steps leading to osteoclast activation. Exemplary Src inhibitors include, but are not limited to, SKI-606 (Wyeth), AZD0530 (AstraZeneca) and BMS-453825 (Dasatinib (SPRYCEL®)).

Cathepsin K inhibitors may be used to block activity of osteoclast-specific collagenase. Exemplary cathepsin K inhibitors include, but are not limited to, balicatib.

A combination therapy can include administering an agent that reduces the side effects of other therapies. In embodiments where the osteolytic disorder is osteotropic cancer, the agent can be an agent that reduces the side effects of anti-cancer treatments. For example, the agent can be leucovorin.

Kits

An MMP-14 or 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-14 or MMP-9 binding protein, e.g., a composition that includes an MMP-14 or MMP-9 binding protein, and, optionally (b) informational material. 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-14 or 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 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 diagnose an osteolytic disorder.

In one embodiment, the informational material can include instructions to administer an MMP-14 or 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 administration described herein). In another embodiment, the informational material can include instructions to administer an MMP-14 or MMP-9 binding protein to a suitable subject, e.g., a human, e.g., a human having, or at risk for, an osteolytic disorder. For example, the material can include instructions to administer an MMP-14 or MMP-9 binding protein to a patient with osteotropic cancer or osteoporosis. 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-14 or MMP-9 binding protein can be provided in any form, e.g., liquid, dried or lyophilized form. It is preferred that an MMP-14 or MMP-9 binding protein be substantially pure and/or sterile. When an MMP-14 or 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-14 or 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-14 or 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 in 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-14 or 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-14 or 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.

EXEMPLIFICATIONS

The following examples provide further illustration and are not limiting.

Example 1: DX-2400 Reduces Osteolytic Lesions in the PC-3 Prostate Cancer Model

PC-3 prostate cancer cells were inoculated intra-tibially into mice. Treatment was initiated 3 days after intra-tibial inoculation of the cells. DX-2400 (10 mg/kg) and a PBS control were administered Q2D for 14 days. X-ray analysis and bone histomorphometric analysis indicated that DX-2400 reduced the area of the osteolytic lesions about 3-fold (FIGURE 2). DX-2400 is a selective inhibitor of MMP-14.

Example 2: Exemplary MMP-9 Binding Antibodies

Experiments were performed to evaluate the *in vitro* effects of 539A-M0240-B03 and 539A-M0237-D02 in bone metastasis models.

Cells. Raw 264.7 cells (Mouse leukemia monocyte/macrophage cell line) were obtained from ATCC (Catalog # TIB-71) and maintained in ATCC recommended complete medium (Catalog # 30-2020). Cells between passage 3-7 were used in this study.

Materials. Osteologic discs were purchased from BD biosciences (Catalog # 354609). Tartrate-resistant acid phosphatase (TRAP) staining kit was obtained from Kamiya Biomedica Company, Seattle WA (Catalog # KT-008). GM6001 was obtained from Millipore.

Methods. Approximately 2000 Raw 264.7 cells per slide were seeded onto osteologic multitest slides with complete growth medium. On the following day, cells were replaced with fresh medium containing 100 ng/ml recombinant murine soluble RANK ligand (Peprotech Inc. UK) along with the

broad spectrum MMP inhibitor GM6001 (5 μM, 10 μM, 25 μM concentrations tested), 539A-M0240-B03 (10 μg/ml, 50 μg/ml concentrations tested), or 539A-M0237-D02 (10 μg/ml, 100 μg/ml concentrations tested). The slides were then incubated at 37°C for 6 days, replacing fresh media on day 3 as described above. At the end of incubation time, one side of the slide was stained for TRAP and the other side of the slide was bleached (10% bleach), washed several times with water, and air dried. The slides were then viewed under the microscope for either multinucleated TRAP positive cells or resorbed areas (pits). Cells incubated with media only and recombinant osteoprotegerin (rH OPG) (100 ng/ml) served as negative and positive controls, respectively.

Conclusion. These *in vitro* experiments suggest that GM6001, 539A-M0240-B03, and 539A-M0237D02 have inhibitory effects on osteoclastogenesis and bone resorption at the concentrations tested (data not shown). The results showed that the inhibitory effect was dose-dependent. As the concentration of GM6001, 539A-M0240-B03, or 539A-M0237D02 increased, the amount of TRAP-positive staining and the number of resorbed areas decreased.

Example 3: Exemplary MMP-9 Binding Antibodies

539A-M0166-F10. An exemplary MMP-9 antibody is 539A-M0166-F10. The amino acid sequences of variable regions of 539A-M0166-F10 sFAB are as follows:

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

FYSHSAQSELTQPPSASAAPGQRVTISCSGSSSNIGSNTVTWYQKLPGTAPKLLIYNNYERPSGVPARFSGSKSGT SASLAISGLQSEDEADYYCATWDDSLIANYVFGSGTKVTVLGQPKANP (SEQ ID NO:12)

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

MKKLLFAIPLVVPFVAQPAMAEVQLLESGGGLVQPGGSLRLSCAASGFTFSPYLMNWVRQAPGKGLEWVSSIYSSG GGTGYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARIYHSSSGPFYGMDVWGQGTTVTVSSASTKGPSVFPLAPS SKS (SEQ ID NO:13)

539A-M0240-B03. Another exemplary MMP-9 antibody is 539A-M0240-B03. 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 complememtarity determining regions (CDRs) of 539A-M0240-B03 light chain (LC) and heavy chain (HC) are as follows:

LC CDR1: TGTSSDVGGYNYVS (SEQ ID NO:14)

LC CDR2: DVSKRPS (SEQ ID NO:15)

LC CDR3: CSYAGSYTLV (SEQ ID NO:16)

HC CDR1: TYQMV (SEQ ID NO:17)

HC CDR2: VIYPSGGPTVYADSVKG (SEQ ID NO:18)

HC CDR3: GEDYYDSSGPGAFDI (SEQ ID NO:19)

Example 4: Exemplary MMP-9/2 Binding Antibody

M0237-D02. An exemplary MMP-9/2 antibody is M0237-D02. The amino acid sequences of variable regions of_539A-M0237-D02 sFAB are as follows:

539A-M0237-D02 (phage/SFAB) VL leader +VL

FYSHSAQDIQMTQSPATLSLSPGERATLSCRASQSISSFLAWYQQKPGQAPRLLIYDASYRATGIPARFSGSGSGT DFTLTISSLEPEDYAVYYCQQRGNWPITFGQGTRLEIKRTVAAPS (SEQ ID NO:20)

539A-M0237-D02 (phage/SFAB) VH leader +VH

MKKLLFAIPLVVPFVAQPAMAEVQLLESGGGLVQPGGSLRLSCAASGFTFSQYPMWWVRQAPGKGLEWVSYIVPSG GRTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKDRAYGDYVGWNGFDYWGQGTLVTVSSASTKGPSVFPLAP SSKS (SEQ ID NO:21)

Example 5: Exemplary MMP-14 Binding Antibodies

DX-2400. An exemplary MMP-14 antibody is DX-2400. The variable domain sequences for DX-2400 are:

VH:

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

CDR2-- FR3----------------------------- CDR3-- FR4---------
DX-2400 **ADSVKG** RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR **GRAFDI** WGQGTMVTVSS
(SEQ ID NO:22)
CDR regions are in bold.

VL:

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

FR3------------------------- CDR3------ FR4-------
DX-2400 RFSGSGSGTDFTLTISSLQPEDFATYYC **QQSYSIPRFT** FGPGTKVDIK

(SEQ ID NO:23)
CDR regions are in bold.

DX-2410. Another exemplary MMP-14 antibody is DX-2410. The variable domain sequences for DX-2410 are:

VH:

```
              FR1-------------------------- CDR1- FR2----------- CDR2-------
DX2410        EVQLLESGGGLVQPGGSLRLSCAASGFTFS VYGMV WVRQAPGKGLEWVS VISSSGGSTWY

              CDR2-- FR3----------------------------- CDR3------- FR4--------
DX2410        ADSVKG RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR PFSRRYGVFDY WGQGTLVTVSS
```

(SEQ ID NO:24)
CDR regions are in bold.

VL:

```
             FR1-------------------- CDR1------- FR2------------ CDR2---
DX2410       DIQMTQSPSSLSASVGDRVTITC RASQGIRNFLA WYQQKPGKVPKLLIY GASALQS

              FR3----------------------------- CDR3----- FR4-------
DX2410        GVPSRFSGSGSGTDFTLTISSLQPEDVATYYC QKYNGVPLT FGGGTKVEIK
```

(SEQ ID NO:25)
CDR regions are in bold.

Example 6: Additional Exemplary MMP-9 Binding Antibodies

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 WYQQHPGKAPKLMIY DVSKRPS GVPD

        FR3-L                       CDR3-L     FR4-L
 RFSGSKSGNTASLTISGLQAEDEADYYC CSYAGSYTLV FGGGTKLTVL (SEQ ID NO:26)

      -------------------
```

LC-lambda1

```
GQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSHRSYS
CQVTHEGSTVEKTVAPTECS (SEQ ID NO:27)
```

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.

Light Chain

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

                      FR1-L                    CDR1-L           FR2-L         CDR2-L
Antibody A: QYELTQPRSVSGSPGQSVTISC TGTSSDVGGYNYVS WYQQHPGKAPKLMIY DVSKRPS   GVPD

                        FR3-L                       CDR3-L    FR4-L
Antibody A: RFSGSKSGNTASLTISGLQAEDEADYYC CSYAGSYTLV FGGGTKLTVL (SEQ ID NO:28)
```

Heavy Chain

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

                        FR1-H                        CDR1-H   FR2-H            CDR2-H
Antibody A: EVQLLESGGGLVQPGGSLRLSCAASGFTFS TYQMV WVRQAPGKGLEWVS VIYPSGGPTVYADSVKG

                         FR3-H                       CDR3-H          FR4-H
Antibody A:  RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR GEDYYDSSGPGAFDI WGQGTMVTVSS (SEQ ID NO:29)
```

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

```
               Q  Y  E  L  T  Q  P  R  S  V  S  G  S  P  G  Q  S  V  T  I
Antibody A   : CAGTACGAATTGACTCAGCCTCGCTCAGTGTCCGGGTCTCCTGGACAGTCAGTCACCATC

               S  C  T  G  T  S  S  D  V  G  G  Y  N  Y  V  S  W  Y  Q  Q
Antibody A   : TCCTGCACTGGAACCAGCAGTGATGTTGGTGGTTATAACTATGTCTCCTGGTACCAACAG

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

               P  D  R  F  S  G  S  K  S  G  N  T  A  S  L  T  I  S  G  L
Antibody A   : CCTGATCGCTTCTCTGGCTCCAAGTCTGGCAACACGGCCTCCCTGACCATCTCTGGGCTC
```

```
                Q  A  E  D  E  A  D  Y  Y  C  C  S  Y  A  G  S  Y  T  L  V
Antibody A    : CAGGCTGAGGATGAGGCTGATTATTACTGCTGCTCATATGCAGGCAGCTACACTTTGGTG

                F  G  G  G  T  K  L  T  V  L (SEQ ID NO:30)
Antibody A    : TTCGGCGGAGGGACCAAGCTGACCGTCCTA (SEQ ID NO:31)
```

Heavy Variable
Antibody A-Heavy: Parental clone (sFab; IgG in pBh1(f)) Heavy variable

```
                E  V  Q  L  L  E  S  G  G  G  L  V  Q  P  G  G  S  L  R  L
Antibody A    : GAAGTTCAATTGTTAGAGTCTGGTGGCGGTCTTGTTCAGCCTGGTGGTTCTTTACGTCTT

                S  C  A  A  S  G  F  T  F  S  T  Y  Q  M  V  W  V  R  Q  A
Antibody A    : TCTTGCGCTGCTTCCGGATTCACTTTCTCTACTTACCAGATGGTTTGGGTTCGCCAAGCT

                P  G  K  G  L  E  W  V  S  V  I  Y  P  S  G  G  P  T  V  Y
Antibody A    : CCTGGTAAAGGTTTGGAGTGGGTTTCTGTTATCTATCCTTCTGGTGGCCCTACTGTTTAT

                A  D  S  V  K  G  R  F  T  I  S  R  D  N  S  K  N  T  L  Y
Antibody A    : GCTGACTCCGTTAAAGGTCGCTTCACTATCTCTAGAGACAACTCTAAGAATACTCTCTAC

                L  Q  M  N  S  L  R  A  E  D  T  A  V  Y  Y  C  A  R  G  E
Antibody A    : TTGCAGATGAACAGCTTAAGGGCTGAGGACACGGCCGTGTATTACTGTGCGAGAGGGGAG

                D  Y  Y  D  S  S  G  P  G  A  F  D  I  W  G  Q  G  T  M  V  T  V  S  S (SEQ ID NO:32)
Antibody A    : GACTACTATGATAGTAGTGGCCCGGGGGCTTTTGATATCTGGGGCCAAGGGACAATGGTCACCGTCTCAAGC (SEQ ID NO:33)
```

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,
5 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

                FR1-L                   CDR1-L          FR2-L            CDR2-L
Antibody B:     QSALTQPRSVSGSPGQSVTISC  TGTSSDVGGYNYVS  WYQQHPGKAPKLMIY  DVSKRPS  GVPD

                FR3-L                              CDR3-L      FR4-L
Antibody B:     RFSGSKSGNTASLTISGLQAEDEADYYC       CSYAGSYTLV  FGGGTKLTVL (SEQ ID NO:34)
```

Heavy Chain

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

                FR1-H                           CDR1-H  FR2-H           CDR2-H
Antibody B:     EVQLLESGGGLVQPGGSLRLSCAASGFTFS  TYQMV   WVRQAPGKGLEWVS  VIYPSGGPTVYADSVKG

                FR3-H                             CDR3-H            FR4-H
Antibody B:     RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR  GEDYYDSSGPGAFDI   WGQGTMVTVSS (SEQ ID NO:35)
```

Light Variable
Antibody B-Light: Germlined, codon optimized in GS vector

```
Antibody B  : CAGAGCGCCCTGACCCAGCCCAGAAGCGTGTCCGGCAGCCCAGGCCAGAGCGTGACCATC
               Q  S  A  L  T  Q  P  R  S  V  S  G  S  P  G  Q  S  V  T  I

Antibody B  : AGCTGCACCGGCACCAGCAGCGACGTGGGCGGCTACAACTACGTGTCCTGGTATCAGCAG
               S  C  T  G  T  S  S  D  V  G  G  Y  N  Y  V  S  W  Y  Q  Q

Antibody B  : CACCCCGGCAAGGCCCCCAAGCTGATGATCTACGACGTGTCCAAGAGGCCCAGCGGCGTG
               H  P  G  K  A  P  K  L  M  I  Y  D  V  S  K  R  P  S  G  V

Antibody B  : CCCGACAGGTTCAGCGGCAGCAAGAGCGGCAACACCGCCAGCCTGACCATCTCCGGACTG
               P  D  R  F  S  G  S  K  S  G  N  T  A  S  L  T  I  S  G  L

Antibody B  : CAGGCCGAGGACGAGGCCGACTACTACTGCTGCAGCTACGCCGGCAGCTACACCCTGGTG
               Q  A  E  D  E  A  D  Y  Y  C  C  S  Y  A  G  S  Y  T  L  V
```

Antibody B : TTCGGCGGAGGGACCAAGCTGACCGTGCTG (SEQ ID NO:36)
F G G G T K L T V L (SEQ ID NO:37)

5 **Heavy Variable**
Antibody B-Heavy: Germlined, codon optimized in GS vector

Antibody B : GAGGTGCAATTGCTGGAAAGCGGCGGAGGACTGGTGCAGCCAGGCGGCAGCCTGAGGCTG
E V Q L L E S G G G L V Q P G G S L R L

10

Antibody B : TCCTGCGCCGCCAGCGGCTTCACCTTCAGCACCTACCAGATGGTGTGGGTGCGCCAGGCC
S C A A S G F T F S T Y Q M V W V R Q A

Antibody B : CCAGGCAAGGGCCTGGAATGGGTGTCCGTGATCTACCCCAGCGGCGGACCCACCGTGTAC
15 P G K G L E W V S V I Y P S G G P T V Y

Antibody B : GCCGACAGCGTGAAGGGCAGGTTCACCATCAGCAGGGACAACAGCAAGAACACCCTGTAC
A D S V K G R F T I S R D N S K N T L Y

20 Antibody B : CTGCAGATGAACAGCCTGAGGGCCGAGGACACCGCCGTGTACTACTGCGCCAGGGGCGAG
L Q M N S L R A E D T A V Y Y C A R G E

Antibody B : GACTACTACGACAGCAGCGGCCCAGGCGCCTTCGACATCTGGGGCCAGGGCACAATGGTGACCGTGTCCAGC (SEQ ID NO:38)
D Y Y D S S G P G A F D I W G Q G T M V T V S S (SEQ ID NO:39)

25

>Antibody B: LV+LC dna
CAGAGCGCCCTGACCCAGCCCAGAAGCGTGTCCGGCAGCCCAGGCCAGAGCGTGACCATCAGCTGCACCGGCACCAGCAGCGACGTGGGCGGCTACAACTACGTGTC
CTGGTATCAGCAGCACCCCGGCAAGGCCCCCAAGCTGATGATCTACGACGTGTCCAAGAGGCCCAGCGGCGTGCCCGACAGGTTCAGCGGCAGCAAGAGCGGCAACA
30 CCGCCAGCCTGACCATCTCCGGACTGCAGGCCGAGGACGAGGCCGACTACTACTGCTGCAGCTACGCCGGCAGCTACACCCTGGTGTTCGGCGGAGGGACCAAGCTG
ACCGTGCTGGGCCAGCCCAAGGCTGCCCCCAGCGTGACCCTGTTCCCCCCCAGCAGCGAGGAACTGCAGGCCAACAAGGCCACACTGGTGTGCCTGATCAGCGACTT
CTACCCAGGCGCCGTGACCGTGGCCTGGAAGGCCGACAGCAGCCCCGTGAAGGCCGGCGTGGAGACAACCACCCCCAGCAAGCAGAGCAACAACAAGTACGCCGCCA
GCAGCTACCTGAGCCTGACCCCCGAGCAGTGGAAGTCCCACAGGTCCTACAGCTGCCAGGTGACCCACGAGGGCAGCACCGTGGAGAAAACCGTGGCCCCCACCGAG
TGTAGCTGATGA (SEQ ID NO:40)

35

> Antibody B: HV+HC dna
GAGGTGCAATTGCTGGAAAGCGGCGGAGGACTGGTGCAGCCAGGCGGCAGCCTGAGGCTGTCCTGCGCCGCCAGCGGCTTCACCTTCAGCACCTACCAGATGGTGTG
GGTGCGCCAGGCCCCAGGCAAGGGCCTGGAATGGGTGTCCGTGATCTACCCCAGCGGCGGACCCACCGTGTACGCCGACAGCGTGAAGGGCAGGTTCACCATCAGCA
GGGACAACAGCAAGAACACCCTGTACCTGCAGATGAACAGCCTGAGGGCCGAGGACACCGCCGTGTACTACTGCGCCAGGGGCGAGGACTACTACGACAGCAGCGGC
40 CCAGGCGCCTTCGACATCTGGGGCCAGGGCACAATGGTGACCGTGTCCAGCGCCAGCACCAAGGGCCCCAGCGTGTTCCCGCTAGCACCTTCCTCCAAGTCCACCTC
TGGCGGCACCGCCGCTCTGGGCTGCCTGGTGAAGGACTACTTCCCTGAGCCTGTGACCGTGAGCTGGAACTCTGGCGCCCTGACCTCCGGCGTGCATACCTTCCCTG

CCGTGCTGCAGTCCTCCGGCCTGTACTCCCTGTCCTCCGTGGTGACAGTGCCTTCCTCCTCCCTGGGCACCCAGACCTACATCTGCAACGTGAACCACAAGCCTTCC
AACACCAAGGTGGACAAGCGGGTGGAGCCTAAGTCCTGCGACAAGACCCACACCTGCCCTCCCTGCCCTGCCCCTGAGCTGCTGGGCGGACCCTCCGTGTTCCTGTT
CCCTCCTAAGCCTAAGGACACCCTGATGATCTCCCGGACCCCTGAGGTGACCTGCGTGGTGGTGGACGTGTCCCACGAGGACCCAGAGGTGAAGTTTAATTGGTATG
TGGACGGCGTGGAGGTCCACAACGCCAAGACCAAGCCTCGGGAGGAACAGTACAACTCCACCTACCGGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGACTGGCTG
5 AACGGCAAGGAATACAAGTGCAAAGTCTCCAACAAGGCCCTGCCTGCCCCCATCGAGAAAACCATCTCCAAGGCCAAGGGCCAGCCTCGCGAGCCTCAGGTGTACAC
CCTGCCTCCTAGCCGGGAGGAAATGACCAAGAACCAGGTGTCCCTGACCTGTCTGGTGAAGGGCTTCTACCCTTCCGATATCGCCGTGGAGTGGGAGTCCAACGGCC
AGCCTGAGAACAACTACAAGACCACCCCTCCTGTGCTGGACTCCGACGGCTCCTTCTTCCTGTACTCCAAGCTGACCGTGGACAAGTCCCGGTGGCAGCAGGGCAAC
GTGTTCTCCTGCTCCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCCCTGTCCCTGAGCCCTGGCAAGTGA (SEQ ID NO:41)

10 > Antibody B: LV+LC aa
QSALTQPRSVSGSPGQSVTISCTGTSSDVGGYNYVSWYQQHPGKAPKLMIYDVSKRPSGVPDRFSGSKSGNTASLTISGLQAEDEADYYCCSYAGSYTLVFGGGTKL
TVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTE
CSss (SEQ ID NO:42)

15 > Antibody B: HV+HC aa
EVQLLESGGGLVQPGGSLRLSCAASGFTFSTYQMVWVRQAPGKGLEWVSVIYPSGGPTVYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARGEDYYDSSG
PGAFDIWGQGTMVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPS
NTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWL
NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN
20 VFSCSVMHEALHNHYTQKSLSLSPGKs (SEQ ID NO:43)

REFERENCES

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

EQUIVALENTS

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. A method for treating an osteolytic disorder in a subject, the method comprising administering an effective amount of an MMP-14 binding protein comprising a heavy chain immunoglobulin variable domain sequence and a light chain immunoglobulin variable domain sequence to a subject suffering from an osteolytic disorder.

2. The method of claim 1, further comprising administering an additional therapeutic to said individual.

3. The method of claim 2, wherein the additional therapeutic is selected from the group consisting of: bisphosphonates, hormone-related compounds, RANKL antagonists, $\alpha_{\gamma}\beta_{3}$ antagonists, Src inhibitors, cathepsin K inhibitors and calcitonin.

4. The method of claim 3, wherein the additional therapeutic is a bisphosphonate.

5. The method of claim 2, wherein the additional therapeutic is an MMP-9 binding protein.

6. The method of claim 1, wherein the MMP-14 binding protein inhibits MMP-14.

7. The method of any one of claims 1, 2, 3, 4, 5, or 6, wherein the osteolytic disorder is selected from the group consisting of: osteotropic cancer and osteoporosis.

8. The method of claim 1, wherein the heavy chain immunoglobulin variable domain sequence comprises the heavy chain CDRs of M0038-F01 and the light chain immunoglobulin variable domain sequence comprises the CDRs of M0038-F01.

9. A method for preventing an osteolytic disorder in a subject, the method comprising administering an effective amount of an MMP-14 binding protein comprising a heavy chain immunoglobulin variable domain sequence and a light chain immunoglobulin variable domain sequence to a subject at risk for an osteolytic disorder.

10. The method of claim 9, wherein the osteolytic disorder prevented is selected from the group consisting of: osteotropic cancer and osteoporosis.

11. The method of claim 9, further comprising administering an additional therapeutic to said individual.

12. The method of claim 11, wherein the additional therapeutic is selected from the group consisting of: bisphosphonates, hormone-related compounds, RANKL antagonists, $\alpha_\gamma\beta_3$ antagonists, Src inhibitors, cathepsin K inhibitors and calcitonin.

13. The method of claim 12, wherein the additional therapeutic is a bisphosphonate.

14. The method of claim 11, wherein the additional therapeutic is an MMP-9 binding protein.

15. The method of claim 9, wherein the MMP-14 binding protein inhibits MMP-14.

16. The method of claim 9, wherein the heavy chain immunoglobulin variable domain sequence comprises the heavy chain CDRs of M0038-F01 and the light chain immunoglobulin variable domain sequence comprises the CDRs of M0038-F01.

17. A kit comprising:

a container comprising an MMP-14 binding protein comprising a heavy chain immunoglobulin variable domain sequence and a light chain immunoglobulin variable domain sequence and

instructions for use of said MMP-14 binding protein for the treatment of an osteolytic disorder.

18. The kit of claim 17, wherein the osteolytic disorder is selected from the group consisting of: osteotropic cancer and osteoporosis.

19. The kit of claim 17, further comprising an additional therapeutic.

20. The kit of claim 19, wherein the additional therapeutic is an MMP-9 binding protein.

21. The kit of claim 17, wherein the heavy chain immunoglobulin variable domain sequence comprises the heavy chain CDRs of M0038-F01 and the light chain immunoglobulin variable domain sequence comprises the CDRs of M0038-F01.

22. A method for treating an osteolytic disorder in a subject, the method comprising administering an effective amount of an MMP-9 binding protein comprising a heavy chain immunoglobulin variable domain sequence and a light chain

immunoglobulin variable domain sequence to a subject suffering from an osteolytic disorder.

23. The method of claim 22, further comprising administering an additional therapeutic to said individual.

5 24. The method of claim 23, wherein the additional therapeutic is selected from the group consisting of: bisphosphonates, hormone-related compounds, RANKL antagonists, $\alpha_\gamma\beta_3$ antagonists, Src inhibitors, cathepsin K inhibitors and calcitonin.

25. The method of claim 24, wherein the additional therapeutic is a bisphosphonate.

10 26. The method of claim 23, wherein the additional therapeutic is an MMP-14 binding protein.

27. The method of claim 22, wherein the MMP-9 binding protein inhibits MMP-9.

28. The method of any one of claims 22, 23, 24, 25, 26, or 27, wherein the

15 osteolytic disorder is selected from the group consisting of: osteotropic cancer and osteoporosis.

29. The method of claim 22, wherein the heavy chain immunoglobulin variable domain sequence comprises the heavy chain CDRs of 539A-M0240-B03 and the light chain immunoglobulin variable domain sequence comprises the CDRs of 539A-M0240-

20 B03.

30. A method for preventing an osteolytic disorder in a subject, the method comprising administering an effective amount of an MMP-9 binding protein comprising a heavy chain immunoglobulin variable domain sequence and a light chain immunoglobulin variable domain sequence to a subject at risk for an osteolytic disorder.

25 31. The method of claim 30, wherein the osteolytic disorder prevented is selected from the group consisting of: osteotropic cancer and osteoporosis.

32. The method of claim 30, further comprising administering an additional therapeutic to said individual.

33. The method of claim 32, wherein the additional therapeutic is selected from

30 the group consisting of: bisphosphonates, hormone-related compounds, RANKL antagonists, $\alpha_\gamma\beta_3$ antagonists, Src inhibitors, cathepsin K inhibitors and calcitonin.

34. The method of claim 33, wherein the additional therapeutic is a bisphosphonate.

35. The method of claim 32, wherein the additional therapeutic is an MMP-14 binding protein.

36. The method of claim 30, wherein the MMP-9 binding protein inhibits MMP-9.

37. The method of claim 30, wherein the heavy chain immunoglobulin variable domain sequence comprises the heavy chain CDRs of 539A-M0240-B03 and the light chain immunoglobulin variable domain sequence comprises the CDRs of 539A-M0240-B03.

38. A kit comprising:

a container comprising an MMP-9 binding protein comprising a heavy chain immunoglobulin variable domain sequence and a light chain immunoglobulin variable domain sequence and

instructions for use of said MMP-9 binding protein for the treatment of an osteolytic disorder.

39. The kit of claim 38, wherein the osteolytic disorder is selected from the group consisting of: osteotropic cancer and osteoporosis.

40. The kit of claim 38, further comprising an additional therapeutic.

41. The kit of claim 40, wherein the additional therapeutic is an MMP-14 binding protein.

42. The kit of claim 38, wherein the heavy chain immunoglobulin variable domain sequence comprises the heavy chain CDRs of 539A-M0240-B03 and the light chain immunoglobulin variable domain sequence comprises the CDRs of 539A-M0240-B03.

FIGURE 1

```
  1 M0031-C02 SC=SC-001 Round=SC-001-SR-003
HC
        1    5     0      5     0      5     0      5     0      5     0
      1 EVQLLESGGG LVQPGGSLRL SCAASGFTFS PYPMGWVRQA PGKGLEWVSS

        5    5      6     6      7     7      8 8   8 8   8 8   9   9
        1 a  5      0     5      0     5      0 2abc3 5   7 9   2   5
     51 IVSSGGLTLY ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGG

             1          1 1      1
        9    0          0 0      1
        7    2abcd efghi3 5      0
    101 RLYDILTGQG APFDYWGQGT LVTVSS

LC
      1 QDIQMTQSPL SLPVTPGEPA SISCRSSQSL LHSNGYYYLD WYLQKPGQSP
     51 QLLIYLGSYR ASGVPDRFSG SGSGTDFTLK ISSVEAEDVG VYYCMQALQT
    101 PLTFGGGTRV DIK

------
  2 M0031-F01 SC=SC-001 Round=SC-001-SR-003
HC
      1 EVQLLESGGG LVQPGGSLRL SCAASGFTFS TYEMHWVRQA PGKGLEWVSS
     51 IYSSGGWTGY ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARSQ
    101 QYYDFSSRYY GMDVWGQGTT VTVSS
LC
      1 QSELTQPPSV SGTPGQRVTI SCSGTSANIG RNAVHWYQQL PGTAPKLLIH
     51 SNNRRPSGVP DRFSGSKSGT SASLAISGLQ SEDEADYYCA AWENSLNAFY
    101 VFGTGTKVTV L

------
  3 M0033-H07 SC=SC-001 Round=SC-001-SR-003
HC
      1 EVQLLESGGG LVQPGGSLRL SCAASGFTFS VYGMVWVRQA PGKGLEWVSV
     51 ISSSGGSTWY ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TALYYCARPF
    101 SRRYGVFDYW GQGTLVTVSS
LC
      1 QDIQMTQSPS SLSASVGDRV TITCRASQGI RNFLAWYQQK PGKVPKLLVF
     51 GASALQSGVP SRFSGSGSGT DFTLTISGLQ PEDVATYYCQ KYNGVPLTFG
    101 GGTKVEIK

------
  4 M0037-C09 SC=SC-001 Round=SC-001-SR-003
HC
      1 EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYEMFWVRQA PGKGLEWVSS
     51 ISPSGGQTHY ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCATDR
    101 TYYDFWSGYG PLWYWGQGTL VTVSS
LC
      1 QDIQMTQSPL SLPVTLGESA SVSCRSSQSL LHENGHNYLD WYLQKPGQSP
     51 QLLIYLGSNR ASGVPDRFSG SGSGTDFTLK ISRVEAEDVG VYYCMQSLKT
    101 PPTFGPGTKV EIK
```

FIGURE 1 (cont.)

```
------
  5 M0037-D01 SC=SC-001 Round=SC-001-SR-003
HC
    1 EVQLLESGGG LVQPGGSLRL SCAASGFTFS MYMMIWVRQA PGKGLEWVSS
   51 IYPSGGNTMY ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCATGV
  101 LRYFDWDAGS GMDVWGQGTT VTVSS
LC
    1 QDIQMTQSPS SLSASVGDRV TITCRASQGI RNDLGWYQQK PGKAPKRLIY
   51 VASSLQSGVP SRFSGSGSGT EFTLTISSLQ PEDFATYYCL QHNSYPWTFG
  101 QGTKVEIK

------
  6 M0038-E06 SC=SC-001 Round=SC-001-SR-003
HC
    1 EVQLLESGGG LVQPGGSLRL SCAASGFTFS PYVMHWVRQA PGKGLEWVSS
   51 ISPSGGWTYY ADSVKGRFTI SRDNSKNTLY LQMNSLRAED MAVYYCARGT
  101 GAYGMDVWGQ GTTVTVSS
LC
    1 QDIQMTQSPG TLSLSPGDRA TLSCGASQLV VSNYIAWYQQ KPGQAPRLLM
   51 YAGSIRATGI PDRFSGSGSG TDFTLTISRL EPEDFAIYYC QQRSNWPWTF
  101 GQGTKVEIK

------
  7 M0038-F01 SC=SC-001 Round=SC-001-SR-003
HC
    1 EVQLLESGGG LVQPGGSLRL SCAASGFTFS LYSMNWVRQA PGKGLEWVSS
   51 IYSSGGSTLY ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARGR
  101 AFDIWGQGTM VTVSS
LC
    1 QDIQMTQSPS SLSAFVGDKV TITCRASQSV GTYLNWYQQK AGKAPELLIY
   51 ATSNLRSGVP SRFSGSGSGT DFTLTINTLQ PEDFATYYCQ QSYSIPRFTF
  101 GPGTKVDIK

------
  8 M0038-F08 SC=SC-001 Round=SC-001-SR-003
HC
    1 EVQLLESGGG LVQPGGSLRL SCAASGFTFS RYKMWWVRQA PGKGLEWVSG
   51 IRPSGGLTRY ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARRG
  101 DYVGGFDYWG QGTLVTVSS
LC
    1 QDIQMTQSPG TLSLSPGERA TLSCRASQSV SSSYLAWYQQ KPGQAPRLLI
   51 YGASSRATGI PDRFSGSGSG TDFTLTISRL EPEDFAVYYC QHYGGSQAFG
  101 GGTKVEIK

------
  9 M0039-H08 SC=SC-001 Round=SC-001-SR-003
HC
    1 EVQLLESGGG LVQPGGSLRL SCAASGFTFS AYNMGWVRQA PGKGLEWVSS
   51 ISSSGGYTGY ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARDL
  101 YRGFDYWGQG TLVTVSS
LC
    1 QDIQMTQSPA TLSVSPGERA TLSCRASESV KNNLAWYQQK PGQAPRLLIY
   51 GVSTRAPGIP ARFSGSGSGT DFTLTISSLE PEDFAVYYCQ QRSNWPPVTF
  101 GQGTRLEIK
```

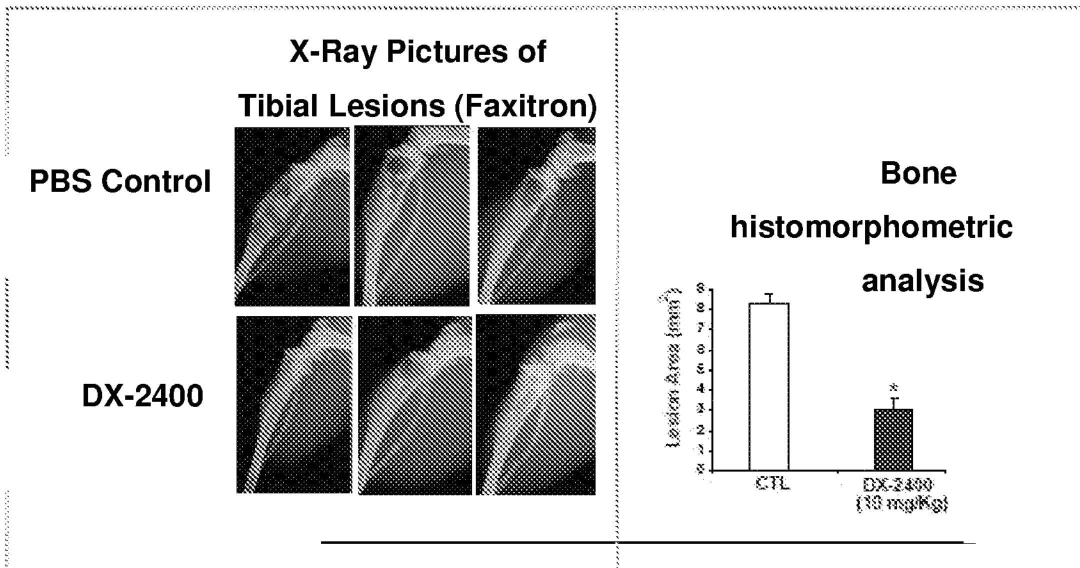
FIGURE 1 (cont.)

```
------
 10 M0040-A06 SC=SC-001 Round=SC-001-SR-003
HC
     1 EVQLLESGGG LVQPGGSLRL SCAASGFTFS YYGMYWVRQA PGKGLEWVSS
    51 ISSSGGYTDY ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARRI
   101 KYYDIEGEGA FDIWGQGTMV TVSS
LC
     1 QDIVMTQTPP SLPVNPGEPA SISCRSSQSL LHRNGYNYLD WYLQKPGQSP
    51 QLLIHLGSYR ASGVPDRFSG SGSGTDFTLK ISRVEAEDVG VYYCMQPLQT
   101 PFTFGPGTKV DIK

------
 11 M0040-A11 SC=SC-001 Round=SC-001-SR-003
HC
     1 EVQLLESGGG LVQPGGSLRL SCAASGFTFS HYVMFWVRQA PGKGLEWVSR
    51 IVPSGGATMY ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARDR
   101 PLYDSSGYVD YWGQGTLVTV SS
LC
     1 QSALTQPPSA SGSPGQSVTI SCTGTSSDVG AYNYVSWYQQ HPDKAPKLII
    51 YNVNERPSGV PDRFSGSKSG NTASLTVSGL QAEDEADYYC TSYAGSNKIG
   101 VSGTGTKVTV L

------
 12 M0043-G02 SC=SC-001 Round=SC-001-SR-003
HC
     1 EVQLLESGGG LVQPGGSLRL SCAASGFTFS WYPMFWVRQA PGKGLEWVSG
    51 IYSSGGPTDY ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAKDT
   101 LGRYYDFWSG YSYGMDVWGQ GTTVTVSS
LC
     1 QDIQMTQSPG TLSLSPGERA TLSCRASQSV SSSYLAWYQQ KPGQAPRLLI
    51 YGASSRATGI PDRFSGSGSG TDFTLTISRL EPEDFAVYYC QSGVTFGGGT
   101 KVEIK
```

FIGURE 2



P value to vehicle control ***, p<0.05***

FIGURE 3

Initial Name	LV-CDR1	LV-CDR2	LV-CDR3	HV-CDR1	HV-CDR2	HV-CDR3
539A-M0072-H10	RASQSVSSYLA	DASNRAT	QQRGNWPIT	PYRMS	SIGSSGGQTSYADSVKG	EPPGYYFDS
539A-M0072-G08	RASQSVSSYLA	DASNRAT	QQRSNWPIT	AYGMV	VIRSSGGPTSYADSVKG	AGGGTYLDY
539A-M0071-E10	RASQSVSSYLA	DASNRAT	QQRSNWPLT	HYRMY	YIGSSGGMTSYADSVKG	
539A-M0076-D07	RASQSVSTFLA	DASNRAT	QQYASPPRT	GYYMS		DSGQTFYYAFDI
539A-M0081-G03	RTSHNVANFLA	DAYNRAT	QQRANWPLS	RYPME	YISSSGGWTSYADSVKG	DGLELFGGWLES
539A-M0075-D06	RTSQSVSDSLA	DASNRAT	QQRGSWPIT	NYRMM	YIGSSGGMTSYADSVKG	ETNWNDLGRYFDY
539A-M0071-D03	RASQSISSSFLA	GASSRAT	QQTYSTPLT	KYSMV	VISPSGGYTGYADSVKG	MRVPAAIGGWLDP
539A-M0072-H07	RASQSVSSNLA	GASTRAT	HQYNDWPLT	PYKMY	YIGSSGGMTSYADSVKG	RGYSSGPLRY
539A-M0081-D05	RASESISRNLA			MYRMS	YIGSSGGPTAYADSVKG	EGDARVPAAIGY
539A-M0071-E02	KSSQNVLLSSNSKNYLA	WASTRES	QQYYSIPWS	NYRMS	SIGSSGGQTMYADSVKG	SHPVSGGVFDF
539A-M0075-B09	KSSQSILYSSNNRNYLA	WASTRES	QHYYTAPYT	GYSMH	SIWPSGGYTRYADSVKG	GNDSDSFAYRF
539A-M0075-G09	KSSQSVLYSSNNKNYLA	WASTRES	QQYYSTPLT	EYRMT	YIGSSGGMTTYADSVKG	GSGSGYDS
539A-M0074-D05	KSSQSVLYSSNNKNYLA	WASTRES	QQSYSTPLT	AYRMH	YIGSSGGMTTYADSVKG	STVTTLDY
539A-M0073-G12	RASQSVSSNLA	GASTRAT	QQYNKWPQT	IYRMH	YIGSSGGNTSYADSVKG	EWVGSSAALDY
539A-M0074-G03	RASQTISSYYLA	GASSRAA	QQYGVSPPYS	YYNMV	VISPSGGWTPYADSVKG	EVGGSGWLGDAFDI
539A-M0075-F03	RASQSVGSDYLA	AASTRAT	QQRSSWPPT	KYYMV	YISPSGGGTYYADSVKG	NYYDSSGTRGAFDI
539A-M0071-G11	RASETVRYGQVA	DASKRAT	QQRSNWPLT	LYRMN	YIGSSGGATAYADSVKG	SMRGGHLDS
539A-M0075-D11	RASHSVGGGYLA	DAFNRAT	QQRSEWPWT	RYKMS	YIGSSGGMTSYADSVKG	DLTATGYFDY
539A-M0072-F05	RASQSISSHLA	GASNRAT	QQRSNWPPT	AYRMQ	YIGSSGGQTSYADSVKG	DPVGAKYYGMDV
539A-M0073-C11	RASQSVSSSYLA	DASNRAT	QQRSNWPIT	NYRMH	WISSSGGPTSYADSVKG	GGSYRHNNVFDI
539A-M0074-E11	RASQSVSSSYLA	GASSR		QYRMF	YIGSSGGMTSYADSVKG	SMGYGDAFDI

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539A-M0073-G10	RASQTVSRNYLA	DASKRAT	QQRSNWPPT			
539A-M0078-G07	RASQSVSSDLA	GVSTKAT	QQYHNWPPLT	SYTME	WISPSGGYTFYADSVKG	GYSYGSIDL
539A-M0071-A06	RSSQSLVSSNGNTYLN	YKVSNRDS	MQGTHWPYT	MYRMM	YIGSSGGMTSYADSVKG	DSVFRGERDAFDI
539A-M0071-D11	RASQNIGKFLA	GASTLQL	QKYDSALWT	GYGMW	SISPSGGWTFYADSVKG	VKVRHGGGFDY
539A-M0071-A04	RSSESLLQSSGHTRFD	LGFNRAS	MHALEPPYT	YYQMM	YISPSGGMTLYADSVKG	GWGYFDY
539A-M0084-E03	RASQSIDTYLN	AASKLED	QQSYSSPGIT	HYDMS	SIWPSGGVTWYADSVKG	GGYNNYYYALDV
539A-M0072-C12	RASQDIRSSLA	AASSLQS	QQANSFPPT	SYRMQ	YIGSSGGMTSYADSVKG	GSWRGGSQYFDY
539A-M0081-E01	RASQSISSYLN	AASSLQS	QQSYSTPRT	HYVMS	SIGSSGGDTHYADSVKG	VWISGSYLDAFDI
539A-M0082-F03	RASQSTSNSLS	AASRLQS	QQSWRTPLT	QYWMT	GIGPSGGPTTYADSVKG	HSTTVTTNFDY
539A-M0076-D03	RASQGIRNDLD	SASNLQS	LQHNSFPLT	LYRMN	YIGSSGGATAYADSVKG	GAWYLDS
539A-M0075-G12	RASQGIRNDLG	AASSLQS	QQTITFPLT	SYRMM	WISSSGGSTGYADSVKG	TTVTRVGSFYFDL
539A-M0072-C04	RASQGIRNDLG	AASSLQS	QQLNSYPPT	PYRMH	RIGSSGGATSYADSVKG	DGIAVAGIAFDI
539A-M0075-A07	RASQGISSALA	DASSLES	QQFHTYPFT	TYRMV	YIGSSGGQTAYADSVKG	HNRAIGTFDY
539A-M0076-H03	RASQGVSNYLA	AASTLQS	QKYNSAPYT	NYSMG	GIYSSGGYTQYADSVKG	GHYVWDSGWYSAFDI
539A-M0085-H01	RASQNIAGLLA	KASTLES	QQYSFNSGT	KYHMH	SISPSGGVTSYADSVKG	
539A-M0085-G04	RASQRISIYLN	AAYNLQS	QQSDSSPzT			
539A-M0071-E12	RASQSISSDLN	AASSLQS	QQSYSTPVT	DYRMF	SISSSGGFTNYADSVKG	DQGGTVVVVATADY
539A-M0072-H08	RASQSISSTITYLN	AASNRAT	QQRSNWPPT	DYKMW	SIRSSGGPIGYADSVKG	ETNQMGMDV
539A-M0071-F10	RASQSISSWLA	KASSLES	QQYNSYPWT	KYKMF	SIGSSGGATSYADSVKG	GGFWSGYYGY
539A-M0083-A05	RASQSISSYLN	AASSLQS	QQSYSTPRT	HYPMS	YIYSSGGDTEYADSVKG	YGSGGWMTYGLDV
539A-M0082-G08	RASQSISSYLN	AASSLQS	QQSYSTPRT	MYYMY	SIRSSGGETQYADSVKG	VWISGSYLDAFDI
539A-M0072-F02	RASQSISSYLN	AASSLQS	QQSYSTPRT	HYVMS	SIGSSGGDTHYADSVKG	VWISGSYLDAFDI
539A-M0071-F09	RASQSISSYLN	GASSLQS	QQSYSIPRT	WYKMA	VIYPSGGPTFYADSVKG	GQRGYNYDRSSYSYHYYYGMDV

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539A-M0082-G09	RATQYISNYVN	AASSLQS	QQANSFPPT	AYSMH	RLGSSGGPTSYADSVKG	RSSYGRGFDY
539A-M0071-A05	RASQGISNYLA	AASNLQS	QQYKTYPFT	PYRMH	YIGSSGGPTAYADSVKG	ARAGTFFDS
539A-M0076-E11	RASQGISRWLA	DASNRAT	QQRSNWPPRLT	FYHMS	SIGPSGGWTNYADSVKG	DGGLEGMDV
539A-M0081-B03	RASQGISSWLA	AASSLQS	QQANSFPYLT	TYMMM	SIWSSGGSTFYADSVKG	GVVVPALDY
539A-M0071-H10	RASQGISSWLA	AASTLQS	QPTYSTSWT	TYSMV	RIGSSGGDTFYADSVKG	DRADTVVTAGGDYYYYYGMDV
539A-M0075-D12	RASQGISSWLA	GASSLES	QQANSFPPT	DYRMT	WIGSSGGQTSYADSVKG	GTPRVASYFDY
539A-M0072-B02	RASQSISSWLA	KASSLES	QQYNSYPWT	NYKMH	SIGSSGGMTSYADSVKG	RDWQHLAGDAFDF
539A-M0071-E03	RASQGISSWLA	YATSSLQS	QQSKSFPPT	RYRMN	YIGSSGGNTAYADSVKG	RRIGVGAKGGGTFDI
539A-M0082-G01	QASQDIGNYLN	DTSILKK	QQANSFzLT	LYNMW	WISSSGGNTKYADSVKG	GAPYYLQL
539A-M0074-D09		DVSNRAT	QQRSNWPLT	MYRMI	WIGSSGGQTSYADSVKG	GLWCDN
539A-M0071-H05	SGDKLGDKYAS	QDRKRPS	QAWDSNTVV	HYDMW	RIVPSGGLTTYADSVKG	HSFWSGYYGAFDI
539A-M0075-H05	TGTSSDVGYYNYVS	DVSARPS	CSYAGSYTYV	MYYMQ	SIRSSGGFTSYADSVKG	GLRLDM
539A-M0113-A08				RYHMF	YISPSGGVTMYADSVKG	GAPSGTFFDY
539A-M0116-E02	RASQSVSSYLA	DASTRAT	HQRSNWPQT	RYRML		RAKKGAFDI
539A-M0131-F06	RASQSVTNNLA	GASTRAT	QQYNNWPRT	EYMMW	RIGSSGGITSYADSVKG	QHYGDYDY
539A-M0131-B05	RASQSISSYLN	AASSLQS		IYNMY	YIYSSGGPTAYADSVKG	RGYYDSSGYWGAFDI
539A-M0133-H11	RASQSVSSSYLA	GASSRAT	QQYGSSHT	HYLMV	GIVSSGGYTAYADSVKG	GAYDSSGIVFDY
539A-M0113-F03	RASQSVSINLA	GASTRAT	QQYDNWWT	QYVMS	SIVPSGGYTSYADSVKG	SLRPGFGELPGY
539A-M0114-D01	RSSQSLLHNNGYNYLD	LGSYRAS	MQALQTPIT	EYAML	YISPSGGSTFYADSVKG	GGTKKSI
539A-M0133-A06	RSSQSLLHRNGQNYLD	LGSNRAS	MQGLQTPRT	MYEMQ	GISPSGGKTGYADSVKG	SRYSGSYFPPGGSHFDY
539A-M0133-H10	RASQSISSYLN	AASSLQS	QQSYSTPWT	PYAMV	WISPSGGDTDYADSVKG	GFGWFDDAFDI
539A-M0133-B09	WASQDVSSFFA	SASTLQG	QQYNTFPWT	MYNMI	VIRSSGGPTSYADSVKG	VVGAAGILQPFDY
539A-M0114-A05	RASQSIDTYLN	AASKLED	QQSYSSPGIT	RYGMG	VIWPSGGSTYYADSVKG	VRDYYDSSGHYFSDAFDI

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539A-M0131-A08	QASQDISNYLN	DASSLQS	EQANSFPLT	SYDMN	GIGSSGGLTEYADSIKG	DRGYNNYYYYGMDV
539A-M0133-C11	RASHGARVDLA	GTSSLQR	LQHNSYPLT	MYDMS	YISSSGGFTMYADSVKG	DLNSSSPPGSNDAFDI
539A-M0113-H02	RASQDISNYLA	AASTLQS	QQSYSAPFT	KYLMN	SISPSGGMTAYADSVKG	MMKETEYDTNWYFAFDY
539A-M0114-F01	RASQGISTFLA	GASTLQS	QKYNSAPFT	KYQMQ	YIVPSGGLTDYADSVKG	GGRGGYTHFDF
539A-M0132-C03	RASQSISRYLN	AAASLQS	QQSYSTPPLT	GYGMA	GIYSSGGWTAYADSVKG	DLSGSYSD
539A-M0132-C05	RASQGISNWLA	FGASNLQS	QQADSFPIT	HYSMV	YIWPSGGTTKYADSVKG	GWFTDAFDI
539A-M0114-E08	RANQRISTYLA	AGSTLQS	QQTITFPLT	SYTMS	SISSSGGFTVYADSAKG	EGGTFPVYYFDN
539A-M0113-G09	RASQGISSWLA	AASSLQS	QQANSFPLT		YIGPSGGYTAYADSVKG	DPSYYDSSGYDAFDI
539A-M0133-B06	RASQGISSWLA	AASSLQS	QQANSFPIT	PYDMH	SIGPSGGVTFYADSVKG	EIPGDSGYDDY
539A-M0133-C02	RASQGITTWLA	SASTLHS	QQANNFPYT	AYSMG	VIGSSGGYTNYADSVKG	RPHSTGTDAFDI
539A-M0113-E04	RASQSIGSWLA	KASSLEG	QQSYSTPLT	GYIMG	SISPSGGITMYADSVKG	DNWNDGAFDI
539A-M0133-D09	RASQYIRNDLG	AASTLQS	LQDYSYPQT	YYPMG	SIYSSGGKTQYADSVKG	GRYGDFDY
539A-M0114-H04	RSSQSLVHSDGNTYLN	KVSNRDS	MQGTHWPWT	NYTMF	VISPSGGNTAYADSVKG	FAGKN
539A-M0132-F12	SGSSSNIGSNTVY	RNNQRPL	AAWDDSLSTWV	VYDMM	GISPSGGYTKYADSVKG	HRLRFLEDAFDI

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